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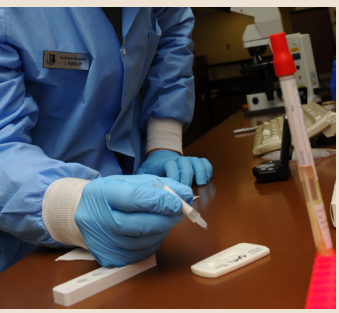

Case Report: Possible Psittacosis in a Military Family Member—Clinical and Public Health Management Issues in Military Settings

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Chlamydia psittaci infection among humans (psittacosis) and pet birds (avian chlamydiosis), also known as parrot disease, parrot fever, and ornithosis, is a zoonotic bacterial disease. Humans most often become infected by inhaling the organism when urine, respiratory secretions, or dried feces of infected birds are dispersed in the air as very fine droplets or dust particles. *C. psittaci* infection of humans can cause influenza-like symptoms, such as fever of abrupt onset, pronounced headache, and dry cough, and can lead to severe pneumonia and non-respiratory health problems. Infection can also be asymptomatic. There is no vaccine for this infection. The disease is treatable with a tetracycline antibiotic, usually doxycycline, or a second-line therapy such as erythromycin or azithromycin. With appropriate treatment, the infection is rarely fatal. This report describes a case of severe, community-acquired pneumonia possibly due to *C. psittaci* in a resident of Colorado and examines significant clinical and epidemiological characteristics of psittacosis that affect confirming the diagnosis and managing the risks of exposure to psittacine (parrot-type) birds.

CASE REPORT

The 24-year-old spouse of an active duty Army service member sought care at the Evans Army Community Hospital (EACH) emergency department (ED) at Fort Carson, CO, several times over the course of 21 days. On 12 February 2018, the patient presented to the ED with fever, gastrointestinal symptoms of diarrhea, vomiting, and cough. Although her gastrointestinal symptoms resolved, the patient returned on 23 February 2018 with persistent cough, fever, chills, lightheadedness, and sinus tachycardia. The patient's chest x-ray was clear, and she was diagnosed with a viral syndrome, not influenza. The patient returned to the ED on 3 March 2018 with dyspnea upon mild exertion (e.g., turning over in bed), occasional wheezing, mild fever (99.3°F), peripheral capillary oxygen saturation (SpO2) of 91% on room air, and lightheadedness. Chest x-ray showed patchy airspace density in the left lung base consistent with early pneumonia, a clear right lung, and no pleural effusion. She was discharged home with a diagnosis of lobar pneumonia with prescriptions for azithromycin 250 mg orally per day for 5 days and the expectorant guaifenesin with codeine. The patient returned to the ED the following day (4 March 2018) with worsening symptoms of dyspnea, fever to 100.7°F, SpO2 of 88% on room air, chest pain, myalgia, pain with breathing, tachycardia, photophobia, and lightheadedness. Chest x-ray revealed an interval increase in the left lower lobe infiltrate, but the right lung remained clear with no pleural effusion. The patient was diagnosed with pneumonia and was administered a course of levofloxacin 500 mg intravenously with O2 therapy. Later that day, she was transported to a local hospital (UCHealth Memorial Hospital Central of Colorado Springs) and admitted for pneumonia and acute respiratory failure with hypoxia. The patient was hospitalized from 5 March through 7 March 2018. The patient was not admitted to EACH because of a brush fire on Fort Carson at the time.

UCHealth Memorial Hospital Central admission screening questions about pets in the home revealed that the patient owned a cockatiel. The civilian hospital collected a serum specimen on 5 March 2018 for a *Chlamydia psittaci* microimmunofluorescence (MIF) immunoglobulin M (IgM) antibody test. Results received on 10 March 2018 included the following values: *C. trachomatis* IgG <1:64, *C. trachomatis* IgM 1:320, *C. pneumoniae* IgG <1:64, *C. pneumoniae* IgM 1:640, *C. psittaci* IgG <1:64, *C. psittaci* IgM 1:320. The reference range for the IgG values was listed as <1:64 for each test. The reference range for the IgM values was listed as <1:20 for each test.

The MIF test was not complete before discharge, but the patient was treated presumptively for *C. psittaci* infection with a course of levofloxacin and doxycycline while hospitalized. Tests for influenza A and B virus antigen, other respiratory viruses, and Group A streptococci were negative. On 7 March the patient was considered medically stable and was discharged with a 3-day course of levofloxacin (750 mg/day), a 4-day course of doxycycline (200 mg/day), and an albuterol inhaler. The patient was instructed to follow up with her primary care manager in 7 to 10 days.

After UCHealth Memorial Hospital of Colorado Springs reported the results of the MIF test to the Colorado Department of Public Health and Environment (CDPHE), the CDPHE state zoonosis veterinarian called the Fort Carson Department of Public Health on 16 March 2018 to report the case and to recommend an interview with the patient and follow-up on her bird. Army Public Health Nursing (APHN) personnel interviewed the patient, provided health education, and advised the patient on treatment options for her bird based on recommendations from the Fort Carson veterinarian. Specific cleaning and disinfecting instructions were provided via

a document created by the National Association of State Public Health Veterinarians titled, "Psittacosis and Avian Chlamydiosis Checklist for Owners of Infected Birds."[1] The patient owned 1 bird, a cockatiel, species *Nymphicus hollandicus.* The patient reported that this was the only bird in the home and that it had been purchased as a chick approximately 3 years previously from a breeder in California. Based on the patient's report, the bird in her home was the most likely exposure. The patient did not work or volunteer at a pet store or shelter and had not visited other homes that housed birds. The bird had not appeared to be sick.

APHN personnel provided the patient, ED personnel, and the Fort Carson veterinarian with educational material referencing the "Compendium of Measures to Control *Chlamydia psittaci* Infection Among Humans (Psittacosis) and Pet Birds (Avian Chlamydiosis), 2017" (hereafter referred to as "the compendium").[2] Two weeks post-hospitalization, on 23 March 2018, the patient was seen again at the ED with pain in her left lung, cough, and dyspnea. Her chest x-ray was clear. She was diagnosed with a recurrence of psittacosis pneumonia and was treated with intravenous doxycycline and dexamethasone. The patient noted that this treatment did not result in a significant improvement in her symptoms, so 5 days later, she was given a second 14-day course of oral doxycycline 200 mg/day because her bird had yet to be examined and treated for psittacosis and because her bird could potentially be shedding the bacteria even though the bird did not appear sick. The patient's husband was evaluated at the ED because of similar symptoms, but no significant diagnosis was made. No family members or other contacts were ill. The patient denied any recent travel. Convalescent testing was performed. Serum for the MIF antibody test was collected at the EACH lab on 2 April 2018. Results available on 5 April 2018 showed that the patient's serological test results specific for *C. psittaci* were negative (IgM <1:16 and IgG <1:10). The laboratory that performed the test during the acute phase of the illness was different from the lab that performed the convalescent test.

APHN personnel consulted with the Fort Carson veterinary clinic, which provided the name of an avian specialist veterinarian in Colorado Springs and recommended the bird be evaluated by that specialist, as military veterinary clinics do not provide clinical care to exotic animals, including avian species. However, the patient took the bird to her regular local veterinarian, an off-post civilian veterinarian, instead of the recommended local civilian avian specialist veterinarian. On 20 March 2018, *Chlamydia* DNA testing was carried out on a choanal/cloacal swab specimen from the bird. On 28 March 2018, the patient's local civilian veterinarian informed her that the DNA test results on her bird's specimen were negative. Because the bird was asymptomatic and the only bird being tested, the recommendations in Appendix 1 of the compendium were indicated. These recommendations state that "diagnosis of avian chlamydiosis can be difficult, especially in the absence of clinical signs. A single testing method might not be adequate. Therefore, use of a combination of culture, polymerase chain reaction (PCR)-based detection, and antibody detection is recommended, particularly when only 1 bird is tested."[1] APHN personnel had initially provided the local civilian veterinary office with the full compendium and sent a highlighted copy of just Appendix 1 after the DNA test results came back negative. Despite having received a copy of the testing protocol for *C. psittaci* in birds,[1] the local veterinarian advised against the recommended testing procedure, stating that the bird appeared well (veterinarian, phone call, March 2018). APHN personnel called the state zoonosis veterinarian again to discuss the issue. The state veterinarian offered to speak to the local veterinarian directly and asked the APHN to convey that offer. Multiple attempts by the APHN to speak to the local civilian veterinarian were unsuccessful. At that point, the state veterinarian and APHN agreed that enough attempts had been made to collaborate with the local treating veterinarian. The patient did not have the funds to have the bird tested elsewhere, so the bird was neither fully tested nor treated.

