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Robert G. Coon
Melinda S. Balansay
Dennis J. Faix
Anthony W Hawksworth
Matthew B. Patterson
Patrick J. Blair

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Naval Health Research Center
140 Sylvester Road
San Diego, California 92106-3521

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Robert G. Coon, BS; Melinda S. Balansay, MS*; CDR Dennis J. Faix, MC USN*; Anthony W. Hawksworth, BS*; LCDR Matthew B. Patterson, MC USN†; CDR Patrick J. Blair, MSC USN**

ABSTRACT Community-acquired pneumonia can compromise readiness of recruits and service members operating in confined spaces. Often respiratory pathogens are implicated in outbreaks. In July 2008, 5 Basic Underwater Demolition/SEAL students entering an intense period of training at Naval Amphibious Base Coronado reported with clinical symptoms and chest radiographs consistent with pneumonia. Throat and nasal swabs were tested for respiratory pathogens. Molecular evidence indicated that they were infected with the atypical bacterium *Chlamydophila pneumoniae.* Thirty contemporaneous Basic Underwater Demolition/SEAL students were tested to determine the extent of *C pneumoniae* infection burden. Five additional cases were captured within this group. The 10 individuals diagnosed with *C pneumoniae* were treated with a course of azithromycin, Avelox (moxifloxacin hydrochloride), and doxycycline. The cases ended following the isolation of cases and prophylaxis with oral antibiotics. This work highlights the importance of rapid respiratory disease diagnoses to guide the clinical response following the emergence of respiratory infections among military trainees.

INTRODUCTION

Excluding tuberculosis, respiratory infections account for more than 6% of the global burden of human disease, causing great mortality and morbidity within all age groups, but with a concentration in the elderly and the very young.[1] Community-acquired pneumonia (CAP) epidemics result in more than 2 million deaths each year in children aged 5 years and younger, predominantly in developing countries.[2] A number of bacterial agents have been implicated in CAP outbreaks, including *Streptococcus pneumoniae, Chlamydophila pneumoniae, Mycoplasma pneumoniae, Legionella pneumophila,* and *Bordetella pertussis*.[3] CAP outbreaks also occur among adults in crowded settings. For example, in 2008, an outbreak of *M pneumoniae* led to 179 cases of acute respiratory illness and 50 cases of radiographically confirmed pneumonia during a 4-month deployment on a U.S. Navy vessel.[4]

Respiratory infections are estimated to cause 25% to 30% of military hospitalizations in the United States.[5] Military recruits experience a high incidence of febrile respiratory illness (FRI), leading to significant morbidity and lost training time. The most common pathogens indicated in respiratory infections of military populations are adenovirus, influenza virus, group A streptococcus, and bacterial pneumonias.[5] Although this phenomenon is not completely understood, contributing factors are believed to include close living conditions, environmental exposures, physical and/or emotional distress, and a constant influx of immunological naïve individuals.[4]

Basic Underwater Demolition/SEAL (BUD/S) training, held at the Naval Special Warfare Center in Coronado, CA, is a tremendously challenging experience. On July 28, 2008, 5 BUD/S students at the Naval Amphibious Base, Coronado, reported to the medical department with FRI symptoms. Chest radiographs (CXRs) demonstrated moderate consolidation and shortness of breath in some of the recruits, leading to the suspicion of a mild pneumonia. Given the time of year, clustering of the cases, intensity of training, and radiological findings, subsequent laboratory analysis was performed. This analysis elucidated the atypical obligate gram-negative bacterium *C pneumoniae* as the etiological agent. Herein, we describe the investigation and response.

METHODS

Sample Collection

On July 28, 2008, throat and nasal swabs were obtained from 5 BUD/S students with suspected pneumonia. Sputum samples were not obtained. Following the identification of the causative agent on July 29, sampling of an additional 30 contemporaneous students was done on July 30 and 31. Although the work described herein constitutes an outbreak investigation, oral consent was obtained from participants. Following collection in viral transport media (Remel, Lemexa, KS), respiratory swabs were transported on ice. Cold chain was maintained at −20°C or below once samples arrived at Naval

*Department of Respiratory Diseases Research, Naval Health Research Center, San Diego, CA 92106.

†Naval Special Warfare Center, Coronado, CA 91915.

