

# Changes in Clinical Presentation and Epidemiology of Respiratory Pathogens Associated With Acute Respiratory Illness in Military Trainees After Reintroduction of Adenovirus Vaccine

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**Background.** Adenovirus (Ad) has long been the predominant cause of acute respiratory illness (ARI) in military trainees. In 2011, live oral Ad vaccines for serotypes 4 and 7 were reintroduced into US basic military training populations. This study evaluated the impact on clinical presentations and other respiratory pathogens.

**Methods.** The Center for Advanced Molecular Detection at Joint Base San Antonio-Lackland prospectively collects demographic, clinical, and polymerase chain reaction data from respiratory specimens (throat swab and nasal wash) among Air Force trainees presenting for care of ARI.

**Results.** From June 2008 to August 2013, 2660 trainees enrolled and were tested for selected respiratory pathogens. Post-vaccine introduction (VI), reported systemic symptoms were less frequent, including fever (38% vs 94%) and myalgia (37% vs 67%;  $P < .01$ ). Median temperature and heart rate decreased (98.4 vs 101.3°F, 81 vs 96 beats per minute;  $P < .01$ ). Ad detection decreased for all Ad (3% vs 68%), Ad4 (1% vs 70%), 7 (0% vs 8%), 14 (0% vs 5%), and 3 (0.1% vs 2%);  $P < .01$ . Rhinovirus and cases with no pathogen identified increased in frequency (35% vs 18%, 51% vs 14%;  $P < .01$ ).

**Conclusions.** Acute respiratory illness in military trainees post-VI is associated with decreased severity of systemic symptoms and reduced fever and heart rate. Marked reductions in frequency of Ad serotypes are seen, including those in the vaccine, with no serotype shift. However, detection of several other respiratory pathogens, most notably rhinovirus, is observed in increasing proportions, and a majority are now undiagnosed clinical syndromes.

**Keywords.** adenovirus; febrile respiratory illness; military trainees; upper respiratory infection; vaccine.

Acute respiratory infections (ARIs), particularly those caused by adenovirus (Ad), are extremely common during military basic training, where they are the leading cause of medical encounters, time lost from training,

and hospital bed-days [1]. Trainees are uniquely vulnerable for numerous reasons, including the stress of training, close living and working conditions, environmental persistence of Ad, decreases in time for personal hygiene, and constant introduction of immunologic naives [2, 3]. Seminal studies performed in the 1950s demonstrated that approximately 80% of military personnel became infected with Ad over the course of basic training, with 40% experiencing clinical illness and 20% requiring hospitalization [4].

In 1971, a military vaccination program began with live, oral vaccines for Ad serotypes 4 and 7. During this time, 50%–60% reductions in the rate of febrile respiratory illness (FRI) were reported, as well as a >95%

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reduction in FRI due to Ad [5]. However, the military was the only purchaser of vaccine, which was produced by a sole manufacturer, and vaccine production ceased in 1995 when funding requirements for updating manufacturing facilities were not met. Attempts by the Department of Defense to find an alternative solution were unsuccessful, and existing vaccine stocks were depleted in 1999. Ad quickly re-emerged as the major cause of morbidity, causing numerous outbreaks with both vaccine and nonvaccine serotypes, and directly resulting in approximately 1 death per year [6–10]. From 1997 to 2012, Ad (primarily serotype 4) caused 68% of all FRI in the basic training environment [11]. It is estimated that associated medical care and time lost from training resulted in costs of \$10–\$26 million per year [12, 13].

In 2001, the US Food and Drug Administration's (FDA) new-product approval process was initiated for resumption of production of Ad4 and Ad7 vaccines by a new manufacturer [14]. Phase 3 vaccine trials demonstrated (1) 99% efficacy for Ad4 and (2) Ad7 (which was not circulating at the time) seroconversion rates of 95% [15]. In 2011, the 2 vaccines were approved by the FDA and reintroduced nearly simultaneously at all 8 US military basic training locations in October/November of that year. Since then, surveillance reports have consistently demonstrated great reductions in FRI and Ad-related illness. Early data indicated a 75% decrease in FRI, and proportions of collected specimens positive for Ad decreased from 75% to 1% in the months surrounding vaccine introduction (VI) [11]. Nearly all of this was attributable to Ad4, with rare detections of serotypes 7, 3, 14, and 21, and 3 cases involving vaccine-type 4p. Follow-up data published in late 2014, evaluating surveillance data from 1996 to 2013, reported reductions in Ad disease burden from 5.9 to 0.02 cases/person-week [16]. The authors estimated that the current vaccines prevent 13 000 cases of FRI, 1100–2700 hospitalizations, and 1 death per year. After VI, Ad14 became the most prevalent circulating serotype, although actual number of cases detected decreased from approximately 610 per year to 44 in 2013. This large study reported comprehensive surveillance data for overall Ad and FRI, but clinical data were not captured. Whether the clinical presentation of those trainees who do present for care with a respiratory illness has changed post-VI is unclear. Because respiratory illness remains a leading cause of presentation for care among military trainees, understanding of trends in clinical presentation and emerging, non-Ad respiratory pathogens in the post-VI era requires evaluation.

