

# Symptomatic Respiratory Syncytial Virus Infection in Previously Healthy Young Adults Living in a Crowded Military Environment

Matthew K. O'Shea,<sup>1</sup> Margaret A. K. Ryan,<sup>2</sup> Anthony W. Hawksworth,<sup>2</sup> Bryan J. Alsip,<sup>3</sup> and Gregory C. Gray<sup>4</sup>

<sup>1</sup>Institute of Naval Medicine, Alverstoke, Gosport, Hampshire, United Kingdom; <sup>2</sup>Department of Defense Center for Deployment Health Research, The Naval Health Research Center, San Diego, California; <sup>3</sup>Community Health Practices Branch, US Army Academy of Health Sciences, Fort Sam Houston, Texas; and <sup>4</sup>Center for Emerging Infectious Diseases, Department of Epidemiology, College of Public Health, University of Iowa, Iowa City

(See the editorial commentary by Hall on pages 318–9)

**Background.** Respiratory syncytial virus (RSV) infection is a potentially important cause of acute respiratory illness in many populations, including military recruits receiving basic training. Understanding the full impact of RSV infection is challenging because of difficulties in diagnosis and the limitations of past epidemiologic studies. In this study, we set out to determine the prevalence and clinical characteristics of RSV infection and infection caused by other common viral agents in a population of previously healthy young adults, namely, military recruits receiving basic training.

**Methods.** In addition to standard viral culture techniques, we employed serologic testing and a recently described, novel, highly sensitive real-time PCR and a molecular beacon probe assay for the detection of RSV infection.

**Results.** Among 256 military trainees with respiratory symptoms, RSV infection was identified in 11% by means of serologic testing and real-time PCR. Viral culture identified adenovirus in 48% of symptomatic recruits, influenza viruses in 11%, parainfluenza virus 3 in 3%, and enterovirus in <1%. The majority of recruits with RSV infection experienced a nonproductive cough, sore throat, and nasal congestion, and almost half reported symptoms of wheeze or shortness of breath. Almost all (94%) of the recruits lost  $\geq 1$  day(s) from training because of illness.

**Conclusions.** This study demonstrates the challenges of diagnosis and clinical significance of RSV infection in symptomatic young adults. RSV may account for 11% of clinically important respiratory illnesses in this population, which is as much as 25% of previously undiagnosed illness. These results have implications for treatment and prevention of RSV in young adults.

Human respiratory syncytial virus (RSV) is an enveloped, single-stranded, negative-sense RNA pneumovirus. Since its initial isolation from infants with respiratory disease in the late 1950s, epidemiological studies have led to the recognition that RSV is the single most important viral pathogen and leading cause of

acute lower respiratory tract infection in infants and young children throughout the world [1–3].

RSV infection in young children has been studied extensively and was rarely considered important in adults. However, during the past decade, RSV has been increasingly recognized as a cause of severe community-acquired lower respiratory tract illness associated with significant morbidity and mortality in certain susceptible adult populations [4].

Acute respiratory disease caused by RSV is not, however, restricted to pediatric and specific high-risk adult populations. Acquired immunity to RSV is both partial and transient, and natural reinfection occurs repeatedly throughout life, despite the presence of specific and neutralizing antibodies [5, 6]. Studies have shown that RSV is a significant pathogen in previously healthy immunocompetent individuals and that although the

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Reprints or correspondence: Prof. Gregory C. Gray, Center for Emerging Infectious Diseases, Dept. of Epidemiology, College of Public Health, University of Iowa, 200 Hawkins Dr., C21K GH, Iowa City, IA 52242 (gregory-gray@uiowa.edu).

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clinical severity of RSV infection or subsequent reinfection in adulthood is typically diminished, the spectrum of disease varies widely [7–14].

The study of RSV and the diagnosis of acute infection in adults have been hindered by the lack of availability of rapid and sensitive methods of detection [4]. Viral culture and antigen detection exhibit greatly reduced sensitivity in adults, even when used to process specimens under optimal conditions, because of the transient shedding of virus at significantly lower titers in the secretions of the upper respiratory tract of adults, the highly thermolabile nature of RSV, and the occurrence of pre-existing antibodies in nasal secretions [4, 14]. Although the use of serologic testing to identify specific anti-RSV antibodies in adults demonstrates greater sensitivity, the requirement for acute-phase and convalescent-phase serum samples allows only a retrospective diagnosis of RSV infection and limits the use of this method.