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Health Research Center until the time when nucleic acid was extracted from the samples.

Polymerase Chain Reaction and Reverse Transcription Polymerase Chain Reaction Amplification

DNA was extracted from nasal and throat swabs using the QIAamp DNA Blood Mini Kit (QIAGEN, Valencia, CA) according to the manufacturer's instructions and stored at −70°C. Samples underwent a number of tests for the detection of viral and bacterial respiratory pathogens. Polymerase chain reaction (PCR) assays were performed to test for adenovirus, rhinovirus, three strains of coronavirus (NL63, 229E, and OC43), and influenza A and B. In addition, a multiplex assay detecting 4 bacterial pathogens was conducted on the samples. Reagents and reaction conditions for each of these tests have been previously described.[6] Thermal cycling was performed on either a Bio-Rad iCycler or a DNA Engine (Bio-Rad Laboratories, Hercules, CA). Resulting amplicons were subjected to electrophoresis on either a 2% agarose gel at 120 V for 90 minutes or a 2% E-Gel (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. Gels were visualized and recorded using a Gel Doc 2000 (Bio-Rad Laboratories).

RESULTS

Over a 2-week period in mid-July 2008, 5 BUD/S students were reported with fever, shortness of breath, and cough. Medical examinations were conducted at the training center's clinic. All had recorded temperatures between 99.0°F and 100.5°F and 3 of the 5 students exhibited shortness of breath. One student reported pharyngitis. CXR of the lungs in the 5 patients revealed patchy diffuse interstitial bilateral infiltrates with no predominance to any lobe. These observations led to an initial diagnosis of pneumonia.

On July 28, respiratory swabs were taken from the 5 cases. Genomic material was extracted and molecular diagnostic assays were conducted to test for possible viral and bacterial pathogens, including adenovirus, influenza, rhinovirus, coronavirus, *B pertussis*, *C pneumoniae*, *L pneumophila*, or *M pneumoniae*. Although the samples proved to be negative for viral etiologies, testing against the bacterial panel demonstrated infection with *C. pneumoniae* (Fig. 1), an obligate gram-negative, nonmotile, coccoid bacteria.[7] On the basis of this presumptive diagnosis, the 5 afflicted trainees were treated with either oral azithromycin (500–1000 mg) or 400 mg of Avelox (moxifloxacin hydrochloride).

These initial findings prompted a sampling of additional 30 contemporaneous students on July 30 and 31. These students were midway through an intense training period and all reported mild to moderate respiratory and other ailments. Neither CXR nor temperatures were recorded at the time when respiratory swabs were taken from members in the group. Subsequently, 5 (17%) individuals proved positive for *C pneumoniae* (Table I). CXR taken following swabbing and laboratory diagnosis exhibited patchy bilateral infiltrates in 2 of the 5 identified cases in the second group. Additionally, rhinovirus was diagnosed in 2 students and coronavirus OC43 in another. No bacterial co-infections were found.

Following diagnosis, the 10 afflicted patients were treated with a course of antibiotics that included, on a case-by-case basis, oral azithromycin and/or doxycycline and/or Avelox (Table II). Before the next stage of training, students were administered 1 mg of azithromycin. Among the 10 individuals with *C pneumoniae* infections, 5 were rolled back into training, 3 were "dropped on request," and 2 completed BUD/S training. These actions likely stemmed the budding *C pneumoniae* outbreak.