The purpose of this study was to evaluate (1) changes in clinical presentations of ARI pre- and post-VI, and (2) reductions in proportions of disease due to Ad. We also sought to further evaluate for evidence of nonvaccine type serotype shift and to determine whether the frequencies of common non-Ad respiratory pathogens have changed after VI, in trainees presenting for care of ARI, which have not previously been described in the published literature.

## METHODS

### Setting

Joint Base San Antonio (JBSA)-Lackland, Texas, is the sole basic military training site for the US Air Force. Training lasts 8.5 weeks, with 6000–7000 recruits present at any given time, and approximately 43 000 training per year. Units consisting of 50–60 individuals train together and live in open bay dormitories. The population is approximately 80% male. Ill or injured trainees present for care at an outpatient medical clinic; those with respiratory illness and fever are then cohorted until well enough to return to training. Those requiring hospitalization are admitted to the local military tertiary care hospital.

Vaccines, including Ad vaccine beginning week 48 of 2011, and influenza vaccine seasonally, are administered during the first week. In 2009, influenza vaccine against pdm09H1N1 influenza was available and administered after December 1, 2009. Oseltamivir was used for prophylaxis of close contacts of confirmed influenza cases throughout the study period. Prophylaxis for *Streptococcus pyogenes* is also administered to all trainees during the first week, consisting of benzathine penicillin, or azithromycin for penicillin allergic individuals.

### Study Design and Procedures

Since 2003, the Center for Advanced Molecular Detection (59th Medical Wing/Science and Technology, Air Education and Training Command) has prospectively evaluated epidemiology of respiratory pathogens and novel technologies for detection. For the purposes of this substudy, data were evaluated from June 2008 to August 2013. Ill recruits presenting for clinical care of ARI at the outpatient clinic or hospital were approached by study personnel regarding participation. Inclusion criteria were met if the trainee was  $\geq 17$  years of age and endorsed any symptom of respiratory infection, including cough, coryza, sore throat, or nasal or sinus congestion. For those consenting to enroll in the study, demographic information was collected, along with a symptom questionnaire, including self-reported stress levels, and clinical signs, including vital signs and physical examination findings recorded during the medical encounter. Provider diagnoses given at the time of the visit were also recorded when available. Provider clinical diagnosis extracted from the note, if any, associated with the visit, was also explored with reference to diagnoses of upper respiratory tract infection (URTI) vs lower respiratory tract infection (LRTI). The diagnoses, "rhinitis, conjunctivitis, otitis, sinusitis, pharyngitis, sore throat" were considered to be representative of URTI, and the terms "bronchitis, pneumonia" were representative of LRTI. Terms including "common cold", "viral syndrome", "fever", or "cough" were not included in the URTI vs LRTI analysis given their lack of anatomical description. Nasal washes and throat swabs were collected for polymerase chain reaction (PCR) assays. Duplicate presentations for multiple

ARI-related visits were excluded; each case represents an individual subject.

Specimen processing was performed as previously described [17]. Respiratory specimens were characterized daily by PCR on Applied Biosystems (ABI) 7500 and 7900HTFast (Applied Biosystems, CA) and the ViiA 7 real-time PCR systems. Specimens were tested for Ad (panAd and serotypes 4, 7, 14, 3, 11, 21, 2, and 5); rhinovirus; influenza A, including H1 and H3; influenza B, enterovirus, human coronaviruses OC43 and 229E, bocavirus, human metapneumovirus (HMPV), parainfluenza virus type 3 (HPIV), *S pyogenes*, *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*, and *Chlamydomphila pneumoniae*. All primer and probe sequences used have been previously reported (Association of Public Health Laboratories guidelines for real-time reverse transcription-PCR assays of influenza respiratory viruses; non-influenza respiratory viruses from clinical respiratory specimens; respiratory bacterial pathogens) [18, 19]. Influenza A and B, enterovirus and rhinovirus thermocycling conditions were 30 minutes at 50°C, 10 minutes at 95°C, followed by 15 seconds at 95°C and 35 seconds at 60°C for 45×. Coronaviruses, HMPV, and HPIV thermocycling conditions were 10 minutes at 48°C, 10 minutes at 95°C, followed by 15 seconds at 95°C and 1 minute at 60°C for 45×. *Streptococcus pneumoniae*, *S pyogenes*, and bocavirus thermocycling conditions were 10 minutes at 95°C followed by 15 seconds at 95°C

