MAJOR ARTICLE

Transmission Dynamics and Prospective Environmental Sampling of Adenovirus in a Military Recruit Setting

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(See the editorial commentary by Gray, on pages 871–3.)

Background. High levels of morbidity caused by adenovirus among US military recruits have returned since the loss of adenovirus vaccines in 1999. The transmission dynamics of adenovirus have never been well understood, which complicates prevention efforts.

Methods. Enrollment and end-of-study samples were obtained and active surveillance for febrile respiratory illnesses (FRIs) was performed for 341 recruits and support personnel. Environmental samples were collected simultaneously. Classic and advanced diagnostic techniques were used.

Results. Seventy-nine percent (213/271) of new recruits were seronegative for either adenovirus serotype 4 (Ad-4) or adenovirus serotype 7 (Ad-7). FRI caused by Ad-4 was observed in 25% (67/271) of enrolled recruits, with 100% of them occurring in individuals with enrollment titers <1:4. The percentage of recruits seropositive for Ad-4 increased from 34% at enrollment to 97% by the end of the study. Adenovirus was most commonly detected in the environment on pillows, lockers, and rifles

Conclusions. Potential sources of adenovirus transmission among US military recruits included the presence of adenovirus on surfaces in living quarters and extended pharyngeal viral shedding over the course of several days. The introduction of new recruits, who were still shedding adenovirus, into new training groups was documented. Serological screening could identify susceptible recruits for the optimal use of available vaccines. New high-throughput technologies show promise in providing valuable data for clinical and research applications.

Unique among respiratory viruses, certain serotypes of adenovirus have found an ecological niche among US military recruits in training, resulting in high morbidity and occasional mortality in this vulnerable population [1–4]. In the United States, highly effective enteric vaccines were available from the early 1970s until early 1999 for serotypes 4 and 7 (Ad-4 and Ad-7, respectively), which greatly decreased the morbidity caused by these viruses [5, 6]. With the loss of these vaccines, efforts to control endemic spread and epidemics have been largely unsuccessful [2, 7–9]. Although some evidence exists that emphasis on frequent and thorough hand washing can decrease rates of infection [10], the implementation of this measure alone has been insufficient

Efforts are currently under way to resume the production of adenovirus vaccines. Although the epide-

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miological characteristics of adenovirus-associated illness were well studied in the 1960s and 1970s and have been studied using modern techniques since 1995, the transmission dynamics of adenovirus have never been well understood [2]. Given our understanding of molecular shifts in the predominant circulating strain of adenovirus in recent years [11, 12], careful studies of the transmission dynamics of adenovirus in the recruit setting are needed, including serological testing to elucidate the percentage of recruits who are vulnerable to infection on arrival and seroconversion rates during training.

Meanwhile, in this era of bioterrorism concerns, environmental surveillance is being implemented in various locales for the detection of pathogen release or the presence of pathogens before the onset of human illness. Early detection could result in pharmacological or defensive intervention. Presymptomatic detection of infections could also result in early clinical intervention. Given its incubation period of 5–8 days, it was hypothesized that viral shedding of adenovirus into the environment might be detected before large numbers of individuals become symptomatic, thus potentially predicting outbreaks.

Evaluation of the potential role of environmental sampling in predicting respiratory illness has been previously hampered by the labor-intensive nature of the laboratory testing required. However, newer technologies are now available that allow high-throughput automated processing, making a study such as this timely in understanding its potential applications. One such technology, triangulation identificatio for the genetic evaluation of risks (TIGER), was developed by Ibis Biosciences, Inc., with Defense Advanced Research Program Agency sponsorship. This high-throughput technique and the accuracy of its detection rate have been described elsewhere [13, 14].

SUBJECTS, MATERIALS, AND METHODS

The training schedule at the study recruit training site—the Marine Corps Recruit Training Command, San Diego—was 12 weeks, with up to 90 recruits in each squad bay. This schedule was interrupted by several weeks of wilderness training in the middle of the course. Given the epidemiological evidence that respiratory outbreaks usually occur during the firs weeks of training [15, 16] and the logistical challenges of environmental sampling during the wilderness experience, only the firs 4 weeks of training, starting in October 2004, were monitored during the study.