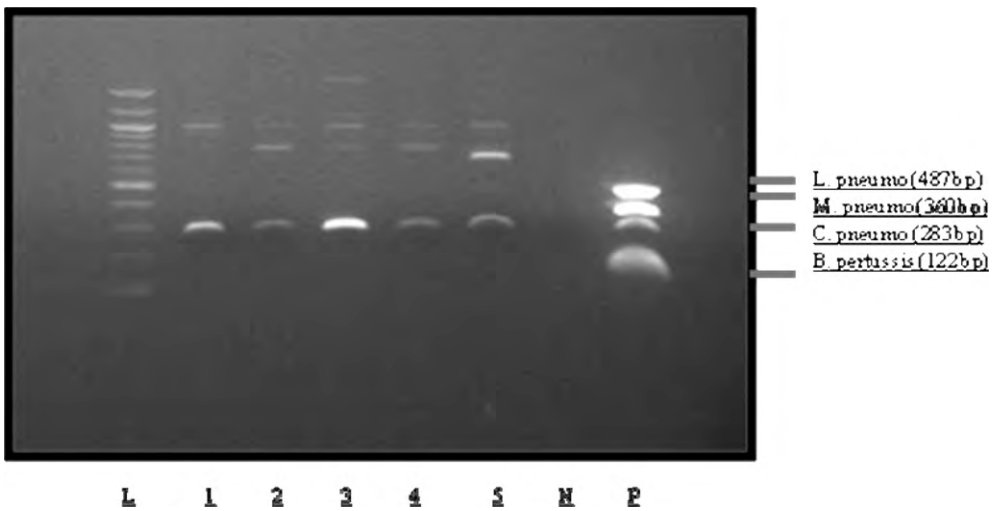

FIGURE 1. Gel agarose electrophoresis showing results of initial 5 BUD/S respiratory samples taken on July 28, 2008. The lane designated L contains the 100-bp ladder. Lanes 2–6 contain BUD/S samples 1–5. Lane N represents a negative control and lane P represents the positive controls for *L pneumophila* (487 bp), *M pneumoniae* (360 bp), *C pneumoniae* (283 bp), and *B pertussis* (122 bp).

TABLE I. Results of Pathogen Testing on Respiratory Swabs in BUD/S Candidates

	Initial Group[a] (N = 5)	Contemporaneous Sampling[b] (N = 30)
Male	5	30
Pathogen testing		
Influenza A	0	0
Influenza B	0	0
Adenovirus	0	0
C pneumoniae	5 (100%)	5 (18%)
M pneumoniae	0	0
L pneumophila	0	0
B pertussis	0	0
Coronavirus	0	1 (3%)
Rhinovirus	0	3 (7%)

[a]Sample collected on July 28, 2008. [b]Sample collected on July 30 and 31, 2008.

TABLE II. Treatment and Outcome of *C pneumoniae*-infected Subjects

PIN	Sample Date	Treatment[a]	Training Result
01	July 28	Azithromycin; 500 mg day 1; 250 mg days 2–5	Rolled back
02	July 28	Azithromycin; 1,000 mg day 1 Doxycycline; 200 mg day1; 100 mg days 2–10	DOR[b]
03	July 28	Azithromycin; 1,000 mg day 1 Avelox; 400 mg days 1–10	Rolled back
04	July 28	Avelox; 400 mg days 1–10	Rolled back
05	July 28	Avelox; 400 mg days 1–10 Azithromycin; 1,000 mg day 1	Rolled back
14	July 31	Avelox; 400 mg days 1–10 Azithromycin; 1,000 mg day 1	DOR[b]
24	July 31	Azithromycin; 1,000 mg day 1 Doxycycline; 200 mg day 1; 1,100 mg days 2–10	DOR[b]
30	July 31	Avelox; 400 mg days 1–10 Azithromycin; 1,000 mg day 1	Passed
32	July 31	Avelox; 400 mg days 1–10 Azithromycin; 1,000 mg day 1	Passed
33	July 31	Azithromycin; 500 mg day 1; 1,250 mg days 2–5 Azithromycin; 1,000 mg day 1	Released

[a]All treatments were given orally. [b]Subjects dropped on request.

DISCUSSION

Herein, we present the results of a laboratory investigation following cases of pneumonia among BUD/S students in July 2008. This work demonstrates the synergy of on-site clinical observations with laboratory diagnosis in the development of a corrective prophylactic response.

Our observations are striking in that, although *C pneumoniae* infection has been implicated in 6% to 22% of lower respiratory infections in pediatric patients reporting for care,[8] the disease is more rare and less mild in adults who often present with a mild "walking pneumonia."[9–11] *C pneumoniae* infections can be severe, with fatalities associated with acute respiratory distress syndrome.[12] The pathogen is believed to be transmitted via respiratory secretions.[13] Our findings suggest that *C pneumoniae* should be included in the differential diagnosis when pneumonia is suspected in military trainees undergoing significant environmental and physical stresses.

