DOI:10.1111/j.1750-2659.2007.00029.x
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Original Article

Respiratory syncytial virus: an important cause of acute respiratory illness among young adults undergoing military training

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Accepted 08 October 2007. Published online 10 December 2007.

Background Military recruits receiving training are vulnerable to acute respiratory disease and a significant proportion of illness is caused by unidentified pathogens. While some countries use surveillance programmes to monitor such illness, few data exist for recruits of the British Armed Forces.

Objectives Through active surveillance of approximately 1000 Royal Navy trainees during 2001, we sought to describe and determine the aetiology of acute respiratory illness.

Methods Standard viral culture was used together with serology and a novel highly sensitive real-time PCR and molecular beacon probe assay for respiratory syncytial virus (RSV) detection.

Results Among 54 Royal Navy recruits with respiratory symptoms adenovirus was identified in 35%, influenza viruses in 19% and RSV in 14%. All recruits were absent from training for almost a week, most of whom were confined to the sickbay.

Conclusions This study is the first to document adenovirus and RSV as important causes of acute respiratory illness among Royal Navy trainees. The study findings demonstrate the clinical significance and challenges of diagnosing RSV infection in young adults.

Key words Adults, human RSV, military personnel, respiratory tract infections.

Please cite this paper as: O'Shea *et al.* (2008) Respiratory syncytial virus: an important cause of acute respiratory illness among young adults undergoing military training. Influenza and Other Respiratory Viruses 1(5-6), 193–197.

Introduction

Military recruits receiving training have, historically, been vulnerable to acute respiratory disease (ARD), their increased susceptibility being attributed to demanding physical training schedules and crowded habitation.[1]

Adenoviruses, influenza A and B viruses, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, Epstein–Barr virus, coronavirus and rhinoviruses have previously been identified as causes of ARD among military populations.[1] These infective agents have been extensively studied in this setting; however, a significant proportion of illness (over 40%) has been attributed to unknown causative agents, probably unidentified respiratory viruses.[1]

Human respiratory syncytial virus (RSV) is an enveloped, single-stranded, negative-sense RNA virus of the genus *Pneumovirus*. RSV infection has long been acknowledged as the single most important viral pathogen and the leading cause of severe lower respiratory tract infection in infants and young children.[2,3] However, for over a decade, RSV has been increasingly recognized as a cause of severe community-acquired lower respiratory tract illness in certain susceptible adult populations including the elderly, the immunocompromised and those with severe underlying pathology.[4,5] RSV infection is not, however, limited to paediatric and certain high-risk adults as acquired immunity to RSV is partial and transient, and natural re-infection occurs repeatedly throughout life.[6] Acute respiratory illness resulting from RSV infection has been identified in previously healthy immunocompetent adults and although the clinical severity of infection or subsequent re-infection is typically diminished, the spectrum of disease varies widely.[7,8]

The role of RSV as a cause of respiratory disease among recruits in training has yet to be fully determined. Previous epidemiological studies have been limited and the study of RSV in acute respiratory infection in adults has remained challenging because of difficulties in diagnosis, primarily due to the shedding of low viral titres in the upper respira-

tory tract secretions of adults over short durations, the thermolabile nature of RSV and the presence of pre-existing nasal antibodies.[4,6,7]

In this preliminary study we set out to estimate the prevalence of clinically significant RSV infection, to characterize the illness associated with RSV, and to determine the prevalence and clinical characteristics of other common viral agents in a population of Royal Navy recruits undergoing basic training. In addition to conventional viral culture techniques, serology and a recently described novel, highly sensitive real-time PCR assay were used for the detection of RSV infection.

Methods

Study participants were symptomatic military recruits receiving basic training at HMS Raleigh, a large Royal Navy new entry training establishment on the south-west coast of Britain. Recruits were eligible for enrolment into the study upon seeking medical attention with nonspecific influenza-like symptoms, an oral temperature of ≥38°C and either a cough or sore throat. Those recruits meeting the case definition received a written and verbal briefing and were invited to give voluntary and informed consent to participate. The study was designed to enrol subjects over a period of 6 months, from the beginning of November 2000 until the end of April 2001, concurrent with annual epidemics of RSV infections among civilian populations.[8]

Bilateral nasopharyngeal swabs and an acute-phase blood sample were collected from participants upon enrolment. In addition, each study participant completed a case report that captured demographic and clinical data. Samples were stored at 2–8°C for up to 48 hours after collection and thereafter nasopharyngeal swabs were stored at −70°C as were serum samples following separation by centrifugation. At 2–4 weeks following initial presentation, participants were recalled and a convalescent serum sample was collected and a follow-up questionnaire regarding the nature, severity and duration of specified symptoms and their effect on training was completed.