Recruit and Support Personnel Sampling

Within 48 h of arrival for training, 271 recruits and 13 support personnel were enrolled after they provided written, informed consent. Enrollment samples were collected using moist, sterile Dacron swabs (Hardy Diagnostics) of the oropharynx and the dominant hand. Throat samples were collected with vigorous swabbing for a minimum of 5 s. Hand samples were collected by rubbing the swab, moistened with viral transport medium (VTM), over the dorsal and palmar aspects and between each finge of the dominant hand. Samples were stored in 5.0 mL of VTM (Reme). In addition, 7 mL of blood was drawn into an SST tube (Fisher).

All participants and support personnel were housed together in 3 distinct squad bays or rooms of 90 recruits and 4 support personnel each. Note that 100% of the recruits and support personnel included in the study provided informed consent. Although nearly one-half of all recruits asked to take part in the study declined, only those recruits who agreed were assigned to the 3 squad bays included in the study, which allowed a thorough examination of the transmission dynamics of adenovirus.

During the firs 4 weeks of recruit training, active surveillance was performed among the participants for febrile respiratory illnesses (FRIs), 5 days per week. Research assistants questioned participants for symptoms of respiratory illness. If symptoms existed, the oral temperature was determined. All participants who met the case definitio of a fever (temperature, $\geqslant$38°C) with a respiratory symptom such as cough or sore throat provided additional throat- and hand-swab samples. Additional samples were obtained from any identifie ill participant every other day, for a total of 3 sampling days over the course of a 5-day period. Six weeks after enrollment, end-of-study samples identical to enrollment samples were collected, and each participant completed a questionnaire of symptoms experienced during training. This 2-week delay before the fina collection was intended to allow the development of antibodies reflectiv of exposure during the 4-week active surveillance period.

During the study, 67 recruits were dropped from training and became no longer available for surveillance. Likewise, some recruits were added to each squad bay after they recycled through a physical-conditioning squad bay or a medical-rehabilitation squad bay. An additional 56 recruits were enrolled into the study in this manner during the 4 weeks subsequent to initial enrollment, and baseline samples were collected.

An ongoing clinic-based surveillance for adenovirus FRI is conducted by our laboratory at this same training site [17, 18]. This clinic-based surveillance requires that recruits present for health care for their data to be captured. We recognize, however, that recruits may be motivated to not present for health care for fear of jeopardizing ongoing training by placement in a medical facility for recovery. This active-surveillance study afforded the opportunity to understand approximately how often febrile recruits choose not to seek medical care.

Environmental Sampling

Surfaces. Sites for surface environmental sampling were chosen on the basis of results of a pilot environmental survey and the perception of frequently touched sites that could harbor adenoviruses. The 9 chosen surface sites were sampled 4 days

before the recruits arrived and 6 times per week thereafter, until the day after the recruits vacated the premises. These sites included toilet handles, sink handles, bedposts on the left and right side of each room, pillows, drinking fountains, clothing lockers on the left and right side of each room, and rifles Samples were collected using the same type of swabs and VTM as those used for participants. In each squad bay, a random selection of 4 different representative surfaces for each of the chosen sites was sampled daily, to avoid comprehensive "cleaning" of the sites. For example, 15 toilet handles were present in the bathrooms of each squad bay. Of these, 4 were randomly selected as representative of the "toilet handles" site and were sampled on that given day.

Air. In addition to surface samples, 2 different air samples were collected in each squad bay, using 2 different techniques. The firs was via dry filte units (DFUs) consisting of high-flow sampling pumps that pulled air through 2 dry filter (DAAD13-03-P-00021, model 1000; Battelle). The second sample was collected on a commercially available electrostatic air-filtratio system (Ionic Breeze Quadra, model SI637; Sharper Image). The DFU operated for 12 h daily, during the evening, night, and early morning hours, when recruits were present. Two polyester filter (DFU-P-24; Lockheed Martin) were used in each DFU. Each morning, both filter were removed from the DFU. One was stored in 20 mL of 1× PBS plus 0.1% Triton X-100 for molecular testing, and the other was stored in 2.5 mL of VTM for growth in cell cultures. Two of these same filter were used to wipe opposite sides of the electrostatic collector filamen and were stored in the same manner as the DFU filters

Laboratory Processing

All throat samples and a small subset of environmental samples ($n = 48$) were processed for viral isolation in A549 cells (human epidermoid lung carcinoma cells; Diagnostic Hybrids) using standard culture techniques and immunofluo escence detection [19, 20]. Serotyping of adenovirus isolates was performed using molecular techniques described and validated elsewhere [21]. Adenovirus isolates were further characterized by restriction-enzyme analysis of viral DNA with the endonucleases *Bam*HI, *Dra*I, and *Sma*I, as described elsewhere [22], and genome type identificatio and denomination was performed as described by Li and Wadell [23].

