Eur J Clin Microbiol Infect Dis (2007) 26:481–483
DOI 10.1007/s10096-007-0312-6

CONCISE ARTICLE

High isolation rate of adenovirus serotype 7 from South Korean military recruits with mild acute respiratory disease

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Published online: 1 June 2007

Abstract Adenovirus is a major cause of acute respiratory disease (ARD) in military recruits. When South Korean military recruits with ARD were surveyed, adenovirus was identified in 122 (61.0%) of the 200 recruits studied. Moreover, all cases of ARD involving adenovirus were caused by serotype 7.

Introduction

Acute respiratory disease (ARD) has long been a problem for military populations [1]. Because of crowded living conditions and a stressful working environment, military trainees are at a particularly high risk for respiratory disease epidemics [2, 3]. Studies of young military recruits during the 1950s and 1960s clearly identified adenovirus as the major cause of ARD in the military [4]. Live oral vaccines were subsequently developed and have served as the primary means of controlling adenovirus-induced ARD in the US military since the early 1970s [5]. This has resulted in a 50–60% reduction in cases of ARD and a greater than 95% reduction in adenovirus-specific respiratory illness compared with rates seen during the pre-vaccine era [5]. However, the only contemporary manufacturer of these vaccines halted production in 1996, resulting in a resurgence of adenovirus-induced ARD among military recruits undergoing basic training in the US [1]. In a survey of ARD performed at a military training facility in South Korea from October 2004 to May 2005, 43,983 cases of ARD were identified in 460,707 recruit-weeks (rate=9.5 cases per 100 recruit-weeks, unpublished data). Although the high incidence rate of ARD was reported, no epidemiologic data were gathered to ascertain the etiology of ARD. In addition, no system of vaccination against ARD was introduced. Since little is known about ARD in Korean military personnel, a survey was performed of respiratory viruses in military recruits with symptoms of ARD during the four-week basic training period.

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Materials and methods

Between February 2006 and May 2006, a survey was conducted to identify the microorganisms present in the upper airways of South Korean military recruits with ARD at a military training facility. Only those recruits seeking medical care were considered and a diagnosis of ARD was made for those recruits presenting with fever, plus any of the following respiratory symptoms: rhinorrhea, cough or sore throat.

Patients with chronic diseases or those who were admitted to a hospital with severe pneumonia were not included in the study group. A convenience sample of 200 recruits consisting of approximately 25 recruits arbitrarily selected every two weeks for the investigating period of 16 weeks from the trainees presenting at the medical clinic who met the ARD definition was recruited. After obtaining informed consent, a throat swab was taken via direct observation of the posterior throat and tonsil area using a

commercial rigid cotton-tipped swab applicator. The posterior oropharynx was scraped vigorously for 2 to 3 seconds and the swab was immediately placed in a refrigerated cell culture media and stirred. All throat samples (n=200) were processed for viral isolation in A549 cells (human epidermoid lung carcinoma cells; Diagnostic Hybrids) using standard culture techniques [6–8]. Cells were grown in Dulbecco's minimum essential medium (DMEM) supplemented with 5% foetal bovine serum (FBS), glutamine and antibiotics and maintained with DMEM supplemented with 2% FBS. Stocks of each virus isolate were grown in the cells and kept frozen at −70°C for further analysis. Each isolate was used to inoculate confluent monolayers of A549 cells in 75-cm^2 plastic flasks. When an extensive cytopathic effect was evident, we harvested three times with frozen and thawing. DNA to be used in polymerase chain reaction (PCR) analysis was purified by the QIAamp Blood Mini Kit protocol (QIAGEN GmbH, Hilden, Germany). The primer pair, ADVF (5′- ATG TGG AAI CAG GCI GTI GAC AG -3′) and ADVR (5′- CGG TGG TGI TTI AAI GGI TTI ACI TTG TCC AT -3′), which created a 458-bp product, was used for PCR diagnosis. All primer sequences are found between base pair position 21 and position 322 in the coding region of the hexon gene. PCR amplifications were carried out in 100-μl reaction mixtures containing 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 3 mM MgCl2, 0.1% Triton X-100, each deoxynucleoside triphosphate at a concentration of 100 mM, each primer at a concentration of 0.5 mM and 1 U of Taq DNA polymerase (Cosmo GeneTech, Korea). A total of 5 μl of QIAGEN-eluted DNA was added to each reaction mixture. The reaction tubes were placed in a 9700 thermal cycler (Applied Biosystems, USA) and were held at 94°C for 3 min, immediately followed by 35 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 1 min. The final cycle had a prolonged extension time of 5 min [9]. Serotyping of adenovirus isolates was performed by type-specific multiplex PCR using molecular techniques described and validated elsewhere [10, 11]. Human coronavirus (HCoV), human rhinovirus (HRV), influenza virus and human bocavirus (HBoV) was detected by PCR or reverse transcription (RT) PCR with RNA and DNA which was directly extracted from throat swabs [12].