Virus isolation and identification were performed using standard procedures.[9] Six tissue culture cell lines were used, including, HEp-2 human epithelial cells, primary rhesus monkey kidney cells, MRC-5 human lung fibroblasts, NCI-H292 human lung mucoepidermoid cells, A549 human lung adenocarcinoma cells and R-Mix [mixed monolayer of mink lung (MvLu1) and A549 cells]. Multiple cell lines were used to facilitate the detection of a variety of respiratory viruses including RSV, influenza viruses A and B, parainfluenza viruses (PIV) 1, 2 and 3, adenovirus, enterovirus and herpes simplex viruses (HSV) 1 and 2. Cultures were examined by light microscopy for characteristic viral cytopathic effect. Immunofluorescence using a panel of monoclonal antibodies directed against each of the respiratory viruses provided viral identification.

An ELISA modified from standard methods was used to determine serum IgG antibodies to RSV.[10] Pooled RSV A2 and B virus-infected cell lysates (positive antigens) and mock-infected control cell lysates (negative antigens) were prepared, optimal antigen dilutions were determined, and incubation times, wash conditions and detection conditions were as previously described.[10] Paired acute- and convalescent-phase sera were assayed at two screening dilutions (1:1000 and 1:2000) and subsequently samples demonstrating a 1.5-fold or greater convalescent-to-acute optical density ratio were subsequently assayed over several serial doubling dilutions (1:500–1:64 000). An antibody response to RSV was considered positive when paired samples possessed an acute-phase serum optical density of ≥0.2 and demonstrated a ≥4-fold increase in RSV-specific IgG antibodies between acute and convalescent samples.[10]

A highly sensitive, semi-quantitative real-time PCR assay employing a molecular beacon probe was used for the detection of RSV RNA in respiratory secretions derived from nasopharyngeal swab samples. Following RNA isolation, cDNA synthesis and nested PCR, molecular beacon real-time PCR and product analysis were performed as previously described.[11] Real-time PCR was performed in triplicate on samples positive for the presence of RSV to determine a mean cycle threshold (C_t) value. Quantification of RSV titre was calculated by interpolation of mean specimen C_t values using previously generated standard reference curves.

Results

Over an 11 week period (early January to late March 2001), 54 symptomatic trainees were enrolled into the study from a group of 134 recruits who met the case definition. The average resident population of recruits at HMS Raleigh was approximately 1000 during this period. Nasopharyngeal swabs, an acute phase blood sample and a completed case report were obtained from each recruit at presentation. A follow-up questionnaire was completed by 37 (69%) of the recruits. Participant details at presentation and subsequently gathered general clinical information are summarized in Table 1.

One or more viruses were isolated and identified by viral culture in 29 of the 54 participants (54%). Adenovirus was isolated from 19 recruits (35%) and influenza viruses from 10 recruits (19%; 20% influenza A and 80% influenza B). Each virus occurred alone (no co-infections were identified), and no other respiratory viruses (i.e. RSV, PIV, enterovirus or HSV) were isolated by viral culture.

Paired acute- and convalescent-phase sera were available for 38 (70%) of the participants and the determination of anti-RSV IgG antibody titres was performed on these

Table 1. Characteristics at presentation and clinical information at follow-up collected from military trainees enrolled into the study

Characteristics	*n* (%) [range]	
	n = 54	
Male	50 (93)	
Mean age (years)	19.8 [16.4–33.0]	
Mean symptom duration (days)	2.5 [1–10]	
Mean training week	4.6 [2–20]	
Influenza vaccination	0	
Clinical details	***n* (%)**	**Duration* [range, days]**
	n = 37 (69)	
Symptoms		
Cough	34 (92)	6.6 [1–27]
Sore throat	32 (86)	4.0 [1–14]
Nasal congestion	30 (81)	6.7 [1–27]
Wheeze	11 (30)	3.0 [1–7]
Dyspnoea	8 (22)	3.1 [1–9]
Clinical impact		
Absence from training (≥1 days)	36 (97)	3.8 [1–7]
Sickbay confinement	19 (51)	3.8 [1–8]
Training recycling	10 (27)	

n = number of trainees.
*Mean days.