RT-PCR for use in viral RNA detection and in the diagnosis of RSV infection has recently been described and has been shown to be especially suited to the detection of low viral titers or nonviable viral RNA in respiratory secretions [15]. Several studies have found RT-PCR and nested RT-PCR to be more sensitive than culture and antigen detection for the diagnosis of RSV infection in infants and adults [15–20].

The development of a novel, highly sensitive, semiquantitative real-time PCR and molecular beacon probe assay for the detection of RSV in respiratory secretions has recently been described [21]. Here, we describe the application of this assay, in comparison with conventional viral culture and serological methods, to estimate the prevalence of clinically significant RSV infection in a symptomatic group of previously healthy young adults and to determine both the clinical presentation and the severity of illness associated with RSV infection in this population. In addition, the prevalence and clinical manifestations of symptomatic disease associated with other common respiratory viral pathogens in this group shall be described and compared with those of RSV infection.

## METHODS

**Subjects and study design.** Subjects for the study were symptomatic military recruits receiving basic training at Fort Benning, a large US Army Infantry training installation located southeast of Columbus, Georgia. This site was selected because of the large population of trainees present at any one time, the existence of infrastructure supporting the enrollment of subjects and the collection, processing, and storage of clinical samples, and because there is evidence, both anecdotal and documented, of previous epidemics of acute upper and lower respiratory tract illnesses among trainees (indeed, the site maintains a specific ward for the isolation of symptomatic trainees). Trainees were considered for inclusion in the study if they

sought medical attention with nonspecific respiratory symptoms and met a case definition, namely, if they possessed an oral temperature of  $\geq 38^{\circ}\text{C}$  and either a cough or a sore throat. In addition, any trainee possessing clinical evidence of acute, nonbacterial pneumonia was considered for the study. Trainees meeting the case definition were briefed on the subject of the study, provided with an information sheet describing the study, and invited to provide voluntary, informed consent to participate (this research, performed under Naval Health Research Center (NHRC) Institutional Review Board–approved protocol #32220, has been conducted in compliance with all applicable Federal Regulations governing the protection of human subjects in research). It was proposed that eligible trainees would be enrolled in the study from November 2000 for a 6-month period (concurrent with annual epidemics of RSV infection among civilian populations).

**Sample collection.** An acute-phase blood sample and bilateral nasopharyngeal swab specimens were collected from participants. Swab specimens from each patient were collected and inserted into a single tube of viral transport medium. A case report form, which collected information on demographic characteristics (i.e., sex, date of birth, week of training, and training unit) and clinical information (i.e., date of sample collection, oral temperature at the time of sampling, duration of symptoms, hospitalization status, and influenza vaccination history), was completed for each symptomatic trainee at presentation. Specimens were promptly transported to an on-site facility and nasopharyngeal swabs were immediately stored at  $-70^{\circ}\text{C}$ , as were serum samples, after fractionation by centrifugation. Participants were recalled 2–4 weeks after initial presentation, when they were asked to complete a follow-up questionnaire and to permit collection of convalescent-phase serum samples.

**Respiratory virus isolation and culture.** Virus isolation and identification were performed using standard procedures [22]. To facilitate the detection of a variety of respiratory viruses, including RSV; influenza viruses A and B; parainfluenza viruses 1, 2, and 3; adenovirus; enterovirus; and herpes simplex viruses 1 and 2, we employed 6 tissue culture cell lines: 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 a mixed monolayer of mink lung (MvLu1) and A549 cells (R-Mix; Diagnostic Hybrids). Tissue culture monolayers were inoculated with a 200- $\mu\text{L}$  sample derived from viral transport medium containing nasopharyngeal swab specimens. Cultures were subsequently examined by light microscopy for characteristic viral cytopathic effect, and viral identification was confirmed by immunofluorescence with a respiratory panel of monoclonal antibodies directed against each of the respiratory viruses previously indicated. Smears of cell suspensions were

examined by fluorescence microscopy, fluorescence was graded on a scale of 0 to 4+, and an estimate of the number of fluorescing cells, as a proportion of the total number of cells, was made. Only smears demonstrating  $\geq 75\%$  fluorescent cells and a fluorescence grade of  $\geq 3+$  were considered positive for a particular respiratory virus.