The Fort Carson Department of Environmental Health (EH) was consulted to discuss the ramifications of an infected bird located in on-post housing. EH recommended that the bird's owners clean the home as indicated in "Part III. Recommendations for Controlling Infection Among Humans and Birds,"[2] replace the carpeting in the home, and keep the birdcage on a non-porous surface that could be more easily disinfected. These recommendations were made for the patient's asymptomatic bird, which was believed to be associated with a human case of psittacosis before the bird was treated. The house was carpeted and the housing office stated the occupants would have to pay out of pocket to have the flooring replaced. The patient and her husband did not have the funds to have the flooring replaced in their rental home. The patient's husband vacuumed the area around the birdcage while the patient was being treated for and recovering from pneumonia. APHN personnel informed the patient and her husband to use the vacuum cleaner with caution because vacuuming may aerosolize infectious *C. psittaci* particles, as noted in the compendium.[2] The patient's condition improved over the course of the next few months, although she continued to have a chronic cough. The patient revisited the ED in June and July 2018 with symptoms of cough, lightheadedness, and nosebleeds that resulted in a diagnosis of allergic rhinitis and epistaxis.

EDITORIAL COMMENT

Avian chlamydiosis, called psittacosis when it occurs in humans, is a zoonotic disease caused by the obligate intracellular gram-negative bacterial pathogen called *C. psittaci,* which is distinct antigenically and genetically from other *Chlamydia* species.[3] Because several diseases affecting humans can be caused by other species of *Chlamydia*, the disease resulting from the infection of humans with *C. psittaci* frequently is referred to as psittacosis rather than chlamydia.

Psittacosis has a worldwide distribution and can occur sporadically or in epidemic fashion at any time of the year.[3] In the U.S., from 2003 through 2014, 112 psittacosis cases were reported to the Centers for Disease Control and Prevention (CDC) through the Nationally Notifiable Diseases

Surveillance System.[2] This number is likely an underestimate of the actual number of cases because psittacosis is difficult to diagnose. The disease is also known as parrot disease, parrot fever, and ornithosis because most psittacosis cases result from exposure to infected pet birds, particularly the Psittaciformes, the order of birds that includes parrots, macaws, cockatiels, and parakeets.[2,4–7] However, psittacosis is prevalent in poultry, pet birds, and wild birds and causes economic losses to the poultry industry and the pet trade.[8,9]

Humans most often become infected by inhaling the organism when urine, respiratory secretions, or dried feces of infected birds are dispersed in the air as very fine droplets or dust particles. Once a bird is infected, the feathers and feces may be contagious many months after the acute illness has resolved.[10] Other sources of exposure include mouth-to-beak contact, a bite from an infected bird, the handling of infected birds' plumage or tissues, and the dissection of dead birds or evisceration in slaughterhouses. Even short-term exposures can lead to symptomatic infection[11,12]; therefore, some patients with psittacosis may not recall or report having any contact with birds.[2]

The symptoms and severity of psittacosis can vary significantly. Some individuals are asymptomatic or only have a very mild infection; others can develop serious widespread infection that affects other parts of the body. The onset of symptoms can be characterized as occurring suddenly or insidiously. *C. psittaci* infection of humans most commonly presents in young or middle-aged adults as fever of abrupt onset, pronounced headache, and dry cough.[2] Affected individuals also may develop chills, myalgia, and malaise. Pneumonia, which is often evident on chest x-ray, commonly occurs and can be severe. Pulse–temperature dissociation (fever without elevated pulse), enlarged spleen, and rash are sometimes observed and suggest a diagnosis of psittacosis for patients with community-acquired pneumonia. Auscultatory findings may underestimate the extent of pulmonary involvement; also, radiographic findings may include lobar or interstitial infiltrates.[2] Frequent epistaxis and hepatomegaly are also familiar occurrences. Although the lungs are the organ most often affected by psittacosis, the disease can potentially affect many organ systems in the body, including the gastrointestinal tract, heart, liver, skin, and central nervous system. The incubation period is usually between 7 and 14 days but can be as long as 39 days.[4]

The differential diagnosis of psittacosis is wide ranging but can be limited based on the specific clinical presentation of the patient. For a patient with atypical pneumonia, the other etiologies to consider include *C. pneumoniae, Mycoplasma pneumoniae,* and *Legionella pneumophila.*[2,13] If the affected patient primarily has a febrile illness without any localizing signs, then, besides psittacosis, other systemic conditions such as influenza, endocarditis, myocarditis, septicemia, vasculitis, *Coxiella burnetii* infection, leptospirosis, and brucellosis should also be considered.[2,14] When extrapulmonary manifestations predominate, the patient should be evaluated for the causes of the most prominent manifestation, such as gastroenteritis, hepatitis, meningitis, or encephalitis.[2,14] Infection with *C. psittaci* has been reported to affect organ systems other than the respiratory tract, resulting in conditions including endocarditis, myocarditis, hepatitis, arthritis, keratoconjunctivitis, encephalitis, and ocular adnexal lymphoma.[15]

CDC and the Council of State and Territorial Epidemiologists have recognized national case definitions for epidemiologic surveillance of psittacosis that healthcare providers can use as part of their clinical evaluation in ascertaining a diagnosis of psittacosis.[13] It is important for healthcare providers to use these case classifications as an element in the overall analysis for confirming a clinical diagnosis or determining medical management **(Table 1)**.[13] Because psittacosis in humans is a nationally notifiable disease, the CDPHE zoonosis veterinarian called the EACH preventive medicine department to report the case and recommend follow-up care and treatment for the patient and her pet bird.

Diagnosis of psittacosis can be challenging. For example, because the patient's initial test IgM titer results appeared to be positive for all 3 types of *Chlamydia* (*C. trachomatis* IgM 1:320; *C. pneumoniae* IgM 1:640; and *C. psittaci* IgM 1:320), the definitive diagnosis was open to several interpretations. Specificity challenges with the 3 types of *Chlamydia* could lead the clinician to the plausible interpretation that the patient had either an infection with *C. pneumoniae* with cross-reactive antibodies to *C. psittaci* or an infection with *C. psittaci* with cross-reactive antibodies to *C. pneumoniae*. Because the medications for both types of pneumonia are similar, the patient's response to the treatments given multiple times does not clarify the diagnosis. The reported convalescent serological results do not make the diagnosis any clearer either. Also, because acute and convalescent sera should be analyzed simultaneously at the same laboratory,[2] the reliability of serological results was diminished, as 2 different labs performed the acute and convalescent tests for the patient. While the presence of the cockatiel in the household suggested a possible diagnosis of psittacosis, it is conceivable that this patient may have had community-acquired pneumonia due to *C. pneumoniae* and she, by chance, happened to own a cockatiel. It is entirely possible that the cockatiel was not infected with *C. psittaci*, as demonstrated by its apparent health and the set of negative test results. On the other hand, a single testing method on the bird might not be adequate, and the use of a combination of culture, PCR-based detection, and antibody detection is recommended, particularly when only 1 bird is tested.[2] A diagnosis of psittacosis could have been made or ruled out if the cockatiel was examined and tested properly by an avian specialist veterinarian because a confirmed psittacosis diagnosis on the bird would have indicated a more definitive source of exposure for the patient. In this analysis, with no other substantial laboratory evidence to narrow down the diagnosis to 1 of these 2 *Chlamydia* species (*C. pneumoniae* or *C. psittaci)*, it appears questionable to definitively diagnose the patient as having psittacosis.