Serum samples from the enrollment and fina collections were tested for the presence of anti–Ad-4 and anti–Ad-7 antibodies by a colorimetric serum microneutralization assay [24], using the prototype strains of serotype 4 (RI-67) and serotype 7 (Gomen C4). A serum neutralization test was considered positive if the titer was $\geq$1:4.

The TIGER technology was used to process all samples (throat, hand, surface, and air) for evidence of adenovirus DNA. Although it is beyond the scope of the present article, a thorough description of this technology has been presented elsewhere [13]. Briefl , the TIGER technology uses broad polymerase chain reaction (PCR) priming teamed with determination of base composition of the resultant amplicons by electrospray ionization and time-of-fligh mass spectrometer analysis (ESI-TOF). These resulting base compositions can be used to identify and characterize the nature of the pathogen(s) present in relevant samples. Genomic DNA was prepared using the QiaAmp Virus BioRobot MDx kit (Qiagen). After broad PCR amplificatio designed to identify the presence of adenovirus in the sample and the specifi identity of a number of specifi serotypes, the samples were desalted and then subjected to ESI-TOF mass-spectrometer analysis. Signal processing of the mass-spectrometry data then determined the quantity of PCR product, which is reported as the "calibrated amplitude" (the estimated number of input genomes) for each primer set.

Statistical Analysis

A binomial probability distribution was used for each squad bay to compare the proportions of recruits with Ad-4–positive

Table 1. Adenovirus-related illness among recruits in each squad bay.

Squad bay	No. of recruits[a]	Participants with FRI, % (no./total)	Ad-4–positive FRI, % (no./no. with FRI)	Sent to MRP,[b] % (no./no. with Ad-4–positive FRI)
A	108	31 (34/108)	94 (32/34)	28 (9/32)
B	113	21 (24/113)	92 (22/24)	36 (8/22)
C	108	24 (26/108)	73 (19/26)	21 (4/19)
All	329	26 (84/329)	87 (73/84)	29 (21/73)

NOTE. Ad-4, adenovirus serotype 4; FRI, febrile respiratory illness; MRP, medical rehabilitation platoon.

[a] No. enrolled in each squad bay, including recruits who were dropped and recruits who were added during the 4 weeks of active surveillance.

[b] Ill recruits are dropped from their training squad bay and sent to MRP—a squad bay where sick recruits convalesce—until they are able to return to training with a different group in a different training squad bay.

Table 2. Adenovirus serotype 4 (Ad-4) and adenovirus serotype 7 (Ad-7) serological results and subsequent outcomes.

	Anti–Ad-4 titer						Anti–Ad-7 titer					
Measurement, outcome	Total	<1:4	1:4	1:8	1:16	>1:16	Total	<1:4	1:4	1:8	1:16	>1:16
Blood draw												
Enrollment[a]		66 (180/271)	1 (3/271)	3 (9/271)	4 (12/271)	25 (67/271)	271	39 (106/271)	11 (29/271)	10 (28/271)	15 (41/271)	25 (67/271)
Final[b]		3.2 (8/251)	0.4 (1/251)	1.2 (3/251)	6.0 (15/251)	89.2 (224/251)	251	38 (96/251)	14 (35/251)	11 (28/251)	14 (34/251)	23 (58/251)
Recruits with listed enrollment titers												
Subsequent Ad-4–positive FRI[c]	25 (67/271)	37 (67/180)	0 (0/3)	0 (0/9)	0 (0/12)	0 (0/67)	25 (67/271)	27 (29/106)	24 (7/29)	25 (7/28)	20 (8/41)	24 (16/67)
Seroconversion[d]	70 (141/201)	98 (126/129)	50 (1/2)	60 (3/5)	60 (6/10)	9 (5/55)	22 (44/201)[e]	43 (35/82)[e]	21 (4/19)[e]	9 (2/22)[e]	7 (2/29)[e]	2 (1/49)[e]

NOTE. Data are % (no./total). FRI, febrile respiratory illness.