**Real-time molecular beacon PCR.** We employed a recently described, highly sensitive, semiquantitative real-time PCR and molecular beacon probe assay for the detection of RSV in respiratory secretions for the analysis of clinical specimens derived from nasopharyngeal swab specimens [21]. Following RNA isolation, we performed cDNA synthesis, primary PCR, qualitative conventional nested PCR, semiquantitative molecular beacon real-time PCR, and product analysis, as described elsewhere [21]. For all samples that were deemed positive for the presence of RSV, real-time PCR was performed in triplicate and on different occasions to obtain a mean cycle threshold ( $C_t$ ) value (the cycle number at which the fluorescence generated within the reaction, representing amplicon synthesis, crosses the threshold). Quantification of viral titer in RSV-positive samples was calculated by interpolating mean specimen  $C_t$  values with standard reference curves that had been previously generated.

To minimize the risk of carry-over of contaminating nucleic acids, all working areas were decontaminated with a 0.5% sodium hypochlorite solution before use, pipetting was performed with aerosol-resistant tips, and RNA extractions and the preparation of PCR reagents and amplification mixtures were performed in different biosafety cabinets decontaminated with UV light irradiation before and after use.

**Serological testing.** Serum titers of IgG antibodies against RSV were measured with an ELISA modified from standard methods [23]. Positive antigens (pooled, high-titered lysates of multiply-passaged RSV A2 and B-infected cells) and negative antigens (mock-infected control cell lysate) were prepared in 0.5% Nonidet P40 (vol/vol). Optimal antigen dilutions were determined and incubation times, wash conditions, and detection conditions were all as described elsewhere [23].

Paired acute- and convalescent-phase serum samples (50  $\mu$ L) were assayed on the same 96-well microtiter plate at 2 screening dilutions (1/1000 and 1/2000), in triplicate, against both positive and negative RSV antigens. Sample pairs demonstrating a  $\geq 1.5$ -fold increase, after background correction, in the optical density from the convalescent-phase sample to the acute-phase sample, were further assayed over a range of serial doubling dilutions (1/500–1/64,000), in triplicate, against positive and negative RSV antigens, and on the same microtiter plate.

A positive antibody response against RSV, indicative of RSV infection during the study period, was defined as a paired serum sample in which the acute-phase serum had an optical density of  $\geq 0.2$  and in which there was a  $\geq 4$ -fold increase in the level

of RSV-specific IgG antibodies between the acute-phase and the convalescent-phase serum samples [23].

## RESULTS

**Participants and clinical manifestations.** From November 2000 to April 2001, the average resident population of recruits at Fort Benning was  $\sim 7000$ , and more than 23,000 recruits trained there during this period. Over 8 weeks (late January–March 2001), 256 male symptomatic trainees receiving basic training were enrolled in the study. Nasopharyngeal swab specimens, an acute-phase blood sample, and a completed case report were obtained from each enrolled recruit at presentation. Thereafter, follow-up questionnaires were obtained from 167 of the participants. The demographic characteristics of participants at presentation and general clinical information gathered subsequently are summarized in table 1.

**Analysis of clinical specimens.** Nasopharyngeal swab specimens obtained from each of the 256 symptomatic trainees were analyzed by viral culture. One or more viruses were isolated and identified from 161 (63%) of the total population. Adenovirus was isolated from 123 trainees (48%), influenza viruses from 28 trainees (11% [influenza A and B viruses were isolated in equal numbers]), parainfluenza virus 3 from 8 trainees (3%), and enterovirus from 2 trainees ( $< 1\%$ ). No other respiratory viruses, including RSV, were isolated by viral culture.