The best method to confirm *C. psittaci* infection in the patient is serologic testing, namely a MIF antibody test.[2] Ideally, most diagnoses are determined by clinical presentation and positive antibodies against *C. psittaci* in paired sera using MIF methods. Convalescent serum testing for MIF was

collected at the EACH lab on 2 April 2018. Results available on 5 April 2018 showed that the patient's serological test results were IgM <1:16 and IgG <1:10. While the MIF antibody test is generally more sensitive and specific than complement fixation tests,[16] cross-reactivity with other chlamydiae (*C. pneumoniae, C. trachomatis,* and *C. felis*) may occur. For this reason, the compendium recommends that a titer less than 1:128 should be interpreted with caution, and true acute (obtained as close to the onset of symptoms) and convalescent (ideally taken 2–4 weeks later) specimen tests are required for accurate interpretation.[2] Because antimicrobial treatment can postpone or weaken the antibody response, a third serum sample 4–6 weeks after the acute sample is recommended.[2] Although serologic testing is more commonly used and available than molecular testing, results can often be unclear, subjective in their interpretation, and ambiguous because of intrinsic limitations of this methodology. If feasible, serology should be considered a supportive test that augments the findings of other more dependable assays, such as nucleic acid–based tests.[17] Information about laboratory testing is available from state public health departments and the compendium.[2] PCR-based testing and assistance can be requested via CDC's Respiratory Diseases Division, and assistance with case investigation can be requested via the U.S. Army Public Health Center and/or the U.S. Army Public Health Command Central.

Tetracycline antibiotics are the drug of choice for treating *C. psittaci* infection in humans.[18] The first choice of therapy for treating mild-to-moderate illnesses is doxycycline 100 mg administered orally twice daily or tetracycline 500 mg 4 times daily for at least 10 to 14 days to prevent relapse. Severely ill patients typically require treatment with intravenous doxycycline hyclate at 4.4 mg/kg/day, divided into 2 infusions per day. Most *C. psittaci* infections are responsive to antibiotics within 1–2 days; however, relapses can occur. Erythromycin is less effective than doxycycline but can be used in cases where tetracyclines are contraindicated (e.g., tetracycline allergy, during pregnancy, for children). The prognosis for treated psittacosis is excellent, with a mortality rate of less than 1%.[19] As has been recommended for the treatment of Rocky Mountain spotted fever, if the benefits outweigh the risks (particularly if the alternative medicine is not effective and it is a life-threatening situation), a tetracycline, such as doxycycline, could be considered in children.[20] Prophylactic antibiotics are not routinely administered after a suspected exposure to *C. psittaci* but may be considered in some circumstances.[20]

According to data from the Armed Forces Health Surveillance Branch (AFHSB), 87 cases of *C. psittaci* infection were reported in U.S. service members between 1 January 2007 and 31 December 2017 (AFHSB, unpublished data, 2018) **(Table 2)**. Over half (n=48) of the total *C. psittaci* cases occurred among non-Hispanic whites. The majority of cases (n=77) occurred in the continental U.S. Slightly more than half of the cases occurred among Army service members (n=45); far fewer cases were diagnosed in members of the Navy (n=16), Air Force (n=13), and Marine Corps (n=13). Of the total cases, 49 were male (56.3%) and 38 were female (43.7%). In addition, seven-eighths (87.4%) of the cases were active duty service members and the remaining cases were Reserve or National Guard members.

TABLE 1. A summary of diagnostic characteristics of psittacosis/ornithosis (*Chlamydia psittaci*) for healthcare providers

Clinical description	Psittacosis is an illness characterized by fever, chills, headache, myalgia, and a dry cough with pneumonia often evident on chest x-ray. Severe pneumonia requiring intensive-care support, endocarditis, hepatitis, and neurologic complications occasionally occur.
Laboratory criteria	Isolation of *C.psittaci* from respiratory specimens (e.g., sputum, pleural fluid, or tissue) or blood, *OR* Four-fold or greater increase in antibody (IgG) against *C. psittaci* by CF or MIF between paired acute- and convalescent-phase serum specimens obtained at least 2–4 weeks apart, *OR* Supportive serology (e.g., *C. psittaci* antibody titer [IgM] of greater than or equal to 32 in at least 1 serum specimen obtained after onset of symptoms), *OR* Detection of *C. psittaci* DNA in a respiratory specimen (e.g., sputum, pleural fluid, or tissue) via amplification of a specific target by PCR assay.
Case classification	Probable An illness characterized by fever, chills, headache, cough, and myalgia that has either supportive serology (e.g., *C. psittaci* antibody titer [IgM] of greater than or equal to 32 in at least 1 serum specimen obtained after onset of symptoms) *OR* detection of *C. psittaci* DNA in a respiratory specimen (e.g., sputum, pleural fluid, or tissue) via amplification of a specific target by PCR assay. Confirmed An illness characterized by fever, chills, headache, cough, and myalgia that is laboratory confirmed by either isolation of *C. psittaci* from respiratory specimens (e.g., sputum, pleural fluid, or tissue) or blood *OR* four-fold or greater increase in antibody (IgG) against *C. psittaci* by CF or MIF between paired acute- and convalescent-phase serum specimens obtained at least 2–4 weeks apart.

Adapted from Centers for Disease Control and Prevention. National Notifiable Diseases Surveillance System (NNDSS). Psittacosis/ornithosis (*Chlamydophila psittaci*) 2010 case definition. https://wwwn.cdc.gov/nndss/conditions/psittacosis/case-definition/2010/. Accessed 26 June 2019.

IgG, immunoglobulin G; CF, complement fixation; MIF, microimmunofluorescence; IgM, immunoglobulin M; DNA, deoxyribonucleic acid; PCR, polymerase chain reaction.

When *C. psittaci* is recognized in a beneficiary of the Military Health System, the case should be promptly reported to local civilian and military public health authorities as soon as laboratory and clinical information are available. Although it is not a requirement to report *C. psittaci* infection in the Disease Reporting System internet for surveillance purposes per the Armed Forces Reportable Medical Events Guidelines and Case Definitions, 2017,[21] the condition can be reported in this system under "Any Other Unusual Condition Not Listed," with "*C. psittaci* infection" entered in the comment field along with a psittacine bird exposure history and other pertinent information. The number of diagnosed and reported human infections likely underestimates the actual (true) number of cases because psittacosis is difficult to diagnose, is treatable with antimicrobials (which may be employed empirically for therapy of community-acquired pneumonia), and often is not reported. Timely, accurate reporting of probable, suspected, or confirmed cases ensures proper identification, treatment, control, and follow-up of cases.

This possible case of psittacosis presented some significant challenges in that recommended avian testing was not performed and there were confounding circumstances that potentially impacted the health of the patient and her bird. Moreover, this case highlighted some issues related to privatized housing and health concerns that can have a public health impact on military installations. The fact that the patient lived in on-post privatized housing posed a barrier to best public health practice. Despite the strong recommendations from Fort Carson EH, the private housing company refused to replace the carpeting/flooring of the patient's home unless paid for by the patient. The financial limitations of this patient, who is the spouse of a junior enlisted soldier, further impacted the ability to disinfect their home environment. The Fort Carson Department of Public Health may have had more authority if on-post housing were owned by the government. Financial limitations also impeded the patient from having her bird properly evaluated by an avian specialist veterinarian even though one was available in town. These factors, along with the lack of adherence to the Fort Carson APHN and EH public health recommendations, potentially left the patient and those that came in contact with her bird at potential risk for infection. It is unclear if the chronic cough the patient reported was related to *C. psittaci* infection. The costs to have the bird adequately tested and to replace the carpeting in the patient's home were minimal compared to the costs associated with the multiple visits to the ED and the subsequent hospitalization of the patient. Appropriate diagnostic testing and interpretation as well as treatment of the patient's bird, which was the suspected source of her infection, should have been overseen by an experienced avian specialist veterinarian.