and 45 seconds at 60°C for 45×. Thermocycling conditions for *M pneumoniae* and *C pneumoniae* were 2 minutes at 50°C, 10 minutes at 95°C, followed by 15 seconds at 95°C and 1 minute at 60°C for 40×.

### Statistical Analysis

Analyses were performed using SPSS (SPSS, version 19.0, SPSS). Dichotomous variables were compared using  $\chi^2$  or Fisher's exact test as applicable. Continuous variables were analyzed using Mann-Whitney *U* test for nonparametric data. All reported *P* values are 2-tailed with statistical significance set at <.05.

### Ethics

Subjects provided voluntary, written informed consent in the presence of ombudsmen, and the study was approved by the JBSA-Lackland Institutional Review Board. This research was conducted in compliance with all applicable international and federal regulations regarding protection of human subjects.

## RESULTS

From June 2008 to August 2013, 2660 trainees enrolled and had specimens tested for respiratory pathogens. Ad VI took place week 48, 2011; 72% of this cohort enrolled pre-VI vs 28% post-VI. None of the pre-VI subjects received Ad vaccine, vs

**Table 1. Demographic Information of Recruits Presenting for Care of Acute Respiratory Infection From June 2008 to August 2013, Pre-VI and Post-VI**

	Total (n = 2660)	Pre-VI (n = 1906)	Post-VI (n = 754)	<i>P</i> Value
Age (years, IQR)	20 (19, 22)	20 (19, 22)	20 (19, 22)	.09
Gender				<.01
Male	2295 (86.3%)	1696 (89.0%)	599 (79.4%)	
Female	365 (13.7%)	210 (11.0%)	155 (20.6%)	
Race				<.01
White	1787 (67.1%)	1278 (69.7%)	509 (67.7%)	
Black	359 (13.4%)	275 (15.0%)	84 (11.2%)	
Hispanic	235 (8.8%)	156 (8.5%)	79 (10.5%)	
Asian	76 (2.8%)	54 (2.9%)	22 (2.9%)	
Native American	11 (0.4%)	9 (0.4%)	2 (0.2%)	
Other/Multiple	116 (4.3%)	61 (3.3%)	55 (7.3%)	
Missing	76 (2.9%)	73 (3.8%)	3 (0.3%)	
Year of enrollment				
2008	137 (5.1%)			
2009	757 (27.9%)			
2010	458 (16.9%)			
2011	634 (23.4%)			
2012	437 (16.1%)			
2013	289 (10.7%)			
Perceived stress level <sup>a</sup>	4 (3, 6)	4 (3, 6)	4 (3, 6)	.09
History of tobacco use	379 (14.2%)	274 (14.4%)	105 (13.9%)	.77

Abbreviations: IQR, interquartile range; VI, vaccine introduction.

<sup>a</sup> On 10-point Likert Scale with 1 representing minimal and 10 representing maximal stress.

**Table 2. Clinical Signs and Symptoms Reported Subjectively and Obtained via Physical Examination, Preadenovirus VI and Post-VI**