Molecular beacon real-time nested PCR detected the presence of RSV in 15 (6%) of the 256 samples obtained from participants. Conventional nested PCR detected RSV in 5 (33%) of these samples (table 2). The mean  $C_t$  value, after repetition of real-time PCR in triplicate and on different occasions for all positive samples, was 28.50 (range, 18.39–33.12). Subsequent semiquantitative quantification demonstrated that the viral load of positive samples ranged from  $1.0 \times 10^{-4}$  to 0.57 plaque forming units (pfu)/mL (mean,  $1.0 \times 10^{-3}$  pfu/mL).

Complete sets of samples (i.e., paired acute- and convalescent-phase serum samples) were available for 157 (61%) of the participants, and serological analysis of anti-RSV IgG antibody levels by an ELISA was performed on these samples. Of these

**Table 1. Demographic characteristics at presentation and clinical information obtained at follow-up from military trainees enrolled in the study.**

Characteristic	Value (n = 256)
Male sex	256 (100)
Mean age, years (range)	21.5 (17.4–33.6)
Mean oral temperature, °C (range)	38.8 (38.2–40.3)
Mean symptom duration, days (range)	4.1 (1–25)
Mean training duration, weeks (range)	4.3 (1–28)
Vaccinated for influenza	254 (99)

**NOTE.** Data are no. (%) of subjects, unless specified otherwise.

**Table 2. Mean cycle threshold (C<sub>t</sub>) values and quantification data from molecular beacon real-time nested PCR analysis and results from conventional nested PCR analysis of clinical specimens collected from trainees found to be positive for the presence of respiratory syncytial virus.**

Specimen	Real-time PCR values		Nested PCR result
	Mean C <sub>t</sub>	RSV titer (pfu/mL)	
1	28.43	1.1 × 10 <sup>-3</sup>	+
2	29.36	6.0 × 10 <sup>-4</sup>	-
3	26.56	3.5 × 10 <sup>-3</sup>	+
4	32.52	1.0 × 10 <sup>-4</sup>	-
5	29.61	5.0 × 10 <sup>-4</sup>	-
6	19.01	3.9 × 10 <sup>-1</sup>	+
7	33.12	1.0 × 10 <sup>-4</sup>	-
8	32.30	1.0 × 10 <sup>-4</sup>	-
9	30.18	4.0 × 10 <sup>-4</sup>	-
10	31.90	1.0 × 10 <sup>-4</sup>	-
11	21.35	9.0 × 10 <sup>-2</sup>	+
12	18.39	5.7 × 10 <sup>-1</sup>	+
13	30.35	3.0 × 10 <sup>-4</sup>	-
14	33.08	1.0 × 10 <sup>-4</sup>	-
15	31.33	2.0 × 10 <sup>-4</sup>	-

**NOTE.** RSV, respiratory syncytial virus; C<sub>t</sub>, cycle threshold.

trainees, 12 (8%) demonstrated serological evidence of RSV infection, indicated by a ≥4-fold increase in the titer of RSV-specific IgG antibodies between the acute-phase and the convalescent-phase serum samples. Five trainees (3%) showed a 2-fold increase in anti-RSV IgG antibody titer, 121 trainees (80%) showed no change in titer, and the remaining 14 trainees (9%) demonstrated a reduction in anti-RSV IgG antibody titer. Five pairs of samples were excluded because of indeterminable IgG antibody titers and prefractionation hemolysis.

The results of the analysis of clinical specimens are summarized in table 3. Of the group of 27 trainees in whom RSV was detected by molecular beacon real-time PCR and/or serologic testing, detection occurred concurrently in 4 individuals. Therefore, after combining the data and correcting for concurrent RSV identification, the prevalence of RSV among

the participants was determined to be 11%. There was no correlation between the results of viral culture and the results of molecular/serological methods of analysis of samples for the presence of RSV. Of the 23 trainees with samples found to be positive for RSV by either molecular or serological methods, convalescent-phase serum was unobtainable from 7 trainees (30%), preventing analysis by ELISA. Among the remaining 16 recruits with positive samples on which both PCR and ELISA analyses were performed, congruent results were obtained for 4 cases (25%). The clinical data associated with each isolated virus are summarized in table 4.