This case demonstrated that early identification of the disease can be challenging because of the non-specific clinical signs that occur during an infection with *C. psittaci*. Nonetheless, a history of recurrent contact with psittacine bird species, along with indicative symptoms, should generate further diagnostics for psittacosis in order to initiate treatment in humans and contact birds as soon as possible. Local military public health authorities should continue to identify cases of *C. psittaci* infection and report them as soon as laboratory and clinical information are available. Preventive measures include 1) cleaning cages regularly (dampening cages and other contaminated areas with cleaning solution or disinfectant reduces aerosolization) and keeping birds and cages in well-ventilated areas to prevent the accumulation of infectious dust; 2) employing good hygiene, including frequent hand washing, when handling birds, their feces, and their environments; 3) utilizing gloves, coveralls or disposable gowns, disposable caps, protective eyewear (e.g., goggles), and a properly fitted respirator mask (i.e., a pre-shaped mask that molds firmly around the mouth and nose); and 4) following all instructions provided by the treating veterinarian regarding treatment, isolation and quarantine, follow-up testing, and handling of any ill and exposed birds.

TABLE 2. Incident diagnoses of psittacosis, by demographic and military characteristics, U.S. Armed Forces, 2007–2017

	Total	% of total
Total	87	
Sex		
Male	49	56.3
Female	38	43.7
Race/ethnicity		
Non-Hispanic white	48	55.2
Non-Hispanic black	20	23.0
American Indian/Alaska Native	2	2.3
Asian	3	3.4
Hispanic	10	11.5
Unknown	1	1.1
Other	3	3.4
Age group (years)		
<20	24	27.6
20–24	23	26.4
25–29	21	24.1
30–34	12	13.8
35–39	2	2.3
40+	5	5.7
Service		
Army	45	51.7
Navy	16	18.4
Air Force	13	14.9
Marine Corps	13	14.9
Rank		
Junior enlisted (E1–E4)	53	60.9
Senior enlisted (E5–E9)	24	27.6
Officer (001-005)	10	11.5
Country of military assignment		
U.S.	77	88.5
Germany	2	2.3
Japan	1	1.1
Puerto Rico	1	1.1
South Korea	2	2.3
Unknown	4	4.6

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REFERENCES

1. National Association of State Public Health Veterinarians. Psittacosis and avian chlamydiosis checklist for owners of infected birds. http://www.nasphv.org/Documents/PsittacosisChecklistOwners2017.pdf. Accessed 25 January 2019.
2. Balsamo G, Maxted AM, Midla JW, et al. Compendium of measures to control *Chlamydia psittaci* infection among humans (psittacosis) and pet birds (avian chlamydosis), 2017. *J Avian Med Surg*. 2017;31(3):262–282.
3. American Academy of Pediatrics. *Chlamydia* (*Chlamydophila) psittaci*. In: Pickering LK, ed. *Red Book: 2003 Report of the Committee on Infectious Diseases*. 28th ed. Elk Grove Village, IL: American Academy of Pediatrics; 2003:237–238.
4. Harkinezhad T, Verminnen K, De Buyzere M, Rietzschel E, Bekaert S, Vanrompay, D. Prevalence of *Chlamydophila psittaci* infections in a human population in contact with domestic and companion birds. *J Med Microbiol*. 2009;58(pt 9):1207–1212.
5. Heddema E, van Hannen EJ, Duim B, et al. An outbreak of psittacosis due to *Chlamydophila psittaci* genotype A in a veterinary teaching hospital. *J Med Microbiol.* 2006;55(pt 11):1571–1575.
6. Kaibu H, Iida K, Ueki S, et al. Psittacosis in all four members of a family in Nagasaki, Japan. *Jpn J Infect Dis.* 2006;59(5):349–350.
7. Smith KA, Bradley KK, Stobierski MG, Tengelsen LA; National Association of State Public Health Veterinarians Psittacosis Compendium Committee. Compendium of measures to control *Chlamydophila psittaci* (formerly *Chlamydia psittaci*) infection among humans (psittacosis) and pet birds, 2005. *J Am Vet Med Assoc*. 2005;226(4):532–539.
8. Geigenfeind I, Haag-Wackernagel D. Detection of *Chlamydophila psittaci* from feral pigeons in environmental samples: problems with currently available techniques. *Integr Zool.* 2010;5(1):63–69.
9. Petrovay F, Balla E. Two fatal cases of psittacosis caused by *Chlamydophila psittaci*. *J Med Microbiol.* 2008;57(pt 10):1296–1298.
10. Evans EE. Zoonotic diseases of common pet birds: psittacine, passerine, and columbiform species. *Vet Clin North Am Exot Anim Pract.* 2011;14(3):457–476.
11. Harkinezhad T, Verminnen K, Van Droogenbroeck C, Vanrompay D. *Chlamydophila psittaci* genotype E/B transmission from African grey parrots to humans. *J Med Microbiol*. 2007;56(pt 8):1097–1100.
12. Matsui T, Nakashima K, Ohyama T, et al. An outbreak of psittacosis in a bird park in Japan. *Epidemiol Infect.* 2008;136(4):492–495.
13. Centers for Disease Control and Prevention. National Notifiable Diseases Surveillance System (NNDSS). Psittacosis/ornithosis (*Chlamydophila psittaci*) 2010 case definition. https://wwwn.cdc.gov/nndss/conditions/psittacosis/case-definition/2010/. Accessed 25 January 2019.
14. Schlossberg D. Psittacosis (due to *Chlamydia psittaci*). In: Bennett J, Dolin R, Blaser MJ, eds. *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*. 8th ed. Philadelphia, PA: Elsevier/Saunders. 2015:2171–2173.
15. Beeckman DS, Vanrompay DC. Zoonotic *Chlamydophila psittaci* infections from a clinical perspective. *Clin Microbiol Infect*. 2009;15(1):11–17.
16. Persson K, Boman J. Comparison of five serologic tests for diagnosis of acute infections by *Chlamydia pneumoniae*. *Clin Diagn Lab Immunol*. 2000;7(5):739–744.
17. Bourke SJ, Carrington D, Frew CE, Stevenson RD, Banham SW. Serological cross-reactivity among chlamydial strains in a family outbreak of psittacosis. *J Infect*. 1989;19(1):41–45.
18. Mandell LA, Wunderink RG, Anzueto A, et al. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. *Clin Infect Dis*. 2007;44(suppl 2):S27–S72.
19. Elliott JH. Psittacosis: a flu-like syndrome. *Aust Fam Physician.* 2001;30(8):739–741.
20. American Academy of Pediatrics. Tetracyclines. In: Kimberlin DW, Brady MT, Jackson MA, Long SS, eds. *Red Book: 2015 Report of the Committee on Infectious Diseases*. 30th ed. Elk Grove Village, IL: American Academy of Pediatrics; 2015:873.
21. Armed Forces Health Surveillance Branch in collaboration with the U.S. Air Force School of Aerospace Medicine, Army Public Health Center, and Navy and Marine Corps Public Health Center. Armed Forces reportable medical events: guidelines and case definitions. https://health.mil/Reference-Center/Publications/2017/07/17/Armed-Forces-Reportable-Medical-Events-Guidelines. Published 17 July 2017. Accessed 25 January 2019.