samples by an ELISA. Among these recruits, three (8%) demonstrated serological evidence of recent RSV infection as indicated by a ≥4-fold increase in RSV-specific IgG antibodies between acute and convalescent sera. Two trainees (6%) showed a twofold increase in anti-RSV IgG titre, 26 trainees (72%) showed no change in titre and the remaining five trainees (14%) demonstrated a reduction in anti-RSV IgG titre. Two pairs of samples were excluded from the analysis because of indeterminable IgG titres and pre-fractionation haemolysis.

Molecular beacon real-time nested PCR detected the presence of RSV RNA in 3 (6%) of the 54 samples. Conventional nested PCR failed to detect the presence of RSV RNA in any of these samples. The estimated RNA levels of positive samples ranged from those equivalent to 1.0×10^{-4} to 2.0×10^{-4} pfu/ml (mean, 2.0×10^{-4} pfu/ml). (This assay can detect both virion RNA and viral mRNA.)

Respiratory syncytial virus was detected among the six different recruits by either PCR or serology, therefore after combining the data the prevalence of RSV among the participants was determined to be 14% (an additive prevalence was determined as the three recruits of 38 (8%) positive for RSV infection by serology were different to the three recruits of 54 (6%) positive by PCR). The clinical data associated with each identified virus are summarized in Table 2.

Discussion

Similar to our study, older studies of respiratory illness associated with RSV in military trainees have reported a prevalence of 10–11% and a recent study in a civilian population of young adults determined a prevalence of 7%.[7,12–14] In the present study, most trainees experienced an upper respiratory tract illness lasting several days. In addition, 60% reported the occurrence of wheeze of a few days duration, consistent with recent studies that have noted the involvement of the lower respiratory tract in some adults infected with RSV.[4,7,14,15] All recruits were absent from training for almost a week, most of whom were confined to the sickbay, illustrating the degree of morbidity associated with RSV infection in this population. Among the samples found to be positive for RSV by PCR or serology, viral culture failed to detect the virus, consistent with the present view that viral culture is not the optimum diagnostic test for detecting RSV in adults. The insensitivity of this technique and the poor correlation between the results of molecular and serological methods may be due to numerous factors including the shedding of low viral titres in respiratory secretions over short periods, the presence of inhibitors in such secretions, the extreme lability of the virus and sub-optimal conditions for sample collection, storage and transportation. In addition, it has been shown that the serological response in young healthy adults to RSV may be diminished compared with the elderly, thus further supporting the current consensus that the combination of both PCR and serology is the optimal method for detecting RSV infection in this population.[16] A larger study would undoubtedly yield valuable information about the relationships between true infection, RSV isolation and the presence or absence and magnitude of the immune response as determined serologically. Finally, the overall incidence of RSV infection is likely to have been higher if recruits with mild upper respiratory tract symptoms were included in the study, which did not meet the more stringent case definition here.

The most common respiratory virus isolated from symptomatic recruits was adenovirus (35%). This is the first account of adenovirus-associated respiratory illnesses in British military trainees. The observed prevalence is similar to that noted in recent studies of adenovirus infections at US military training centres,[14,17] but is significantly greater than estimates of such infections in civilian adults (2%).[18] In this study, most recruits experienced upper respiratory tract symptoms and some reported symptoms of lower respiratory tract disease including wheezing and dyspnoea. Furthermore, 21% of those with confirmed adenovirus

Table 2. Clinical characteristics of recruits positive for a respiratory virus*

	Adenovirus (*n* = 10)		Influenza (A & B) (*n* = 10)		RSV (*n* = 5)	
	No. recruits (%)	Duration (mean days)	No. recruits (%)	Duration (mean days)	No. recruits (%)	Duration (mean days)
Symptoms						
Cough	10 (100)	4.5	10 (100)	11.1	4 (80)	8.3
Sore throat	10 (100)	3.1	8 (80)	5.0	5 (100)	6.2
Nasal congestion	10 (100)	3.8	10 (100)	9.9	3 (60)	8.7
Dyspnoea	3 (30)	2.0	2 (20)	5.0	0	
Wheeze	2 (20)	2.0	3 (30)	2.7	3 (60)	2.7
Clinical impact						
Absence from training (≥1 day)	9 (90)	3.2	10 (100)	4.1	5 (100)	4.4
Confinement to sickbay	2 (20)	4.0	8 (80)	4.1	4 (80)	4.8
Training recycling	4 (40)	–	3 (30)	–	1 (20)	–

n = number of recruits positive for a respiratory virus for whom completed follow-up questionnaires were available.
*As determined from completed follow-up questionnaires.