## DISCUSSION

Acute respiratory disease is the leading cause of outpatient illness and hospitalization among US military personnel, with a prevalence exceeding that of the adult civilian population by up to 3-fold [24–26]. Historically, military trainees have been vulnerable to acute respiratory disease and febrile respiratory illness, and their reduced immunity and increased susceptibility have been attributed to demanding physical training schedules and crowded habitation [26]. Although numerous infective agents have been extensively studied in this population, a significant proportion of illness (>40% of cases) has been attributed to unknown causative agents, probably unidentified respiratory viruses [27]. An association between RSV infection and acute respiratory illness in military personnel has previously been made; however, only a few limited studies have been undertaken to investigate this association further [7, 27–29].

In our study, RSV infection was identified in 11% of symptomatic trainees, findings similar to those of the only 2 other studies of RSV infection in military trainees, both of which employed serological methods of detection [28, 29]. A recent study of RSV infection in civilian young adults found a prevalence of 7% [14]. Interestingly, military trainees have previously been shown to be reluctant to seek medical attention. One study demonstrated that as many as 42% of trainees with serological evidence of *Streptococcus pyogenes* failed to present [30]. Therefore, one may speculate that the true prevalence of

**Table 3. Summary of the results of analysis of clinical specimens obtained from symptomatic trainees and of the overall prevalence of identified respiratory viruses.**

Variable	RSV	Adenovirus	Influenza virus	Parainfluenza virus 3	Enterovirus
Assay					
Culture	0/256 (0)	123 (48)/256	28 (11)/256	8 (3)/256	2 (0.8)/256
PCR	15/256 (6)	...	...	...	...
Serological testing	12/157 (8)	...	...	...	...
Prevalence, % of patients	11	48	11	3	0.8

**NOTE.** Data are presented as no. of specimens that tested positive (%) / no. of specimens tested, unless specified otherwise. RSV, respiratory syncytial virus.

**Table 4. Clinical characteristics of trainees who tested positive for a respiratory virus, according to virus type, as determined from completed follow-up questionnaires.**

Characteristic	RSV (n = 17)		Adenovirus (n = 89)		Influenza viruses A and B (n = 16)		Parainfluenza virus 3 (n = 8)		Enterovirus (n = 2)	
	No. (%) of recruits	Mean duration, days	No. (%) of recruits	Mean duration, days	No. (%) of recruits	Mean duration, days	No. (%) of recruits	Mean duration, days	No. (%) of recruits	Mean duration, days
Symptom										
Cough	13 (76)	11.6	77 (87)	10.1	13 (81)	11.6	8 (100)	9.9	1 (50)	10.0
Sore throat	16 (94)	8.2	85 (96)	6.6	15 (94)	5.9	8 (100)	5.4	1 (50)	7.0
Nasal congestion	15 (88)	8.7	81 (91)	7.5	14 (88)	9.5	8 (100)	8.0	1 (50)	14.0
Dyspnea	6 (35)	2.8	54 (61)	3.7	6 (38)	3.5	7 (88)	5.4	1 (50)	5.0
Wheeze	7 (41)	4.3	41 (46)	2.8	5 (31)	2.2	5 (63)	3.0	1 (50)	2.0
Clinical impact										
Absence from training	16 (94)	3.1	88 (99)	2.7	13 (81)	3.2	8 (100)	2.9	2 (100)	1.5
Ward confinement	14 (82)	2.6	78 (88)	2.5	10 (63)	2.8	7 (88)	2.9	1 (50)	2
Training recycling	0	...	2 (2)	...	4 (25)	...	0	...	0	...

**NOTE.** RSV, respiratory syncytial virus. Training recycling refers to recruits repeating a phase of training once returned to fitness.

acute respiratory disease and febrile respiratory illness in this population may be inherently difficult to determine.

The spectrum of clinical disease in adults varies considerably. Early studies of RSV infection in young, previously healthy adults demonstrated only mild disease [7, 31–34]. Recent studies have shown that infection is asymptomatic in 16% of patients and confined to the upper respiratory tract in the majority of cases [14]. However, RSV infection in this population is not limited to mild disease; >80% of infected adults present with a moderately severe, protracted, symptomatic illness characterized by fever, rhinorrhea, nasal congestion, pharyngitis, wheeze, and cough lasting for 7–10 days, and as many as 50% of infected adults are absent from work for 2–14 days [9, 11–14, 22].