Serological Evidence of *Burkholderia pseudomallei* Infection in U.S. Marines Who Trained in Australia From 2012–2014: A Retrospective Analysis of Archived Samples

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Infection with the gram-negative bacterium *Burkholderia pseudomallei* can result in a life-threatening disease known as melioidosis. Historically, melioidosis was a common infection in military forces serving in Southeast Asia, and it has the potential to have a serious impact on force health readiness. With the U.S. Department of Defense's increasing strategic and operational focus across the Pacific Theater, melioidosis is an increasingly important issue from a force health protection perspective. U.S. Marines deploy annually to Darwin, Australia, a "hyperendemic" region for *B. pseudomallei*, to engage in training exercises. In an effort to assess the risk of *B. pseudomallei* infection to service personnel in Australia, 341 paired samples, representing pre- and post-deployment samples of Marines who trained in Australia, were analyzed for antibodies against *B. pseudomallei* antigens. Serological evidence of possible deployment-related infection with *B. pseudomallei* was found in 13 Marines. Future prospective studies are required to further characterize the risk to service members deployed to melioidosis endemic areas.

WHAT ARE THE NEW FINDINGS?

Since 2012, U.S. Marines have participated in training exercises in Darwin, Australia, one of the world's "hyperendemic" regions for *Burkholderia pseudomallei*. Analysis of pre- and post-deployment serum samples obtained from the Department of Defense Serum Repository identified serological evidence of possible infection with *B. pseudomallei* in U.S. Marines who trained in Australia during 2012–2014.

WHAT IS THE IMPACT ON READINESS AND FORCE HEALTH PROTECTION?

Historically, melioidosis was a common infection in military forces serving in Southeast Asia, and it has the potential to have a serious impact on force health readiness today. Given the U.S. Department of Defense's increasing strategic and operational focus across the Asia-Pacific Theater, melioidosis is an increasingly important issue from a force health protection perspective.

Melioidosis is a potentially life-threatening disease caused by the gram-negative bacterium *Burkholderia pseudomallei*. Endemic to tropical regions, melioidosis is especially prevalent in Southeast Asia (Thailand, Malaysia, Singapore, Cambodia, Vietnam, and Myanmar) and northern Australia.[1,2] A recent model estimated an incidence of 165,000 melioidosis cases per year (an incidence rate of 5.0 per 100,000 people at risk), with a predicted mortality of 89,000 per year, among the 3 billion people residing in areas likely to contain *B. pseudomallei.*[3] The true global distribution of *B. pseudomallei* and the incidence of melioidosis remain poorly understood, and it is not yet known if the growing number of melioidosis cases reported worldwide reflects an unmasking of long-standing bacterial presence or the spread of *B. pseudomallei* to previously unaffected areas.[4]

The primary route of infection with *B. pseudomallei* is believed to be through skin inoculation of the soil-dwelling bacterium. Inhalation, aspiration, or ingestion from contaminated water sources is also common.[4,5] The bacterium can infect any organ in the body, precipitating a diverse assortment of clinical presentations. Patients may experience asymptomatic seroconversion; a mild illness manifesting as non-specific febrile symptoms; or a severe, systemic infection resulting in abscess formation, pneumonia, and fatal septic shock.[6] Latent infection with subsequent activation can also occur but has been very uncommon according to data from the Darwin Prospective Melioidosis Study.[7]

The indirect hemagglutination assay (IHA) is the most widely used serological test for the detection of antibodies directed against *B. pseudomallei.*[8–10] While the clinical utility of the IHA in both melioidosis-endemic and non-endemic settings is well described, persistently negative IHA findings in culture-confirmed cases of melioidosis, decreasing titers in serial samples, and high background seropositivity in endemic locations are all well documented.[11–13] In addition, performing the IHA is cumbersome, making it impractical for screening large numbers of samples. The utility of the IHA is further limited by the fact that its results may vary depending on the population being tested and that the reagents are difficult to obtain in non-endemic areas. Expert commentary has acknowledged the need to develop and validate alternative serological tests, especially for pre- vs post-exposure surveillance.[10]

Enzyme-linked immunosorbent assays (ELISAs), when antigens are carefully selected and properly validated, represent a potentially superior method for detecting *B. pseudomallei* infection, especially when

pre- and post-exposure samples can be examined.[10,14,15] Two antigens from *B. pseudomallei* are undergoing clinical validation in an effort to provide accurate, inexpensive, and reproducible assays for the detection of *B. pseudomallei* infection. An O-polysaccharide (OPS)-based ELISA (OPS-ELISA) was recently evaluated using serum samples from culture-confirmed melioidosis patients from Thailand, patients with other bacterial infections, and healthy donors from northeast Thailand and the U.S.[16] The OPS-ELISA displayed a sensitivity of 71.6% among culture-confirmed patients, a specificity of 95.7% for healthy Thai controls, and a specificity of 96.7% for healthy U.S. controls, demonstrating the potential for a superior serological assay for melioidosis.[16] Hemolysin co-regulated protein 1 (Hcp1) is another promising target for serological assays.[17,18] While the Hcp1-ELISA was more sensitive than the OPS-ELISA (83.0% versus 71.6%), both had specificity greater than 95%.[18] Although more studies are needed to determine how broadly these results can be applied, Hcp1- and OPS-based assays represent viable platforms for the detection of antibodies directed against *B. pseudomallei*, especially in an immunologically naïve population.

Historically, melioidosis has been a serious threat to foreign military forces deployed to Southeast Asia. Foreign troops are often immunologically naïve and are potentially exposed to environmental *B. pseudomallei* percutaneously through skin abrasions, burns, and combat wounds or by inhalation through aerosolization of *B. pseudomallei* from blasts, helicopter rotor blade updraft, or severe weather events.[6] For instance, numerous cases have been reported in helicopter crews, likely due to inhalation of dust and aerosolized water during dustoffs.[19,20] Between 1948 and 1954, at least 100 French troops developed melioidosis while serving in Indochina.[19] During the Vietnam conflict, 343 cases of melioidosis were reported in U.S. soldiers by 1973. Furthermore, an estimated 225,000 U.S. military personnel returning from Vietnam exhibited serological evidence of infection.[21] Termed "the Vietnamese time bomb," reactivation from latent foci was well documented among veterans of the Vietnam War years after return to the U.S.[22–27]

Today, melioidosis remains a force health protection challenge to the U.S. military. For example, in May 2006, a previously healthy U.S. Marine developed severe systemic melioidosis following a 2-week military exercise in Thailand.[28] He was treated with an intensive 12-month course of multi-antibiotic therapy and experienced full recovery. As the U.S. Department of Defense (DoD) continues to increase its focus toward the Asia-Pacific Theater, melioidosis has the potential to threaten U.S. military members serving in *B. pseudomallei*-endemic areas.

In April 2012, U.S. Marines began deploying to Darwin, Australia, to participate in joint training exercises with the Australian Defense Force. Marine Rotational Force–Darwin (MRF-D) began with a modest force of approximately 200 Marines and has grown to over 1,500 Marines. It is expected to eventually grow to over 2,500 Marines and sailors. Current MRF-D rotations last approximately 6 months and are timed to avoid the wet season, when the majority of melioidosis cases occur. However, the duration of rotations may increase in the future, and there have been several cases of melioidosis in Australian military personnel undertaking training in the same location in northern Australia over the last 2 decades[7] (also unpublished data, Dr. Bart J. Currie, Royal Darwin Hospital), indicating that U.S. Marines and sailors may be at risk. But, no melioidosis serosurveys have been undertaken by the Australian military, so the seroprevalence is unknown.

This study sought to understand the risks of *B. pseudomallei* infection in U.S. Marines serving in Australia by first characterizing the rates of infection in archived serum samples. This report describes serological evidence of possible asymptomatic *B. pseudomallei* infections in U.S. Marines serving in endemic areas.

METHODS

Serum samples

De-identified serum samples were obtained from the DoD Serum Repository (DoDSR).[29] Samples from 1,124 U.S. Marines with deployment histories that included deployment to Australia between 2012 and 2014 were located in the DoDSR by Armed Forces Health Surveillance Branch staff. Information on demographic and military characteristics, serum collection dates, sex, military occupational specialty, and deployment history was provided. The exact locations of deployment sites (e.g., Darwin) and training areas in Australia were not captured.