	n	Pre-VI	Post-VI	P Value
Days of symptoms (median, IQR)	2660	3.0 (2, 6.3)	6.0 (4, 10)	<.01
<b>Symptoms</b>				
Subjective fever	2659	1798 (94.4%)	287 (38.1%)	<.01
Cough	2659	1659 (87.1%)	695 (92.2%)	<.01
Sore throat	2659	1663 (87.3%)	654 (86.7%)	.70
Sinus congestion	2656	1492 (78.4%)	660 (87.5%)	<.01
Myalgia	2658	1281 (67.3%)	279 (37.0%)	<.01
Coryza	2658	1221 (64.1%)	590 (78.2%)	<.01
Malaise	2654	1111 (58.4%)	377 (50.0%)	<.01
Vomiting	2658	297 (15.6%)	64 (8.5%)	<.01
Diarrhea	2656	211 (11.1%)	42 (5.6%)	<.01
Conjunctivitis	2658	61 (3.2%)	61 (8.1%)	<.01
<b>Vital signs</b>				
Oral temperature		101.3 (100.7, 102.0)	98.4 (98.1, 98.8)	<.01
Heart rate		96.0 (86.0, 104.0)	81.0 (69.0, 91.0)	<.01
Respiratory rate		18.0 (16.0, 18.0)	16.0 (16.0, 18.0)	<.01
Systolic blood pressure		120.0 (114.0, 127.0)	118.0 (111.0, 125.0)	<.01
Diastolic blood pressure		72.0 (66.0, 77.0)	69.0 (62.0, 75.0)	<.01
<b>Physical exam findings</b>				
Sinus tenderness	2066	115 (7.1%)	73 (16.3%)	<.01
Pharyngeal erythema	2253	1157 (70.0%)	218 (36.3%)	<.01
Pharyngeal exudate	2229	276 (15%)	71 (12.0%)	<.01
Tonsillitis	1871	367 (22.8%)	55 (21.0%)	.51
Cervical lymphadenopathy	2242	720 (44.3%)	80 (12.9%)	<.01
Nasal discharge	2247	818 (50%)	321 (52.5%)	.29
<b>Abnormal examination<sup>a</sup></b>				
Lungs	2333	142 (8.6%)	122 (18.1%)	<.01
Tympanic membranes	2255	106 (6.5%)	65 (10.5%)	<.01
Cardiac	2305	12 (0.7%)	2 (0.3%)	.24
Abdominal	497	7 (2.2%)	8 (4.5%)	.15

Abbreviations: IQR, interquartile range; VI, vaccine introduction.

<sup>a</sup> Abnormal examination: any of the following: "decreased breath sounds, rales, crackles, rhonchi, wheezes" for lungs; "abnormal, dull, erythematous, effusion" for tympanic membranes; "gallop, murmur, abnormal rhythm" for cardiac; "abnormal bowel sounds, distended, tender" for abdominal.

93% post-VI. Overall, 86% were male, with a median age of 20 years. Enrollments ranged from 437 to a peak of 757 in 2009 for years with a complete calendar-year of data. Trainees reported a perceived stress level of 4 on a 10-point Likert scale. Post-VI, the male predominance of enrolled subjects decreased from 89% to 79% ( $P < .01$ ) (see Table 1).

Clinical characteristics are presented in Table 2. Before VI, 91% of subjects had a recorded oral temperature  $>100.4^{\circ}\text{F}$ , vs 10% afterward. One or more diagnoses representative of URI, LRTI, or both, were present in 64.3% pre-VI and 74.5% post-VI. Terms associated with lower respiratory tract infection (LRTI) were more commonly included post-VI (26.1% vs 12.9%,  $P < .01$ ) and terms associated with URI were less commonly included post-VI (79.0% vs 89.5%,  $P < .01$ ). Those with the diagnosis of "pneumonia" in the post-VI period were predominantly afebrile (89.5%). Supporting radiographs, if

performed, were unavailable. The term "allergic" or "allergy" was included in none of the diagnoses pre-VI, but was included in 8.1% post-VI ( $P < .01$ ); 75% of these were listed in combination with some other diagnosis of URI or LRTI. Clinical signs and symptoms specifically associated with Ad vs rhinovirus were also compared, with similar findings as those seen in general for pre- and post-VI (data not shown). The exceptions were the loss of statistical significance between those describing malaise and with abnormal tympanic membrane examinations. Those with Ad vs rhinovirus also more often described sore throat (90.8%, 83.1%,  $P < .01$ ) and had more documented tonsillitis (23.5% vs 13.8%,  $P < .01$ ).

Influenza A detections were less frequent post-VI; H1 accounted for the majority of the pre-VI detections, and these were all in 2009. In subjects with documented fever post-VI ( $n = 69$ ), rhinovirus accounted for the largest proportion of

**Table 3. Frequency of Detection of Respiratory Pathogens by PCR of Throat Swab and/or Nasal Wash, Pre-adenovirus Vaccine Introduction (VI) and Post-VI**