We found the majority of trainees experienced an upper respiratory tract illness that lasted for several days and included a nonproductive cough, a sore throat, and nasal congestion. However, a significant number (more than two-thirds) of trainees reported symptoms of lower respiratory tract disease, such as dyspnea and/or wheeze, for up to 4 days in duration, which is in agreement with the findings of more recent studies. Regarding the significance of the morbidity associated with RSV infection, as illustrated by the clinical impact of the illness, almost all of the trainees experienced absence from training and confinement to the febrile respiratory illness ward for a few days.

The diagnosis of acute RSV infection in adult populations has been hindered by the lack of rapid and sensitive diagnostic methods, which has hampered early administration of definitive therapy and the study of RSV infection in this group. We recently described the development of a highly sensitive, semi-quantitative, real-time PCR and molecular beacon probe assay

for the detection of RSV in respiratory secretions [21]. The assay has a detection limit up to the equivalent of  $1 \times 10^{-4}$  pfu/mL of tissue culture–passaged virus and demonstrates high degrees of accuracy, repeatability, and reproducibility. The robustness of this method has been effectively demonstrated in the present study and the assay affords the possibility both of identifying RSV infection in adults in whom extremely low viral titers are identified, in circumstances of suboptimal sample collection, and of providing an estimation of viral load in clinical samples.

Samples from 27 patients were found to be positive for RSV by at least 1 method; of these, 15 were positive by PCR and 12 were positive by serological testing, with agreement between the tests occurring in 4 cases. Viral culture failed to detect RSV in any of the specimens found to be positive by other methods, reinforcing the current consensus that viral culture is not the definitive diagnostic test for detection of RSV in adults. The relatively poor correlation between the results of molecular and serological methods may be attributed to many of the factors that would have resulted in the failure of viral culture to detect RSV, namely, low viral titer or suboptimal sample collection, storage, transportation. In addition, although there are no specific data regarding age-related differences in immunity to RSV, it is well documented that cellular immunity diminishes with age, and, therefore, it is reasonable to suppose that young, healthy adults may respond to infection with RSV more rapidly than elderly persons and may thus be PCR-positive for a shorter period [20, 35]. Also, the presence of inhibitors in samples would hinder viral detection. All of these factors could have contributed to the occurrence of false-negative PCR results. False-negative serologic test results may occur because of both a long illness duration prior to initial presentation (resulting

in high anti-RSV IgG antibody titers obscuring any subsequent increase in antibody titer that would be indicative of recent infection) and either a shortened or prolonged interval between acute- and convalescent-phase serum sample collection. It should be noted, however, that a truly accurate comparison between the results obtained by serologic testing and by PCR is not possible because of the incomplete collection of convalescent-phase serum samples.

The other viruses most commonly identified in this study shall be briefly discussed. Adenoviruses were most commonly isolated, occurring in 48% of participants, which is a prevalence similar to that reported in recent studies of adenovirus infection among trainees at US military training centres (50%–53%) [36], but which is significantly greater than estimates of the prevalence of respiratory illnesses associated with adenovirus in civilian adults (2%) [37]. Most trainees experienced upper respiratory tract symptoms and many also reported symptoms of lower respiratory tract disease. It is interesting to note the significant incidence of respiratory disease associated with adenovirus infection in this particular population, especially in view of the recent cessation of a previously highly effective vaccination program in this group [36, 38].

Symptomatic infections associated with influenza viruses were identified in 11% of trainees, producing an acute illness limited to the upper respiratory tract in the majority of cases. A total of 254 of the trainees (99%) had received a vaccination during the 4 months preceding presentation. Both of the unvaccinated trainees were found to be positive for influenza virus, and the vaccinated trainees found to be positive for influenza virus had received vaccinations, on average, <1 week before presentation. Maximum protection against influenza virus does not fully develop until ~2 weeks after vaccination. These results demonstrate the extremely high protective efficacy of the influenza vaccine that was employed.