Paired sample selection **(Figure 1a)** began by screening post-deployment samples for antibodies to the *B. pseudomallei* type A OPS **(Figure 1b)** using a 1:2,000 dilution as previously described.[16] In the absence of known cutoff values for U.S. samples, a total of 223 individuals representative of the screen results (i.e., highly positive through negative; **Figure 1b**) were selected for further testing. To address potential selection bias associated with the screening results, an additional 118 unscreened individuals whose only deployment was to Australia in 2014 were identified. Pre-and post-deployment samples from each of these 341 individuals were paired and analyzed as described below.

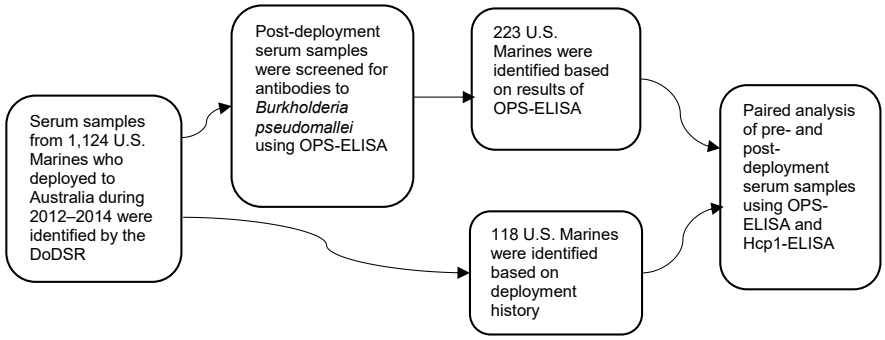
Serological analyses

Antibody capture ELISAs targeting the *B. pseudomallei* Hcp1 and type A OPS antigens were performed as previously described, with minor modifications to the sample preparation.[16] Briefly, 3-fold serial dilutions of the serum were used to generate curves ranging from 1:74 to 1:54,000. Paired titers were run in duplicate and positive results were verified by repeated analysis. Negative controls were commercially obtained serum samples from U.S. sources (Biological Specialty Corporation, Reading, PA 19602), while positive control sera were derived from culture-confirmed melioidosis cases from Cambodia.[30] Pre-deployment and post-deployment sera for each individual were paired for analysis. IHAs were performed at the Royal Darwin Hospital as previously described.[8,9,12]

Statistical analysis

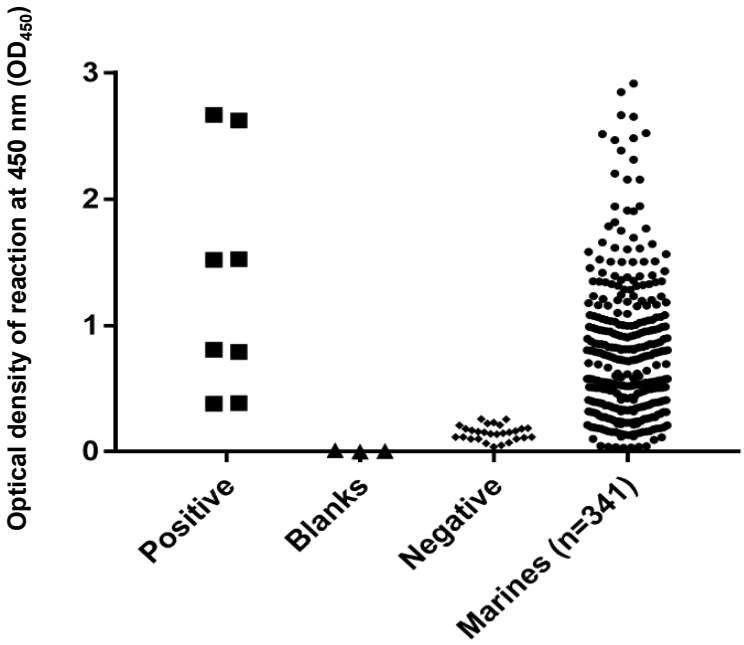
Paired pre- and post-deployment sera were evaluated with a paired 2-tailed *t* test

FIGURE 1a. Overall sample selection strategy



DoDSR, Department of Defense Serum Repository; OPS-ELISA, O-polysaccharide-based enzyme-linked immunosorbent assay; Hcp1-ELISA, hemolysin co-regulated protein 1-based enzyme-linked immunosorbent assay.

FIGURE 1b. Screening ELISA results for study sample (n=341)



Note: Positive controls are 2-fold serial dilutions of pooled sera from culture positive melioidosis patients.[30] Blanks were tested with assay buffer containing no primary antibody. Negative controls were serum from healthy U.S. donors (n=20). The screening ELISA results for each of the 341 post-deployment serum samples selected for further analysis are shown.

ELISA, enzyme-linked immunosorbent assay.

using GraphPad Prism version 6.0 (2013, GraphPad Software, La Jolla, CA) and Stata version 14 (2015, StataCorp LP, College Station, TX). Statistical significance was defined as $p<.05$.

RESULTS

Demographic and military characteristics of the study sample (n=341) are presented in **Table 1**. The individuals were heavily skewed toward white and male (251/341 and 335/341, respectively) junior enlisted Marines (314/341). Australia was the first deployment for over half (51.3%) of the study sample. Of those with prior deployments, 131/166 (78.9%) were deployed to non-endemic areas (e.g., Afghanistan, Iraq, Japan). During the 2012 rotation, it was common practice for Marines to also travel to Thailand for a short period of training (2–4 weeks); more than three-quarters (33/42) of those with an MRF-D rotation in 2012 had travel histories that included Thailand **(Table 1)**.

Serological evidence of infection with *B. pseudomallei* using the OPS-ELISA

Paired sera samples analyzed for antibodies against the OPS-ELISA produced 3 patterns of results **(Figures 2a, 2b, 2c)**. The vast majority (78.9%; 269/341) of the Marines in the study lacked detectable OPS antibodies in both their pre- and post-deployment sera samples **(Figure 2a)**. Fifty-nine Marines displayed elevated antibody titers in both their pre- and post-deployment samples **(Figure 2b)**. Finally, 13 Marines appeared to have seroconverted to *B. pseudomallei* OPS during their deployment to Australia, as their post-deployment serum samples displayed statistically significant increases in antibody titer **(Figure 2c)**.

Hcp1-ELISA–based evidence of infection with *B. pseudomallei*

Paired sera samples analyzed for antibodies against Hcp1 were broadly consistent with OPS-ELISA results and yielded similar categories of results **(Figures 3a, 3b, 3c)**, although with interesting differences. The number of pairs with detectable antibodies in both their pre- and post-deployment samples fell by 51 **(compare Figure 2b to Figure 3a)**, resulting in only 8 Marines with elevated anti-Hcp1 pre- and post-deployment titers **(Figure 3b)**. Using Hcp1-ELISA, paired sera from 12 Marines, including 10 who were OPS-ELISA positive, displayed statistically significant increases in their post-deployment samples relative to their pre-deployment samples **(Figure 3c)**. While

3 individuals demonstrating seroconversion to OPS showed no detectable antibodies to Hcp1, the Hcp1-ELISAs resulted in the identification of 2 additional seroconverters not seen with the OPS-ELISA.

Previous studies employing the OPS- and Hcp1-ELISA have found that the 1:2,000 dilution of serum produces highly consistent and discriminating (i.e., melioidosis vs non-melioidosis) results.[2,16,18] Here, a paired 2-tailed *t* test was used to analyze the results from each pair of samples that exhibited seroconversion across the entire dilution series. These analyses determined that the 1:2,000 dilution provided the most consistent resolution (p=.004 and .0105 for OPS and Hcp1, respectively) for these samples as well **(Figures 2c, 3c)**. The optical density $(OD)_{450}$ values at 1:2,000 for these individuals were then compared by a paired 2-tailed *t* test and found to be significantly different for each antigen **(Figure 4a)**. Finally, the OD_{450} values of paired pre- and post-deployment sera at 1:2,000 were used to calculate fold change in anti-OPS and anti-Hcp1 titers **(Figure 4b)**.