[a] Includes only recruits who entered the study during the initial enrollment (not those recruits added at a later date).

[b] Includes all recruits who gave samples at final collection, regardless of enrollment date.

[c] Seventy-three participants had Ad-4–positive FRI, but only 67 of these were present at initial enrollment.

[d] Includes only participants who gave samples for both enrollment and final collections (i.e., of the 271 participants who gave a sample at initial enrollment, only 201 were captured at the final collection). Seroconversion was defined as a ⩾4-fold increase in titer.

[e] Development of heterotypic antibodies, given the evidence of only Ad-4 in circulation.

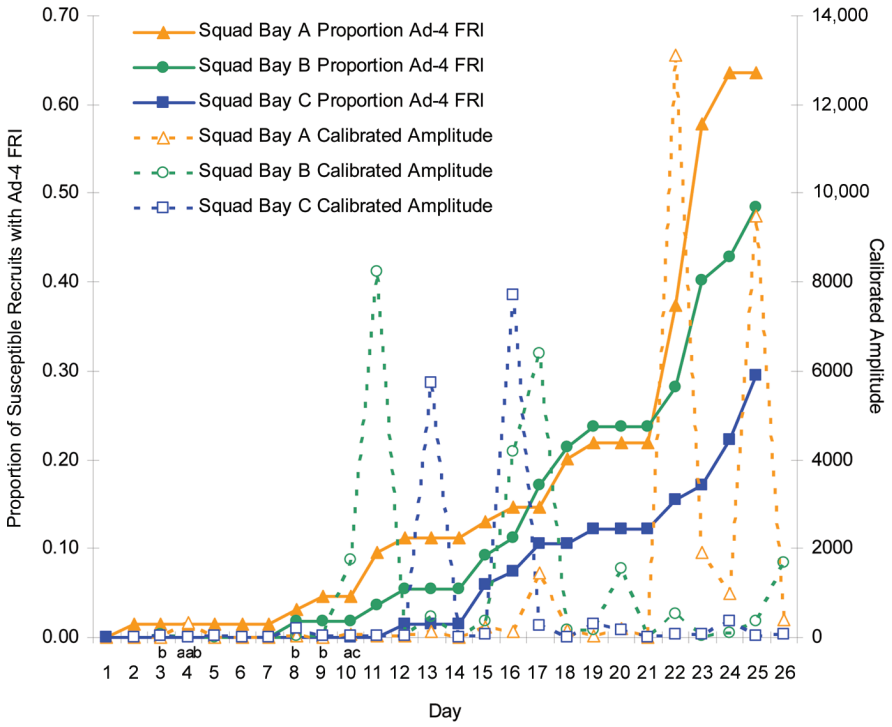

Figure 1. Proportion of susceptible recruits who developed an adenovirus serotype 4 (Ad-4)–positive febrile respiratory illness (FRI), total daily calibrated amplitude from all positive environmental samples, and date of late-entering Ad-4–positive recruits for each squad bay. *Filled markers, left axis,* Proportion of susceptible recruits (Ad-4 titer <1:4) who developed a subsequent Ad-4–positive FRI, as determined by throat culture, for each squad bay and plotted against day of active surveillance. A dramatic increase in the proportion of recruits who developed an Ad-4–positive FRI is demonstrated for weeks 3 and 4. *Empty markers, right axis,* Sums of calibrated amplitudes of each triangulation identification for the genetic evaluation of risks (TIGER)–positive sample found in the squad bay environment, plotted against day of active surveillance. In squad bay A, the days of highest calibrated amplitudes of the TIGER-positive samples in the environment tended to coincide with the days of highest culture-positive counts in the recruits. Throughout training, recruits are often added to squad bays already in training. The lowercase letters ("a," "b," and "c") below dates on the *X* axis indicate Ad-4–positive recruits who were added to a squad bay. There were 3 such positive late enrollments in squad bay A (indicated by "a"), 4 in squad bay B ("b"), and 1 in squad bay C ("c").