infection were hospitalized with pneumonia. This is consistent with the view that infection with adenovirus is typically characterized by upper respiratory disease but may progress to involve the lower respiratory tract.[18] In addition, almost all of the trainees experienced absence from training and a considerable proportion (40%) were 'rolled-back' to another unit for recycled training. Adenoviruses, particularly serotypes 4 and 7, have been recognized as an important cause of ARD among young adults undergoing basic military training since 1954, being consistently isolated from 30% to 70% of US military recruits with ARD.[17,19–21] Large epidemics at training centres could incapacitate commands, halt training programmes and have serious repercussions on military readiness. In 1971, the US Department of Defense began routine administration of live attenuated vaccines against adenovirus types 4 and 7 to all military recruits with dramatic impact on recruit illness, reducing total respiratory disease rates by 50–60%, reducing adenovirus-specific disease by 95–99% and promptly halting disease epidemics in recruits.[17,22–25] In 1995 the sole manufacturer ceased production of adenoviral vaccines.[17,26] Following shelf-life extensions and rationing of existing product, no vaccine has been available for use since early 1999.[17] As supplies gradually declined, outbreaks of adenoviral respiratory illness re-emerged in US military training establishments and adenovirus morbidity has markedly increased. In view of the re-emergence of adenovirus infection the US Department of Defense has contracted a new vaccine manufacturer to produce adenovirus 4 and 7 vaccines. The present study provides preliminary data for debate concerning the relevance of such a programme in British recruits.

Influenza viruses were associated with symptomatic infections in 19% of the trainees. In most cases an acute upper respiratory tract illness resulted, although lower respiratory tract disease did occasionally occur. Almost all of the recruits experienced absence from training and a significant number were confined to the sickbay. Influenza is of particular importance in increased-density populations, and as such, military populations are at high risk of infection. Inactivated influenza vaccines have been shown to be safe, immunogenic and highly efficacious at reducing febrile respiratory illness caused by influenza in adult populations.[27] In addition, influenza vaccination has been shown to be cost-effective relative to providing either supportive care alone or treatment with neuraminidase inhibitors.[28] An influenza vaccination programme for military basic trainees is current policy in the USA (but not in the UK), in which a vaccine is administered within the first days of training. Although the efficacy of this programme in preventing influenzal infections in trainees may be considerable, this is dependent on congruity between vaccine and circulating strains which may, on occasion, be suboptimal.[29]

The present study is unique as it is the first to report respiratory tract illness associated with adenoviral and RSV infections in symptomatic British military recruits. In addition, although involving a relatively small number of subjects, this study provides preliminary data in support of employing adenovirus and influenza vaccines in such populations. Validation and expansion of this study by further research, to include cost-benefit and cost-effectiveness evaluations of vaccines, would seem an appropriate next step.

Ethical approval

Ethical approval was granted by the Defence Medical Services Clinical Research Committee (IRB-approved protocol 034), and the study was conducted in compliance with all applicable regulations governing the protection of human subjects in research.

Disclaimer

The views expressed in this article are those of the authors and do not reflect the official policy or position of the Royal Navy, the Ministry of Defence, or the British Government, or the US Department of the Navy, the US Department of Defense or the US Government.

Conflict of interest

Dr Gray serves on the Data Monitoring Committee for Duramed Inc., the US manufacturer of adenovirus vaccines.

Acknowledgements

The authors thank Dr John Graham (Royal Army Medical Corps) for support in the establishment of this collaborative study, Marie Boot and Laura Owen (HMS Raleigh) for specimen collection, and Marietta Malasig, Dawn Taggett and Kathryn Gabot (DoD Center for Deployment Health Research) for assistance with sample processing and analysis.

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