In summary, 15 Marines demonstrated seroconversion to *B. pseudomallei* antigens; 13 of these Marines showed at least a 2-fold increase in OPS titers, and 12 had increased Hcp1 titers **(Table 2)**. A greater than 4-fold increase in antibody titer occurred in 3 of the 13 for OPS-ELISA and 3 of the 12 for Hcp1-ELISA **(Figure 4b)**. Ten of these Marines overlapped in the 2 groups, 3 Marines had increasing OPS titers but no Hcp1 titer, and 2 Marines who were OPS positive in both pre- and post-deployment samples demonstrated deployment-related seroconversion to Hcp1.

IHA serology results

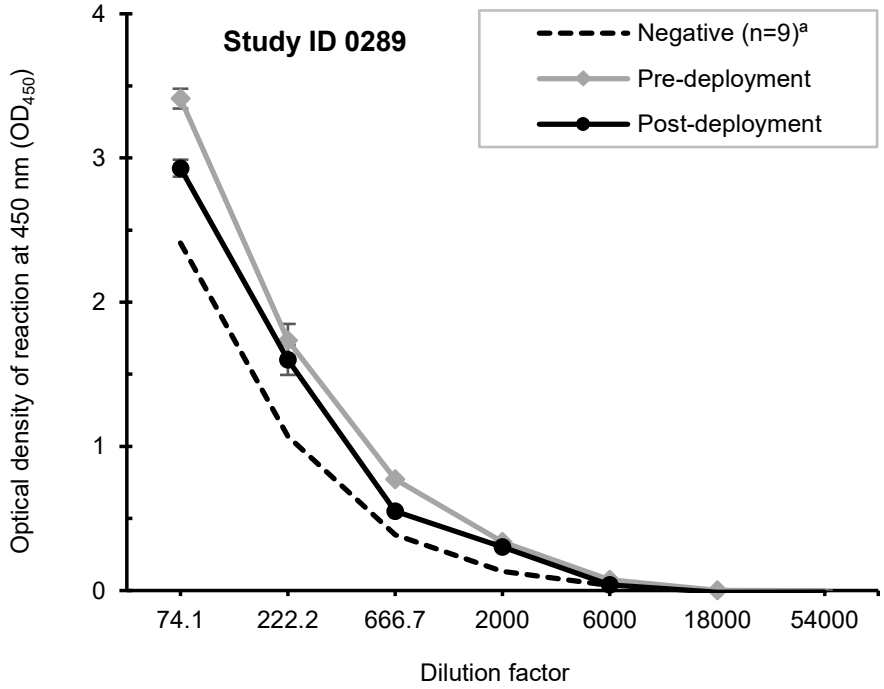
To begin to understand the relationship between IHA and OPS/Hcp1-ELISAs in a U.S. population, samples from 22 individuals were selected to be analyzed by IHA as previously described.[8,31] These samples included pre- and post-deployment sera from 9 Marines who seroconverted to OPS **(Figure 2c)**, 5 Marines who were positive for OPS antibodies in both pre- and post-deployment sera **(Figure 2b)**, and 8 who were negative in both samples **(Figure 2a)**. All IHA titers registered at either <1:20

TABLE 1. Demographic and military characteristics of the study sample (n=341)

	MRF-D rotation			
	2012 (n=42)	2013 (n=118)	2014 (n=181)	Total (n=341)
Sex				
Male	42	118	175	335
Female	0	0	6	6
Race/ethnicity				
Non-Hispanic white	30	93	128	251
Non-Hispanic black	2	7	13	22
Hispanic	5	14	31	50
Asian/Pacific Islander	3	3	5	11
American Indian/Alaska Native	0	1	1	2
Other	1	0	3	4
Unknown	1	0	0	1
Rank				
Junior enlisted (E1–E4)	38	115	161	314
Senior enlisted (E5–E9)	3	1	9	13
Officer	1	2	11	14
No. of individuals with prior deployments[a]	39	55	72	166
No. of individuals with prior deployments to endemic areas[b]	33[c]	1	1	35
Median no. days in Australia (range)	120 (58–152)	152 (89–152)	213 (60–213)	181 (58–213)
Assigned to infantry (%)	31 (73.8)	93 (78.8)	103 (56.9)	227 (66.6)

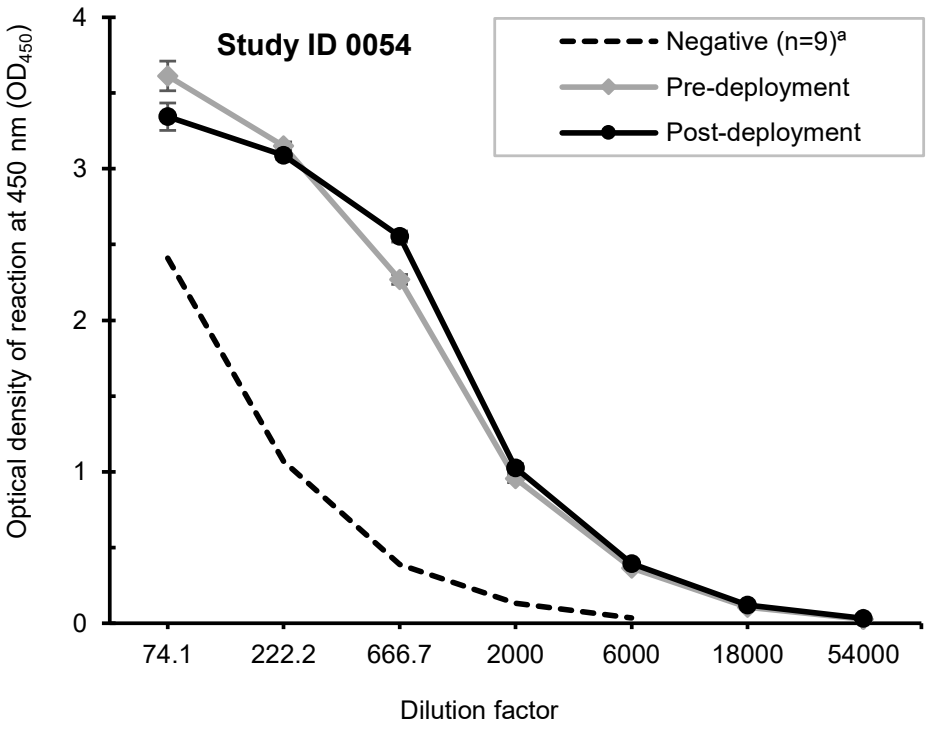
[a]Number of Marine Corps members who were deployed before or during their training in Australia.
[b]Number of Marine Corps members who were deployed to a known *B. pseudomallei* endemic region before or during their training in Australia.
[c]These 33 Marine Corps members trained in an additional endemic region (Thailand) during their deployment to Australia.
MRF-D, Marine Rotational Force-Darwin; No., number.