The present study includes one of the largest cohorts of symptomatic adults to be tested for the presence of RSV infection and is the largest such study of military trainees. The data presented confirm the significance of RSV infection in previously healthy, immunocompetent, young adults, with respect to the prevalence, morbidity, and clinical sequelae associated with RSV infection. These results have implications for the treatment of trainees receiving basic military training and raise important questions regarding the need for immunoprophylaxis against RSV in this population.

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## References

1. Chanock RM, Finberg L. Recovery from infants with respiratory illness of a virus related to chimpanzee coryza agent (CCA). II. Epidemiological aspects of infection in infants and young children. *Am J Hyg* **1957**; 66:291–300.
2. Chanock RM, Roizman B. Recovery from infants with respiratory illness of a virus related to chimpanzee coryza agent. *Am J Hyg* **1957**; 66:281–90.
3. Chanock RM, Parrott RH. Acute respiratory disease in infancy and childhood: present understanding and prospects for prevention. *Pediatrics* **1965**; 36:21–39.
4. Falsey AR, Walsh EE. Respiratory syncytial virus infection in adults. *Clin Microbiol Rev* **2000**; 13:371–84.
5. Glezen WP, Taber LH, Frank AL, Kasel JA. Risk of primary infection and re-infection with respiratory syncytial virus. *Am J Dis Child* **1986**; 140:543–6.
6. Cane PA. Molecular epidemiology of respiratory syncytial virus. *Rev Med Virol* **2001**; 11:103–16.
7. Johnson KM, Bloom HH. Natural re-infection of adults by respiratory syncytial virus. Possible relation to mild upper respiratory disease. *N Engl J Med* **1962**; 267:68–72.
8. Hall CB, Geiman JM, Biggar R, Kotok DI, Hogan PM, Douglas GR Jr. Respiratory syncytial virus infections within families. *N Engl J Med* **1976**; 294:414–9.
9. Hall WJ, Hall CB, Speers DM. Respiratory syncytial virus infection in adults: clinical, virologic, and serial pulmonary function studies. *Ann Intern Med* **1978**; 88:203–5.
10. Henderson FW, Collier AM, Clyde WA, Jr., Denny FW. Respiratory syncytial virus infections, re-infections and immunity. A prospective, longitudinal study in young children. *N Engl J Med* **1979**; 300:530–4.
11. Finger R, Anderson LJ, Dicker RC, et al. Epidemic infections caused by respiratory syncytial virus in institutionalized young adults. *J Infect Dis* **1987**; 155:1335–9.
12. Dowell SF, Anderson LJ, Gary HE Jr, et al. Respiratory syncytial virus is an important cause of community-acquired lower respiratory infection among hospitalized adults. *J Infect Dis* **1996**; 174:456–62.
13. Murry AR, Dowell SF. Respiratory syncytial virus: not just for kids. *Hosp Pract (Off Ed)* **1997**; 32:87–8, 91–4, 98.
14. Hall CB, Long CE, Schnabel KC. Respiratory syncytial virus infections in previously healthy working adults. *Clin Infect Dis* **2001**; 33:792–6.
15. Walsh EE, Falsey AR, Swinburne IA, Formica MA. Reverse transcription polymerase chain reaction (RT-PCR) for diagnosis of respiratory syncytial virus infection in adults: use of a single-tube “hanging droplet” nested PCR. *J Med Virol* **2001**; 63:259–63.
16. van Milaan AJ, Sprenger MJ, Rothbarth PH, Brandenburg AH, Masurel N, Claas EC. Detection of respiratory syncytial virus by RNA-polymerase chain reaction and differentiation of subgroups with oligonucleotide probes. *J Med Virol* **1994**; 44:80–7.
17. Freymuth F, Eugene G, Vabret A, et al. Detection of respiratory syncytial virus by reverse transcription-PCR and hybridization with a DNA enzyme immunoassay. *J Clin Microbiol* **1995**; 33:3352–5.
18. Henkel JH, Aberle SW, Kundi M, Popow-Kraupp T. Improved detection of respiratory syncytial virus in nasal aspirates by seminested RT-PCR. *J Med Virol* **1997**; 53:366–71.
19. Gonzalez IM, Karron RA, Eichelberger M, et al. Evaluation of the live attenuated cpts 248/404 RSV vaccine in combination with a subunit RSV vaccine (PPF-2) in healthy young and older adults. *Vaccine* **2000**; 18:1763–72.
20. Falsey AR, Formica MA, Walsh EE. Diagnosis of respiratory syncytial virus infection: comparison of reverse transcription-PCR to viral culture and serology in adults with respiratory illness. *J Clin Microbiol* **2002**; 40:817–20.