FRI during weeks 1 and 2 with that during weeks 3 and 4. The same procedure was used to detect significan differences between the numbers of positive environmental samples per squad bay during the firs 2 weeks compared with the second 2 weeks. Analysis of variance was used to assess the relationship between rates of Ad-positive FRI and the quantity of DNA for each squad bay, using the rate of Ad-positive FRI as the independent variable and the quantity of DNA as the dependent variable.

RESULTS

Participants. The number of participants from each squad bay identifie as having FRI and the percentage of culture samples that were positive for adenovirus is shown in table 1. Of the 84 identifie FRIs, 87% (73/84) were positive for adenovirus by culture, and molecular serotyping revealed that all were Ad-4. Overall, clinical febrile adenovirus illness was found in 25% (67/271) of the initially enrolled recruits (table 2). Only 31% (21/67) of these Ad-4–positive FRIs, or 8% (21/271) of the total population, were also captured by the clinic-based surveillance system. Figure 1 illustrates the proportion of recruits susceptible to infection who subsequently had an Ad-4–positive FRI during the month of active surveillance in the 3 squad bays.

None (0/271) of the recruits and none (0/13) of the support personnel had throat swabs positive by culture for adenovirus at enrollment. Of the 56 recruits added to the 3 squad bays in the middle of training, 3 of 19, 4 of 23, and 1 of 14 placed in squad bays A, B, and C, respectively, were culture positive for adenovirus on arrival. The date of arrival of these Ad-4–positive recruits is illustrated in figu e 1.

Of recruits who had an Ad-4–positive FRI, 69% (50/73) had evidence of Ad-4 DNA on their hands. Because samples were only collected from recruits identifie as having a temperature ⩾38°C, there were only 11 recruits who had FRI but whose throat swabs were negative for adenovirus. Among hand samples from these recruits with nonadenovirus FRIs, only 18% (2/11) had Ad-4 DNA on their hands.

Among recruits with identifie Ad-4–positive FRIs, 87% (41/

Table 3. Surface and air environmental-sample results as identified by the triangulation identification for the genetic evaluation of risks (TIGER) test.

Site	Samples, total no.	Positive samples, %	Calibrated amplitude of positive samples,[a] mean
Surface samples			
Pillows	69	39	165
Left lockers	71	35	193
Right lockers	71	31	120
Rifles	63	33	180
Sink handles	71	28	15
Toilet handles	71	20	62
Left bedposts	71	17	174
Right bedposts	71	14	20
Drinking fountain	71	17	53
Air samples			
Electrostatic collector	20	50	25
Dry filter unit	19	42	13

NOTE. Mass-spectrometer results were used to estimate the product quantity of polymerase chain reaction.

[a] Mean of all positive samples, using the signal processing from the TIGER electrospray ionization and time-of-flight mass-spectrometer analysis.

47) demonstrated continued shedding at the time of the second throat swab and 32% (6/19) at the time of the third throat swab. The fina specimen collection from 254 participants at 6 weeks revealed 5 positive results (2%). Four of these were from recruits with no previous signs of FRI during the active surveillance period, and 1 was from a recruit who had been captured during the active surveillance as being a nonadenovirus FRI.

Serological results for all enrolled recruits are shown in table 2. Of recruits enrolled initially, 27% (73/271) lacked antibodies to both Ad-4 and Ad-7, and 79% (213/271) lacked antibodies to either Ad-4 or Ad-7. At enrollment, 34% (91/271) of recruits had antibodies to Ad-4; this percentage increased to 97% (243/251) by the end of the study. By contrast, 61% (165/271) had antibodies to Ad-7, and this percentage remained essentially stable at 62% (155/251) at the end of the study. Recruits with enrollment titers to Ad-4 that were $\geq$1:4 were highly protected, with none (0/91) having a subsequent Ad-4–positive FRI. By contrast, titers to Ad-7 $\geq$1:4 were not protective, with 23% (38/165) of these recruits having a subsequent Ad-4–positive FRI. This lack of protection is consistent with our culture data demonstrating that only Ad-4 was in circulation. All support personnel had anti–Ad-4 titers $\geq$1:16 at enrollment.

