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Short communication

# Viral agents responsible for febrile respiratory illnesses among military recruits training in tropical Singapore

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ABSTRACT

*Background:* Military personnel are highly susceptible to febrile respiratory illnesses (FRI), likely due to crowding, stress and other risk factors present in the military environment.
*Objective:* Our objective was to investigate the viral etiological agents responsible for FRI among military recruits training in a tropical climate in Singapore.
*Study design:* From March 2006 through April 2007, a total of 1354 oropharyngeal (throat) swabs were collected from military recruits who reported sick with an oral temperature of ≥38 °C and a cough and/or sore throat. Real-time polymerase chain reaction (PCR) was used to assay for the presence of influenza A and B viruses and adenoviruses (H-AdV), and conventional PCR used for the remaining respiratory viruses in all specimens.
*Results:* Influenza A virus was the dominant infection with a laboratory-confirmed incidence of 24% (326/1354) and a predominance of the H3N2 subtype. The temporal pattern for influenza A virus infections coincided with the nation-wide pattern in the civilian community. Detection rates of 12% (159/1354) and 2.7% (5/1354) were obtained for influenza B virus and other respiratory viruses, respectively.
*Conclusions:* The laboratory findings identified influenza A virus as the primary causative viral agent for FRI in the Singapore military, in strong contrast to findings from temperate countries and countries where recruits are often vaccinated for influenza. Our results suggest that influenza vaccination should be considered as a requirement to reduce the incidence of influenza infections. This is the first report describing respiratory infections in a tropical military setting, in a developed country in Asia.


## 1. Background

Febrile respiratory illness (FRI) patterns in military populations are different than those observed in general civilian populations. This increased vulnerability has been ascribed to high population density, extreme physical and psychological challenges during training and wartime operations.[1] High rates of FRI were consistently observed throughout the history of the US military.[2–4] Although influenza outbreaks and infections have been documented, human adenovirus (H-AdV) has consistently been the predominant cause of FRI with US military.[5–14] In Asia, influenza outbreaks have been reported with Taiwan military and H-AdV infections with South Korea military, but none reported in the tropics.[15,16] Other surveys in Asia were conducted by US military personnel stationed on active duty in Asian countries.[17–19] These reports suggest that acute respiratory infections can occur in military populations indigenous to Asia, but these are either mostly not surveyed, or remained unreported. Clearly, the spread of these diseases can continue to compromise mission capability and security.

## 2. Objectives

The objective of our study was to determine the viral FRI burden, especially by influenza viruses and H-AdV, in the military in Singapore, a tropical Asian city-state. The virological data gathered from this study will inform policy makers of the disease impact and can guide the implementation of public health interventions to reduce the disease burden.

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**Table 1**
Number of clinical specimens that tested PCR positive for respiratory viruses in Camp X.

| Viral agents tested | Number[a] (%) |
|---|---|
| Number of specimens collected | 1354 |
| Adenovirus | 5 (0.4) |
| Influenza A virus | 326 (24) |
| Influenza B virus | 159 (12) |
| Influenza C virus | 4 (0.3) |
| Parainfluenza type 1 | 1 |
| Parainfluenza type 2 | 0 |
| Parainfluenza type 3 | 4 (0.3) |
| Parainfluenza type 4 | 0 |
| Rhinovirus | 15 (1.1) |
| Coronavirus, OC43 | 0 |
| Coronavirus, 229E | 1 |
| Respiratory syncytial virus | 0 |
| Human metapneumovirus | 9 (0.7) |
| Total number tested positive | 524 (38.7) |

[a] The number of clinical specimens that tested PCR positives refer to the specimens that tested positive for each viral agent relative to the total number of specimens collected for the study. The percentage is represented within brackets.

## 3. Study design

### 3.1. Recruitment of participants

Participants were recruited from a military training camp (Camp X) from March 2006 through April 2007. Personnel who reported sick with FRI symptoms, defined as fever (oral temperature $\geq$38 °C) with acute respiratory symptoms of cough or/and sore throat were recruited from Monday through Friday throughout the entire year. Oropharyngeal (throat) swabs (Remel, Lenexa, Kansas, USA) were collected from participants who consented to the study, and asked to fill out a simple self-report form including their demographic and clinical details.

### 3.2. Specimen collection and laboratory processing

Swabs were resuspended in viral transport medium and total nucleic acids were extracted using the RNeasy minikit (Qiagen, Inc., Valencia, CA, USA) according to manufacturer's instructions, and stored at −80 °C before PCR analysis.

### 3.3. PCR analysis

Real-time PCR tests for influenza A and B viruses and H-AdV were performed in a Lightcycler 1.5 (Roche Diagnostics, Mannheim, Germany).[8,20,21] Influenza A viruses were subtyped using a modified nested PCR format in an MJ conventional machine (Promega).[22,23] Multiplex PCR tests for the remaining respiratory viruses listed in Table 1 were used on the same conventional PCR machine.[24] The amplicons for HMPV were genotyped by DNA sequencing using the BigDye Terminator cycle sequencing (Applied Biosystem, Foster City, CA, USA).