Results and discussion

During the four-month study period, 24,004 cases of ARD were identified in 238,068 recruit-weeks (rate=10.0 cases per 100 recruits-weeks) and 200 throat swabs were collected respectively from 200 military recruits with ARD. A total of 160 respiratory viruses were identified in 137 (68.5%) of the 200 military recruits (Table 1). The most common virus identified by the throat swabs was adenovirus (122/160, 76.3%) and molecular serotyping revealed that all of the adenoviral infections involved serotype 7. The other viruses identified occurred at the following frequencies: 17 cases of HCoV (10.6%), 16 cases of HRV (10.0%), four cases of HBoV (2.5%) and one case of influenza A virus (0.6%). Two or more viruses were identified from the same specimen in 21 recruits (13.1%) and these included 12 cases of HCoV with adenovirus, four cases of HRV with adenovirus, three cases of HBoV with adenovirus, one case of influenza A virus with adenovirus and HCoV, and one case of HRV with adenovirus.

Military service is compulsory for all healthy young persons in South Korea; however, to the best of our knowledge, this is the first etiologic study of ARD in Korean military recruits. Overall, respiratory viruses were identified in 68.5% of military trainees with ARD and the predominant pathogen identified was adenovirus (61.0%).

The high isolation rate of adenovirus noted in this study is comparable to isolation rates seen among military recruits during the pre-vaccine era in the US [4]; however, the sole adenoviral serotype identified in the present study was serotype 7. This differs from surveys completed during the prevaccine era indicating serotype 4 to be the predominant strain [5, 13]. The results of our study also differ from a recent report describing a 5-year survey of military recruits at eight training sites throughout the US, in which approximately 95% of the adenoviral isolates were serotype 4 [14]. It is not clear why serotype 7 was predominant in Korean military recruits, differently from serotype 4 in the US military recruits. It is likely that this discrepancy in the predominant serotype could be caused by the prevailing serotype in the general population, since serotype 7 adenovirus is a frequent cause of acute respiratory infections in Korean children [10, 12, 15–17].

A major limitation of this study is that collecting samples from all recruits with ARD was impossible. The study was performed at a military training facility and sampling was dependent on voluntary trainee presentation at a medical clinic; therefore, this study population may not be representative of Korean military recruits. However, of

Table 1 Number of the respiratory viruses isolated from Korean military trainees with acute respiratory illness during four weeks of basic military training

Virus	No. of isolates (%)
Adenovirus	122 (76.4)*
Human coronavirus	17 (10.6)
Human rhinovirus	16 (9.9)
Human bocavirus	4 (2.5)
Influenza A virus	1 (0.6)

*One or more other viruses were isolated in 21 military trainees

30 positive viral culture specimens from 62 military recruits presenting with ARD at other military training facilities in South Korea, 26 (86.7%) specimens involved adenovirus and all were serotype 7 (Dr. C.-H. Yoon, personal communication). Therefore, it appears that the results do not deviate greatly from the actual situation among South Korean military recruits. Additional PCR work suggests that other viruses were less commonly found in the samples processed. However, we did not test the sensitivity/specificity with known positive and negative samples.

The results of this study clearly indicate a high isolation rate of adenovirus from South Korean military recruits with ARD during the four-week basic training period. Standardised surveillance for adenovirus-associated ARD in the military is, therefore, necessary. In addition, more effort is needed to halt the spread of adenoviral infections among high-risk groups, such as military personnel.

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