Environment. A total of 668 samples were collected from 9 surface and 2 air sites in the environment of all 3 squad bays; samples were processed by TIGER. As can be seen in table 3, 14%–50% of collected samples tested positive by TIGER; adenovirus was clearly distributed throughout the environment and in the air. The mean calibrated amplitude of all positive TIGER results from each site during the 4 weeks of the study produced an estimate of the total adenovirus "burden" associated with that particular site. Lockers, rifles bedposts, and pillows appeared to have the largest burden. No samples from the environment tested positive for adenovirus before the arrival of the recruits; however, 9 were positive after the recruits departed, and 5 of these were from squad bay A. Of these 5 samples, 1 was found to be culture positive. Among the total subset of 48 environmental samples for which cell culture was also performed, 6 were found to be positive, indicating viable virus.

Summing the total amplitudes of all adenovirus-positive sites in each squad bay on a given day gave an estimate of the daily burden of adenovirus. This daily burden of adenovirus in each squad bay is shown in f gure 1, graphed together with the identifie proportion of susceptible recruits having a subsequent Ad-4–positive FRI (the proportion failing in the life-table analysis). For all squad bays, the number of Ad-4–positive FRIs was significantl greater during the last 2 weeks than during the firs 2 weeks ($P < .001$). In addition, for squad bay A, the greatest quantity of adenovirus DNA detected in the environment was significantl associated with the largest outbreak of Ad-4–positive FRI ($df_{2,60} = 6.04$; $P < .01$). This occurred during week 4. However, this association did not appear to hold for the other 2 squad bays, even though, for each of the squad bays, the number of positive environmental results was significantly greater during the second 2 weeks than during the firs 2 weeks ($P < .001$).

Isolated virus strains from environmental samples ($n = 6$), actively captured symptomatic recruits ($n = 6$), and asymptomatic recruits added to the squad bays later during training

Table 4. Heterotypic cross-neutralization results.

Group of participants	Ad-4 positive	Seroconversion to Ad-7 (heterotypic cross-neutralization)
Enrollment titers ≤1:4 for both Ad-4 and Ad-7	33 (67/201)	52 (35/67)
Enrollment titers >1:4 for both Ad-4 and Ad-7	18 (36/201)	0 (0/36)
All others[a]	49 (98/201)	9 (9/98)

NOTE. Data are % (no./total no.). Includes only participants who gave samples for both enrollment and final collections (of the 271 participants who gave a sample at initial enrollment, 201 gave a sample at the final collection). Seroconversion was defined as a ≥4-fold increase in titer. Ad-4, adenovirus serotype 4; Ad-7, adenovirus serotype 7.

[a] These participants had either an anti–Ad-4 or anti–Ad-7 titer that was >1:4 but not both.

($n = 5$) were characterized by restriction-enzyme analysis of genomic DNA with the endonucleases *Bam*HI, *Dra*I, and *Sma*I. All samples showed identical patterns with all 3 enzymes and were identifie as corresponding to genome serotype 4a, as described by Li and Wadell [23].

Results of classic cell culture versus TIGER. Of 135 positive throat cultures identifie by cell culture during the study, TIGER identifie 134 as positive (99%). TIGER also identifie an additional 78 throat samples as positive that were negative by cell culture, which is not surprising for a molecular-based technique that identifie nonviable DNA.

DISCUSSION

Ad-4 was the cause of the majority of the respiratory infections prospectively captured in the present study; restriction-enzyme analysis clearly demonstrated that only 1 strain of Ad-4 was in circulation. Since the loss of the adenovirus vaccines, this serotype has caused >98% of all captured adenoviral FRIs among US military recruits [12, 16]. During historical effectiveness studies of the oral Ad-4 vaccines in the late 1960s, it was found that, once this serotype was controlled with vaccination, Ad-7 quickly fille the niche [25]. For this reason, current efforts to resume vaccine production are following the historical precedent; both Ad-4 and Ad-7 are being included in the vaccine formulations. In this new era, the demonstration that >79% of incoming recruits may be vulnerable to Ad-4 or Ad-7 infection is critical information. This is similar to historic rates of seroprevalence at matriculation that were documented during the 1960s [26] and 1990s [27]. Likewise, the clear demonstration that antibody titers are protective is important. All recruits with captured Ad-4–positive FRI had enrollment anti–Ad-4 titers of <1:4. Historically, this was less clearly demonstrated, with at least some individuals with demonstrable titers developing febrile adenoviral illness during outbreaks [26]. The ultimate cost for the adenovirus vaccines currently under development is unclear; however, they will likely be significantl more expensive than those of previous decades. For cost savings and the alleviation of unnecessary vaccination, serological testing is being considered in our recruit camps to guide vaccination efforts for most required vaccines. The present data would support such a policy for the oral adenovirus vaccines.