## 4. Results

### 4.1. Incidence of viral infections

A total of 1354 specimens were collected, representing all recruits matching the inclusion criteria who reported for medical care during weekdays. Twenty-four percent (326/1354) were PCR positive for influenza A virus, 12% (159/1354) for influenza B virus and 0.4% (5/1354) for H-AdV (Table 1). Of the remaining 864 specimens, only 4 influenza C virus, 1 human coronavirus 229E, 9 human metapneumovirus (HMPV) (7 of the genotype A2), 15 rhinovirus, 1 parainfluenza virus (PIV) type 1, and 4 PIV type 3 (Table 1) were PCR-detected. The remaining 830 (61%) specimens were negative by all tests performed, suggesting that these specimens may be collected in ways that precluded viral identification, or that the collected specimens were not stored properly before transport to the diagnostic laboratory.

These results indicate that influenza A virus is the leading identifiable cause of FRI among Singapore military recruits. As shown in Fig. 1, the FRI incidence differed significantly over time. There were 2 distinctive surges of FRI that coincided with the peaks for influenza A virus in the months of June 2006 and January-February 2007. We observed a predominance of H3N2 subtypes at 38.0% (124/326) of all influenza A virus infections, and less H1N1 at 15.6% (51/326) (data not shown). The remaining influenza A-positive samples remained negative from the PCR-subtyping assays, suggesting that these specimens were of relatively low viral load. The pattern for influenza B virus incidence was sporadic throughout the surveillance period (Fig. 1). In May 2006, the incidence of influenza B virus superseded that of influenza A virus, resulting in a noticeable peak (Fig. 1). We speculate that this could represent a small outbreak among the recruits within the same training group, or sharing the same sleeping dormitories.

### 4.2. Demographical and clinical details

The symptoms most frequently reported by participants testing positive for influenza viruses or H-AdV are shown in Table 2. Other than high fever (which was a requirement for inclusion), the most common symptoms were cough, sore throat, headache, body ache and running nose. Participants with influenza A virus infections were more likely to have cough when compared with participants with influenza B virus or H-AdV ($P < 0.05$). From our results, the clinical impact of FRI can be estimated, with at least 2708 training (work) days lost among the 1354 patients recruited for the study.

## 5. Discussions

This is the first comprehensive study that provides data on the viral burden in a military population in Asia with a tropical setting. Throughout the current one-year study, the laboratory findings identified influenza A and B viruses in 36% (485/1354) of the FRI cases, with H3N2 as the predominant annual subtype in Singapore. Two peaks were observed, in June 2006 where the proportion of influenza detected rose above 80%, and January-February 2007 (Fig. 1). This bimodal pattern and the predominant H3N2 subtype concurred with reports at the national level from the Singapore Ministry of Health.[25–27] On the other hand, the sporadic pattern for influenza B virus incidence is in contrast with the cluster of infections reported nationally at the beginning of 2006 and 2007.[25,26] The laboratory-confirmed data suggests that the outbreaks in the military were related to the national outbreaks due to the interactions by the military personnel and the community. In addition, the influenza A cases in our study had significantly more symptoms of cough than other FRI cases (Table 2). The participants in Camp X were new recruits who were exposed to military activities for the first time, and we expected them to demonstrate increased vulnerability to respiratory infections. In the US military, it was reported that new recruits were 29 times more likely to be hospitalized for respiratory infections than other military personnel.[2–4] The laboratory-confirmed incidence of H-AdV infections remained low throughout the study period, at 0.4% (5/1364). This is in contrast to more than 50% reported in South Korean and the US military recruits.[9,14,16] No respiratory syncytial virus (RSV) was detected in the Singapore cohort.[28] Instead 9 cases of HMPV (Table 1) were detected and majority were of the A2 genotype, reported to be associated with an increased severity in respiratory infections.[29,30]