Pathogen	Pre-VI	Post-VI	P Value
<b>Adenovirus</b>			
Any	1266/1850 (68.4%)	21/754 (2.7%)	<.01
4	1242/1778 (69.9%)	9/754 (1.2%)	<.01
7	92/1182 (7.8%)	0/754 (0%)	<.01
14	78/1492 (5.2%)	0/754 (0%)	<.01
3	16/1060 (1.5%)	1/754 (0.1%)	<.01
11	0/793 (0%)	0/754 (0%)	
21	0/1013 (0%)	0/754 (0%)	
2	0/25 (0%)	4/630 (0.63%)	1.0
5	0/25 (0%)	7/741 (9.4%)	1.0
Rhinovirus	335/1880 (17.8%)	262/754 (34.7%)	<.01
Influenza A <sup>a</sup>	69/1854 (3.7%)	7/754 (0.92%)	<.01
H1	11/1111 (9.9%)	0/43 (0%)	.04
H3	7/110 (6.4%)	5/43 (11.6%)	.32
Influenza B	8/1855 (0.4%)	0/754 (0%)	.12
Enterovirus	0/194 (0%)	4/754 (0.5%)	.59
Human coronavirus OC43	11/1639 (0.7%)	47/754 (6.2%)	<.01
Human coronavirus 229E	16/1639 (1.0%)	6/754 (0.8%)	.67
Bocavirus <sup>b</sup>	4/1852 (0.2%)	2/47 (4.3%)	<.01
Metapneumovirus	34/1623 (2.1%)	11/636 (1.7%)	.58
Respiratory syncytial virus	4/1830 (0.2%)	2/754 (0.3%)	.82
Human parainfluenza type 3	19/1859 (1.0%)	18/754 (2.4%)	.01
<i>S pneumoniae</i>	19/1862 (1.0%)	4/754 (0.5%)	.22
<i>S pyogenes</i>	23/1862 (1.2%)	3/754 (0.4%)	.05
<i>M pneumoniae</i>	14/1855 (0.8%)	16/754 (2.1%)	<.01
<i>C pneumoniae</i>	0/453 (0%)	0/741 (0%)	
No pathogen identified	260/1906 (13.6%)	383/754 (50.8%)	<.01
Any coinfection	198/1906 (15.6%)	33/754 (4.4%)	<.01

Abbreviations: *C*, *Chlamydomphila*; *M*, *Mycoplasma*; PCR, polymerase chain reaction; *S*, *Streptococcus pneumoniae*; VI, vaccine introduction.

<sup>a</sup> Numbers of H3 and H1 do not equal total influenza A detections because not all cases were subtyped; in 1 case, both H3 and H1 were detected.

<sup>b</sup> Bocavirus testing had been exploratory and was discontinued shortly post-VI after low rates of detection.

these (n = 24), with influenza detected in 7 cases and any Ad in 5 (4 serotype 4, 1 serotype 3). Twenty-one of these had no pathogen detected. The rate of detection of rhinovirus doubled in the post-VI period; an increase in raw numbers of rhinovirus positive enrollments per month was also seen despite lower enrollments in general (see Table 3). Pre-VI, there were 1.9 rhinovirus positive cases per week, and 10.5 enrolled/week; post-VI, 2.9/week were rhinovirus positive, among 8.5 enrolled/week.

## DISCUSSION

Reintroduction of the Ad vaccine in basic training populations has again been extraordinarily successful in reducing the burden of both Ad-related respiratory illness and the burden of respiratory illness with fever. 75% reductions in FRI have been demonstrated by others, including at Joint Base San Antonio-Lackland where this study was conducted, against the backdrop of a 99.6% reduction in the weekly rate of Ad-related illness [11, 16]. Sustaining the commitment to prevention of Ad-related illness in uniquely susceptible trainees will be necessary if history is not to repeat itself. However, prevention of respiratory illness in this population is a complex task. Risk factors for transmission of respiratory pathogens will continue to be present in conditions inherent to basic military training. Adenovirus, despite its preeminence as a pathogen of interest in this group, has never been the entire story, and large outbreaks of non-vaccine serotype Ads have occurred even while vaccine serotypes were circulating [9]. Influenza causes annual epidemics which, in the context of an effective vaccine program, are typically limited in this population, but which can have considerable impact when new strains emerge. In summer and fall of 2009, influenza was responsible for 20% of FRI in those who were tested [20, 21]. Large outbreaks of pharyngitis caused by *S. pyogenes*, complicated by acute rheumatic fever, pneumonia, necrotizing skin and soft tissue infections, and other suppurative and immunologic complications, have been reported throughout the past century, prompting widespread use of antimicrobial prophylaxis at training sites [22–24]. Pneumococcal outbreaks have also occurred despite such prophylaxis, including pneumonia and fatal meningitis [25, 26]. Others, including *Neisseria meningitidis*, *Bordetella pertussis*, *M. pneumoniae*, and *C. pneumoniae*, have been well-described in this population [27]. Horizontal efforts at respiratory infection prevention, such as promoting hand hygiene, environmental including gas mask disinfection, cohorting of ill trainees, and respiratory etiquette, will require continued emphasis, even with near-elimination of Ad-related illness. However, vertical measures targeting specific organisms have also been demonstrated to have significant impact, with Ad vaccine as the prime example, and ongoing exploration into post-VI causes of illness will be necessary to direct further interventions.