Data from the study comparing active surveillance with clinic-based surveillance suggested that ~69% of recruits with a fever and respiratory symptoms were not captured by clinic-based surveillance. This findin is remarkably consistent with those of historical reports; in one study, >70% of persons with adenoviral disease did not come to the attention of the medical department [28]; in another, 67% (602/899) of recruits with acute respiratory disease did not seek treatment for their infections [29]. The data presented here also suggest that approximately one-third of incoming recruits were immune to Ad-4, nearly one-third developed a febrile infection, and the remainder seroconverted with lesser symptoms (asymptomatic or afebrile infection). This is consistent with historical reports [30, 31]. Nearly all recruits (97%) had positive titers (≥1:4) by the end of the sixth week of training.

All data collected during the present study strongly suggest that Ad-4 was the sole serotype of adenovirus in circulation. Given this, the seroconversion to Ad-7 noted among participants would represent the development of heterotypic cross-neutralizing antibodies. Note that this was observed in 22% (44/201) of all recruits with paired samples (table 2) and was primarily observed among those with low initial anti–Ad-4 and anti–Ad-7 titers. Table 4 demonstrates that more than one-half of recruits with paired serum samples who had undetectable levels of anti–Ad-4 and anti–Ad-7 antibodies at enrollment heterotypically seroconverted to Ad-7; by contrast, no heterotypic seroconversion was observed among participants with demonstrable anti–Ad-4 and anti–Ad-7 antibodies detected at enrollment.

The ability to consistently identify adenovirus DNA on surfaces and in the air was clearly demonstrated in the present study. Despite evidence of an increased environmental burden of adenovirus concomitant with human infection, the prediction of the outbreak was not demonstrated. Air-filte detection of adenovirus concomitant with an outbreak was reported by Echavarria et al. [32] in 2000; during an adenovirus outbreak

among Army recruits, 26 (44%) of 59 filter from the ventilation system were found to be positive by PCR, although none tested positive by cell culture. However, no surface sampling was performed in that study.

It should be emphasized that the environmental sampling conducted in the present study was crude at best, yet it was significantl better than was reasonably feasible before the availability of a high-throughput technology such as TIGER. Nevertheless, it was likely still an underestimate of the actual environmental adenovirus burden.

The data presented in the present article provide clues to the origin of the captured Ad-4–associated illnesses. No recruits or support personnel started the training period with Ad-4–positive throat cultures. Throughout training, recruits were added to each squad bay who had throat cultures positive for Ad-4, and they were a potential source of transmission (figu e 1). Likewise, viable Ad-4 and DNA were detected in the environment, concomitantly with the human infections and after the departure of the recruits. The potential contribution of such environmental contamination to subsequent human infection and transmission can only be hypothesized. However, sites that might be targeted for additional prophylactic cleaning were identified Of note, careful historical studies suggested that environmental contamination with another respiratory pathogen, group A streptococcus, was unimportant in subsequent transmission in this recruit setting, with person-to-person spread proving to be more important [33].

As more sophisticated pathogen-detection technologies become available, additional uses and applications will be sought. The unique environment of military recruit training, combined with the ongoing challenges of adenovirus, afforded the opportunity for preliminary experimentation with such a technology. The TIGER capability used in the present study successfully identifie sites of increased pathogen burden and proved to be implementable in support of environmental surveys.

The military is responsible for doing everything possible to decrease illness experienced by members in training. The expeditious completion of safety and efficac trials for the newly manufactured adenovirus vaccines is a priority and will go far in achieving this end. Nevertheless, taking the opportunity to understand the unique dynamics of adenovirus transmission in military training settings could be very helpful in understanding the transmission of respiratory pathogens in a variety of other settings.

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