21. O'Shea MK, Cane PA. Development of a highly sensitive semi-quantitative real-time PCR and molecular beacon probe assay for the detection of respiratory syncytial virus. *J Virol Methods* **2004**; 118:101–10.
22. Lennette EH, Lennette DA, Lennette ET, eds. Diagnostic procedures for viral, rickettsial and chlamydial infections. 7th ed. Washington DC: American Public Health Association, **1995**.
23. Wilson SD, Roberts DK, Hammond K, Ayres JG, Cane PA. Estimation of incidence of respiratory syncytial virus infection in schoolchildren using salivary antibodies. *J Med Virol* **2000**; 61:81–4.
24. Gray GC, Mitchell BS, Tueller JE, Cross ER, Amundson DE. Pneumonia hospitalizations in the US Navy and Marine Corps: rates and risk factors for 6,522 admissions, 1981–1991. *Am J Epidemiol* **1994**; 139: 793–802.
25. Gaydos CA, Gaydos JC. Adenovirus vaccines in the U.S. military. *Mil Med* **1995**; 160:300–4.
26. Gray GC, Callahan JD, Hawksworth AW, Fisher CA, Gaydos JC. Respiratory diseases among U.S. military personnel: countering emerging threats. *Emerg Infect Dis* **1999**; 5:379–85.
27. Lennette EH, Stallones RA. Pattern of respiratory virus infections in army recruits. *Am J Hyg* **1961**; 74:225–33.
28. Hers JF, Masurel N, Gans JC. Acute respiratory disease associated with pulmonary involvement in military servicemen in The Netherlands: a serologic and bacteriologic survey, January 1967 to January 1968. *Am Rev Respir Dis* **1969**; 100:499–506.
29. Sanford JP. Acute respiratory disease in the United States Army in the Republic of Vietnam, 1965–1970. *Yale J Biol Med* **1975**; 48:179–84.
30. Gray GC, Escamilla J, Hyams KC, Struewing JP, Kaplan EL, Tupponce AK. Hyperendemic *Streptococcus pyogenes* infection despite prophylaxis with penicillin G benzathine. *N Engl J Med* **1991**; 325:92–7.
31. Johnson KM, Chanock RM. Respiratory syncytial virus. IV. Correlation of virus shedding, serologic response, and illness in adult volunteers. *JAMA* **1961**; 176:663–7.
32. Kravetz HM, Knight V. Respiratory syncytial virus. III. Production of illness and clinical observations in adult volunteers. *JAMA* **1961**; 176: 657–63.
33. Mills J, Van Kirk JE, Wright PF, Chanock RM. Experimental respiratory syncytial virus infection of adults. Possible mechanisms of resistance to infection and illness. *J Immunol* **1971**; 107:123–30.
34. Hall CB, Douglas RG Jr, Schnabel KC, Geiman JM. Infectivity of respiratory syncytial virus by various routes of inoculation. *Infect Immun* **1981**; 33:779–83.
35. Miller RA. The aging immune system: primer and prospectus. *Science* **1996**; 273:70–4.
36. Gray GC, Goswami PR, Malasig MD, et al., for the Adenovirus Surveillance Group. Adult adenovirus infections: loss of orphaned vaccines precipitates military respiratory disease epidemics. *Clin Infect Dis* **2000**; 31:663–70.
37. Yang E, Rubin BK. “Childhood” viruses as a cause of pneumonia in adults. *Semin Respir Infect* **1995**; 10:232–43.
38. Barraza EM, Ludwig SL, Gaydos JC, Brundage JF. Re-emergence of adenovirus type 4 acute respiratory disease in military trainees: report of an outbreak during a lapse in vaccination. *J Infect Dis* **1999**; 179: 1531–3.