Furthermore, although widespread efforts exist to monitor FRI rates and conduct surveillance for common respiratory viruses, not all acute respiratory illness is febrile. Clearly, trainees are still presenting for illness, but those without fever, which now represent >90% of those presenting for care, will not have respiratory pathogen analysis via DoD-directed surveillance mechanisms. Few prior data inform clinical differences between those presenting with Ad vs other respiratory pathogens. Recent comparisons of pdm(09)H1N1 influenza and Ad, including an analysis from this cohort, corroborated a predominance of coryza and cough presentations for influenza, vs



pharyngitis for Ad [20, 21]. This evaluation again emphasizes a classic presentation of Ad-related illness: fever, systemic complaints, and pharyngitis, distinct from the afebrile, coryza/cough presentations of those presenting post-VI and with rhinovirus. Interestingly, documentation of abnormal lung examination findings increased post-VI, as did use of clinical diagnostic terms suggesting LRTI, including both bronchitis and pneumonia. It is likely that most of those labelled “pneumonia” were never confirmed with radiographs, and absence of fever with these argues against that diagnosis in this young, otherwise healthy population. The predominant organism identified among these was rhinovirus, which, while not classically associated with lower respiratory disease in healthy adults, has been described including within military training populations [28, 29]. Nevertheless, the combination of increased LRTI diagnoses, and the increase in cough as well as physical examination findings of the same, provide signal of an increase in LRTI post-VI which should be explored with targeted research. It is also considerable that, despite a relatively broad panel of respiratory pathogens targeted with molecular methods, >50% post-VI had no pathogen detected. Some of these may have been non-infectious, as suggested by the increase in clinical diagnostic terms relating to allergies, but this represents a significant research gap. The nature of respiratory illness itself in basic military trainees has changed after reintroduction of Ad vaccine, transitioning from a febrile pharyngitis marked by systemic signs and symptoms, to an afebrile, cough and coryza predominant illness.

The ecologic niche occupied by vaccine-serotype Ad in this population was remarkable, causing approximately 70% of all FRI historically and with 80% of trainees infected by the end of training [4, 11]. During Ad VI in 1971, molecular methods for pathogen surveillance were not available; despite this, serotype shift was observed. Initially, Ad4 was the only serotype included in the vaccine program, but Ad7 was later added after this emerged and replaced Ad4 as the predominant cause of FRI [5]. Since that time, dozens of additional Ad serotypes and other respiratory viral pathogens such as bocavirus and human metapneumovirus have been identified. Respiratory pathogens cause outbreaks, which may come and go independently of a vaccine program’s effect, so changes in frequency must be interpreted with caution. However, it is reassuring that Ad14 has not yet reemerged in this population and, in fact, decreased in frequency since VI, a finding which has been corroborated by others, and potentially related to cross-protection with Ad7 immunity [9, 16]. The decrease in frequency of influenza A was driven by the unusually high number of influenza A cases in 2009, which contributed 63 to the total of 76 during the entire study. The trend toward a decrease in *S. pyogenes* (with no changes in antibiotic prophylaxis during the study period) is potentially biologically plausible with viral coinfection increasing the likelihood of streptococcal illness, although specific associations between Ad and *S. pyogenes*

have not been established, and rates of *S. pyogenes* illness are not known to have changed during the first iteration of the Ad vaccine program. The small increases seen in detection of *M. pneumoniae*, bocavirus and coronavirus OC43 may be due to chance alone or natural variation, but bear further observation. Most significant was the increase seen in detections of rhinovirus, which increased as a proportion of detected pathogens, in rates of positive tests among those tested for rhinovirus, and in raw numbers despite fewer overall enrollments. Rhinovirus may be associated with decreased probability of detecting other respiratory viral pathogens, including Ad. An evaluation of virus pairs in a PCR-based analysis of co-detections demonstrated a negative association between Ad and rhinovirus. While multiple additional respiratory viruses also demonstrated a negative association with rhinovirus, no others correlated either positively or negatively with Ad [30]. A negative interaction between these 2 pathogens has previously been reported in military recruits with and without symptoms of respiratory illness, and with Ad, but not rhinovirus, clearly associated with illness [31].