Among the 10 trainees diagnosed with *C pneumoniae*, 1 was found to be co-infected with rhinovirus and another with coronavirus OC43. Within human populations, there is a high microbial burden imposed by human rhinoviruses, the cause of the common cold, and coronaviruses.[14] However, the resultant disease is generally mild. We believe that these pathogens were commensal and not the cause of the outbreak of respiratory symptoms and pneumonia within the class.

The sensitivity and specificity of clinical symptoms alone is quite low in diagnosing CAP.[15] Additionally, radiological findings have a limited capacity to differentiate the etiologic agents of CAP.[16] Because treatment modalities are often tied to specific disease pathogens, the importance of rapid diagnostics should not be underappreciated. Upon clinical recognition of FRI, *C pneumoniae* was identified by PCR within 12 hours of sample arrival at the laboratory. Others have shown that PCR for *C pneumoniae* is superior to conventional cell culture in the diagnosis of acute-phase individuals.[17]

The incidence of *C pneumoniae* infections within hospitalized populations or during community outbreak has previously been determined upon analysis of serum antibodies.[18,19] In cases of acute respiratory *C pneumoniae* infection, combinations of PCR and single-serum IgM measurement have been recommended[20] for laboratory-confirmed diagnosis.

Historically, respiratory pathogens have adversely affected U.S. military operations. Adenoviruses and influenza remain the primary threats to military students,[5] although outbreaks of pneumococcal pneumonia have led to attack rates as high as 5.2% in a Marine Corps training company.[18] Epidemics of *C pneumoniae* have also afflicted military students. A study in Izmir, Turkey, found a high prevalence of military students with *C pneumoniae* infections.[19] In addition, outbreaks were reported among military populations in Finland and Thailand and among U.S. recruits.[20–22]

Antibiotic prophylaxis has been moderately successful in stemming bacterial outbreaks in military populations.[23] Results from a 1998 randomized, placebo-controlled clinical trial of azithromycin prophylaxis in BUD/S students indicated that oral azithromycin (1 g/week) was more successful in the prevention of respiratory infections than benzathine penicillin G prophylaxis.[24] Immunity to *C pneumoniae* is often incomplete, and recurrence can occur with some regularity, necessitating long-term macrolide therapy.[25] Fluoroquinolones such as moxifloxacin have shown excellent activity against gram-negative and gram-positive bacilli and have been used in the treatment of atypical pneumonias including *M pneumoniae* and *C pneumoniae*.[26]

The need to minimize the impact of morbidity from infectious pathogens in military personnel is especially critical among students, highly specialized units, and those in confined environments. The incidence of *C pneumoniae* documented herein suggests the importance of added surveillance

in training and other groups from which BUD/S students are determined. Rapid diagnosis of respiratory infections aids military health care providers in making treatment decisions and limits the emergence of potentially mission-compromising epidemics.

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Robert G. Coon; Melinda S. Balansay; CDR Dennis J. Faix, MC USN, Anthony W. Hawksworth; LCDR Matthew B. Patterson, MC USN, CDR Patrick J. Blair, MSC USN

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Naval Health Research Center
140 Sylvester Rd
San Diego, CA 92106-3521

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Commanding Officer
Naval Medical Research Center
503 Robert Grant Ave
Silver Spring, MD 20910-7500

Commander
Navy Medicine Support Command
P.O. Box 140
Jacksonville, FL 32212-0140

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14. ABSTRACT

Community-acquired pneumonia can compromise readiness of recruits and service members operating in confined spaces. Often, respiratory pathogens are implicated in outbreaks. In July 2008, 5 Basic Underwater Demolition/SEAL (BUD/S) students entering an intense period of training at Naval Amphibious Base Coronado reported with clinical symptoms and chest radiographs consistent with pneumonia. Throat and nasal swabs were tested for respiratory pathogens. Molecular evidence indicated they were infected with the atypical bacterium *Chlamydia pneumoniae.* Thirty contemporaneous BUD/S students were tested to determine the extent of *C. pneumoniae* infection burden. Five additional cases were captured within this group. The 10 individuals diagnosed with *C. pneumoniae* were treated with a course of azithromycin, Avelox (moxifloxacin hydrochloride), and/or doxycycline. Cases ended following the isolation of cases and prophylaxis with oral antibiotics. This work highlights the importance of rapid respiratory disease diagnoses to guide the clinical response following the emergences of respiratory infections among military students.

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