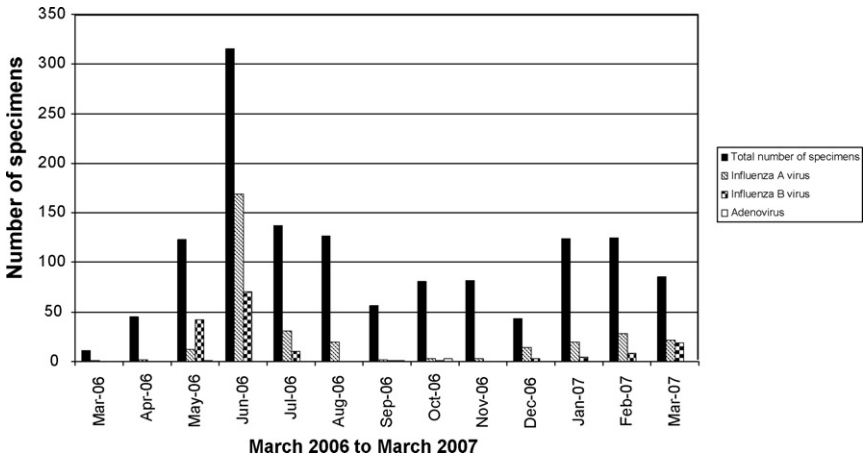


**Fig. 1.** Number of FRI specimens collected showing PCR positive for the laboratory-confirmed incidence of influenza A and B viruses, and H-AdV. The real-time PCR testings were performed on the LightCycler, and the results for the presence of influenza viruses were read on the F1 channel and that for H-AdV were differentiated from non-specific products such as primer-dimers via the different melting points. The laboratory-identified incidences of FRI, measured by the total number of specimens collected, were monitored on a monthly basis together with the specific incidences of influenza viruses and H-AdV. The disease data are presented as incidence, as rate data could not be collected due to the classified nature of recruit population data. (■) represents total number of specimens collected that fulfilled the FRI criterion, (▧) PCR positive for the presence of influenza A virus, (▪) for influenza B virus and (□) for H-AdV.

The data from the current study further suggests that in the event an outbreak happens in the Singapore military, the attack rate is likely to be high because vaccination is not mandatory for the Singapore military. In contrast, influenza outbreaks in US trainees are rare because they are vaccinated against circulating influenza strains.[6–8] Vaccine effectiveness against influenza A virus was reported to be at 92% for the US military, and 38.1% and 41.6% in asymptomatic and symptomatic Israeli soldiers respectively.[31,32] These reports support reductions of influenza-associated illness in young adults by the use of influenza vaccination. In conclusion, this prospective surveillance study demonstrated that influenza viruses are the most common identifiable viral agents causing FRI in the Singapore military. Compulsory vaccination to reduce influenza infections should be considered upon enlistment of personnel for the Singapore military.

**Table 2**
Demographic and clinical characteristics of participants.

| Characteristic | Influenza A virus $N^a$ = 326 | Influenza B virus $N^a$ = 215 | Adenovirus $N^a$ = 39 | *P*-value[b] |
|---|---|---|---|---|
| *Age* | | | | |
| Range | 16.8–43.4 years | 16.8–43.4 years | 19.0–21.4 years | [f]0.635 |
| Median | 20.2 years | 19.8 years | 20 years | |
| Gender | Male | Male | Male | |
| *Symptoms duration*[c] | | | | |
| Range | 1–16 days | 1–7 days | 1–2 days | [f]0.11 |
| Median | 2 days | 2 days | 1 day | |
| *Highest temperature* | | | | |
| Range | 36.5–40.3 °C | 37–40.2 °C | 38.2–38.7 °C | [f]0.2 |
| Median | 38.6 °C | 38.6 °C | 38.6 °C | [f]0.93 |
| *Current temperature* | | | | |
| Range | 35.4–40.2 °C | 37–40 °C | 37.9–38.6 °C | |
| Median | 38.5 °C | 38.5 °C | 38.4 °C | |
| Sore throat | 235/326 (72.1) | 116/159 (73.0) | 5/5 (100.0) | [f]0.38 |
| Cough | 305/326 (93.6) | 137/159 (86.2) | 2/5 (40.0) | [d,e]<0.05 |
| Shortness of breath | 80/326 (24.5) | 41/159 (25.8) | 1/5 (20) | [f]0.93 |
| Congestion | 216/326 (66.3) | 94/159 (59.1) | 3/5 (60) | [f]0.30 |
| Headache | 233/326 (71.5) | 115/159 (72.3) | 3/5 (60) | [f]0.83 |
| Pink eyes | 19/326 (5.8) | 13/159 (8.2) | 1/5 (20) | [f]0.31 |
| Body ache | 158/326 (48.5) | 78/159 (49.1) | 2/5 (40) | [f]0.92 |
| Nausea | 83/326 (25.5) | 44/159 (27.7) | 1/5 (20) | [f]0.83 |
| Asthma | 75/326 (23.0) | 26/159 (16.4) | 1/5 (20) | [f]0.24 |
| Vaccinated for influenza viruses | 5/326 (1.5) | 6/159 (3.8) | 0/5 (0) | [f]0.28 |
| Smoking | 75/326 (23) | 33/159 (20.8) | 1/5 (20) | [f]0.85 |

[a] N represents number of respondents who answered all the questions in the case form, and whose specimens tested PCR positive.
[b] For each viral agent, a positive characteristic was scored and this is expressed relative to the number of respondents. The percentage is represented within brackets. Data analysis was performed using the open-source statistical software R (http://www.r-project.org/) and univariate analysis with the Pearson's Chi-squared test to examine categorical measurements of illness characteristics, physical examination findings and laboratory results.
[c] Refers to the number of days reported sick before recruitment.
[d] Influenza A virus versus influenza B virus.
[e] Influenza A virus versus adenovirus.
[f] NS represents not significant.

## Conflict of interest

None.

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