The most significant strength of the study is the collection of a wide array of respiratory pathogen data alongside a detailed collection of clinical and demographic data, allowing thorough evaluations for ecologic niche replacement, as well as changes in clinical illness, throughout a major change in vaccine administration, in >2600 trainees. Others include the high uptake of Ad vaccine, and the consistency in access to care, living and training conditions, and preventive medicine measures throughout the study period. The study also has a number of limitations. This is a single center which, although spanning several years, cannot account for natural variability of respiratory pathogens here or at other training sites, and some pathogens were tested for only from a subset of samples. These represent a convenience sample of the overall burden of trainees presenting to medical care for respiratory illness. Trainees, for a number of reasons, may be reluctant to self-identify when ill and present for care. While we have no reason to believe this limitation changed over the course of the study, this limits the ability to extrapolate frequencies of detection to rates of disease. Since asymptomatic subjects are not captured, it also limits the ability to determine colonization vs correlation with clinical illness. However, study procedures and approaches to enrolling trainees were unchanged over the course of the study period. There may have been increases in healthcare-seeking behavior in 2009 during the influenza pandemic, and this year saw the highest number of enrollments over the course of the study. Finally, comorbidities in this group were not captured, although significant known comorbidities are typically disqualifying for military accession.

## CONCLUSIONS

In conclusion, reintroduction of Ad vaccine in military trainees has markedly reduced the frequency of Ad detection in trainees presenting for care of respiratory illness, and been associated

with a 10-fold reduction in the proportion presenting with objective fever. Acute respiratory illness has transitioned away from a febrile pharyngitis with systemic signs and symptoms to a predominantly afebrile coryza and cough illness. No evidence of Ad serotype shift has been demonstrated, but rhinovirus is emerging as a potential pathogen of importance, and despite a broad array of tested viruses and bacteria, a majority now results with no pathogen identified. Respiratory infections are no longer the leading cause of medical encounters among military recruits; now they are the second-leading cause [32]. Their ongoing importance epidemiologically requires continued surveillance and research into additional preventive measures.

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## References

1. Armed Forces Health Surveillance Center (AFHSC). Surveillance snapshot: illness and injury burdens among U.S. military recruit trainees, 2011. *MSMR* **2012**; 19:23.
2. Cohen S, Tyrrell DA, Smith AP. Psychological stress and susceptibility to the common cold. *N Engl J Med* **1991**; 325:606–12.
3. Russell KL, Broderick MP, Franklin SE, et al. Transmission dynamics and prospective environmental sampling of adenovirus in a military recruit setting. *J Infect Dis* **2006**; 194:877–85.
4. Hilleman MR. Epidemiology of adenovirus respiratory infections in military recruit populations. *Ann N Y Acad Sci* **1957**; 67:262–72.
5. Top FH Jr, Dudding BA, Russell PK, Buescher EL. Control of respiratory disease in recruits with types 4 and 7 adenovirus vaccines. *Am J Epidemiol* **1971**; 94:142–6.
6. Gray GC, Goswami PR, Malasig MD, et al. Adult adenovirus infections: loss of orphaned vaccines precipitates military respiratory disease epidemics. For the Adenovirus Surveillance Group. *Clin Infect Dis* **2000**; 31:663–70.
7. Gray GC. Adenovirus transmission—worthy of our attention. *J Infect Dis* **2006**; 194:871–3.
8. Ryan MA, Gray GC, Smith B, et al. Large epidemic of respiratory illness due to adenovirus types 7 and 3 in healthy young adults. *Clin Infect Dis* **2002**; 34:577–82.
9. Tate JE, Bunning ML, Lott L, et al. Outbreak of severe respiratory disease associated with emergent human adenovirus serotype 14 at a US air force training facility in 2007. *J Infect Dis* **2009**; 199:1419–26.
10. Potter RN, Cantrell JA, Mallak CT, Gaydos JC. Adenovirus-associated deaths in US military during post vaccination period, 1999–2010. *Emerg Infect Dis* **2012**; 18:507–9.
11. Hawksworth A. Surveillance snapshot: adenovirus among U.S. military recruit trainees. *MSMR* **2012**; 19:5.
12. Howell MR, Nang RN, Gaydos CA, Gaydos JC. Prevention of adenoviral acute respiratory disease in Army recruits: cost-effectiveness of a military vaccination policy. *Am J Prev Med* **1998**; 14:168–75.
13. Hyer RN, Howell MR, Ryan MA, Gaydos JC. Cost-effectiveness analysis of reacquiring and using adenovirus types 4 and 7 vaccines in naval recruits. *Am J Trop Med Hyg* **2000**; 62:613–8.
14. Hoke CH Jr, Snyder CE Jr. History of the restoration of adenovirus type 4 and type 7 vaccine, live oral (Adenovirus Vaccine) in the context of the Department of Defense acquisition system. *Vaccine* **2013**; 31:1623–32.
15. Kuschner RA, Russell KL, Abuja M, et al. A phase 3, randomized, double-blind, placebo-controlled study of the safety and efficacy of the live, oral adenovirus type 4 and type 7 vaccine, in U.S. military recruits. *Vaccine* **2013**; 31:2963–71.
16. Radin JM, Hawksworth AW, Blair PJ, et al. Dramatic decline of respiratory illness among US military recruits after the renewed use of adenovirus vaccines. *Clin Infect Dis* **2014**; 59:962–8.
17. Metzgar D, Skochko G, Gibbins C, et al. Evaluation and validation of a real-time PCR assay for detection and quantitation of human adenovirus 14 from clinical samples. *PLoS One* **2009**; 4:e7081.
18. Heim A, Ebnet C, Harste G, Pring-Akerblom P. Rapid and quantitative detection of human adenovirus DNA by real-time PCR. *J Med Virol* **2003**; 70:228–39.
19. Dare RK, Fry AM, Chittaganpitch M, et al. Human coronavirus infections in rural Thailand: a comprehensive study using real-time reverse-transcription polymerase chain reaction assays. *J Infect Dis* **2007**; 196:1321–8.
20. Padin DS, Faix D, Brodine S, et al. Retrospective analysis of demographic and clinical factors associated with etiology of febrile respiratory illness among US military basic trainees. *BMC Infect Dis* **2014**; 14:576.
21. Yun HC, Fugate WH, Murray CK, et al. Pandemic influenza virus 2009 H1N1 and adenovirus in a high risk population of young adults: epidemiology, comparison of clinical presentations, and coinfection. *PLoS One* **2014**; 9:e85094.
22. Gunzenhauser JD, Longfield JN, Brundage JF, et al. Epidemic streptococcal disease among Army trainees, July 1989 through June 1991. *J Infect Dis* **1995**; 172:124–31.
23. Centers for Disease Control and Prevention (CDC). Outbreak of group A streptococcal pneumonia among Marine Corps recruits—California, November 1–December 20, 2002. *MMWR Morb Mortal Wkly Rep* **2003**; 52:106–9.
24. Centers for Disease Control and Prevention (CDC). Acute rheumatic fever among Army trainees—Fort Leonard Wood, Missouri, 1987–1988. *MMWR Morb Mortal Wkly Rep* **1988**; 37:519–22.
25. Dawood FS, Ambrose JF, Russell BP, et al. Outbreak of pneumonia in the setting of fatal pneumococcal meningitis among US Army trainees: potential role of *Chlamydia pneumoniae* infection. *BMC Infect Dis* **2011**; 11:157.
26. Crum NF, Wallace MR, Lamb CR, et al. Halting a pneumococcal pneumonia outbreak among United States Marine Corps trainees. *Am J Prev Med* **2003**; 25:107–11.
27. Gray GC, Callahan JD, Hawksworth AW, et al. Respiratory diseases among U.S. military personnel: countering emerging threats. *Emerg Infect Dis* **1999**; 5:379–85.
28. Minosse C, Selleri M, Zaniratti MS, et al. Frequency of detection of respiratory viruses in the lower respiratory tract of hospitalized adults. *J Clin Virol* **2008**; 42:215–20.
29. George RB, Mogabgab WJ. Atypical pneumonia in young men with rhinovirus infections. *Ann Intern Med* **1969**; 71:1073–8.
30. Greer RM, McErlean P, Arden KE, et al. Do rhinoviruses reduce the probability of viral co-detection during acute respiratory tract infections? *J Clin Virol* **2009**; 45:10–5.

31. Wang Z, Malanoski AP, Lin B, et al. Broad spectrum respiratory pathogen analysis of throat swabs from military recruits reveals interference between rhinoviruses and adenoviruses. *Microb Ecol* **2010**; 59:623–34.
32. Armed Forces Health Surveillance Center (AFHSC). Surveillance snapshot: illness and injury burdens among U.S. military recruit trainees, 2013. *MSMR* **2014**; 21:22.