FIGURE 2a. OPS-ELISA results representative of negative antibody titer



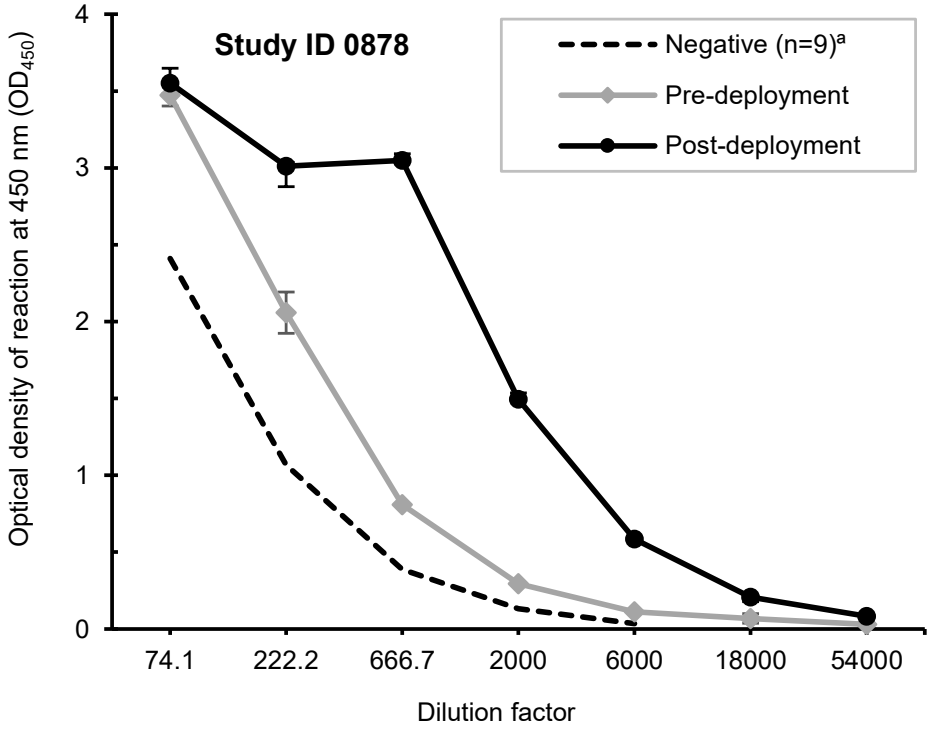
[a]Pooled sera from 9 individuals (negative controls).
OPS-ELISA, O-polysaccharide-based enzyme-linked immunosorbent assay.

FIGURE 2b. OPS-ELISA results representative of positive antibody titer



[a]Pooled sera from 9 individuals (negative controls).
OPS-ELISA, O-polysaccharide-based enzyme-linked immunosorbent assay.

FIGURE 2c. OPS-ELISA results representative of seroconversion



[a]Pooled sera from 9 individuals (negative controls).
OPS-ELISA, O-polysaccharide-based enzyme-linked immunosorbent assay.

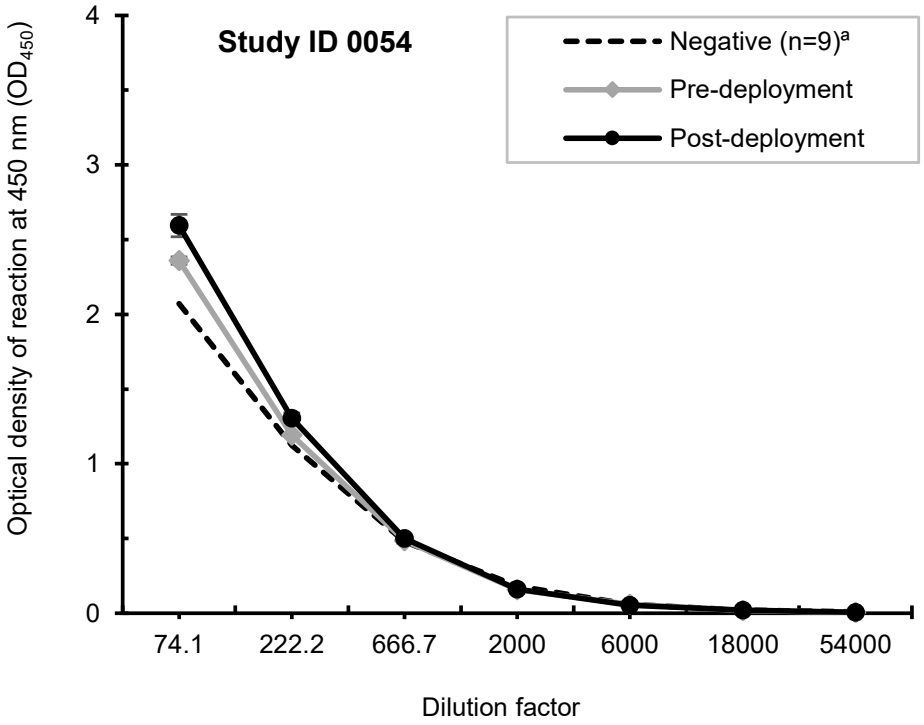
or 1:20, titers considered negative in Darwin, Australia.[12] However, 4 individuals who seroconverted to OPS and/or Hcp1 were <1:20 in their pre-deployment sample and 1:20 in their post-deployment sample, potentially indicating low-level seroconversion by IHA.

EDITORIAL COMMENT

Melioidosis among deployed military forces is well described,[9–21,27,28,32–34] and it is an emerging public health problem in tropical regions, with increases in incidence reported in multiple regions of Southeast Asia[3] and in northern Australia.[35] Even in non-resource–limited settings, the reported mortality of acutely diagnosed melioidosis is high and ranges between 9–18% in recent reports from Australia and Singapore.[36,37] The diagnosis of melioidosis can often be delayed given its heterogeneous presenting syndrome and the fact that empiric sepsis therapy in non-endemic regions often excludes the antimicrobial agents used in melioidosis treatment (i.e., ceftazidime, imipenem, and meropenem).[38] With the DoD's increasing strategic and operational focus throughout melioidosis-endemic areas, this disease is important for force health protection, and deployment history should be considered by treating physicians.

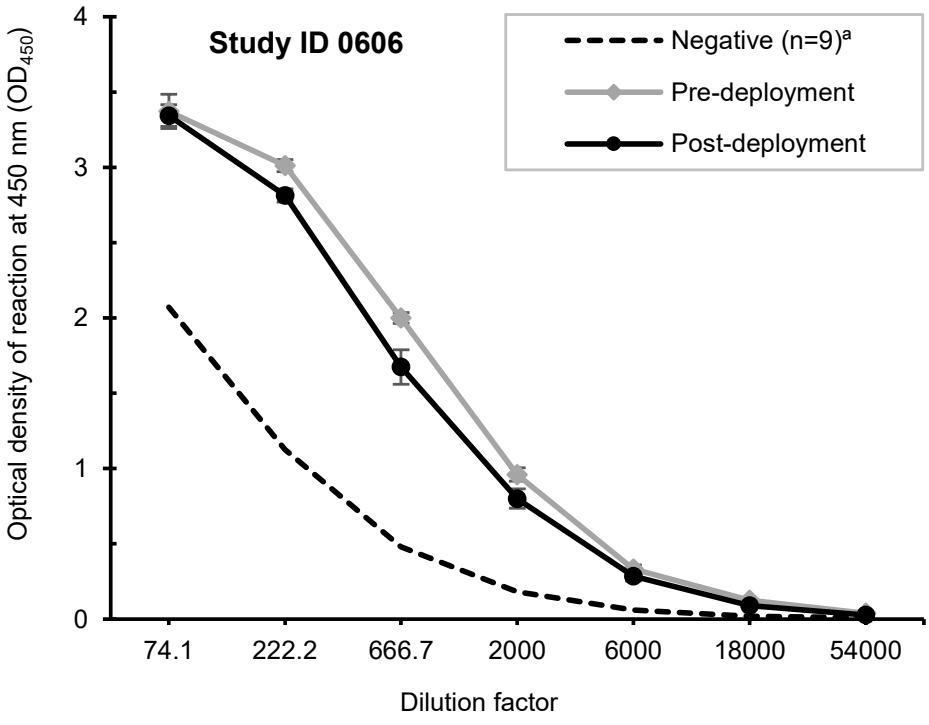
OPS is a major structural component of lipopolysaccharide and has been shown to be a dominant antigen recognized by antibodies in melioidosis patient sera.[15,39,40] Three serologically distinct OPS types (A, B, and B2) have been described for *B. pseudomallei* strains.[41,42] Of these, type A OPS is the predominant antigen expressed by the majority of strains described to date. Interestingly, the proportion of strains expressing the different OPS types can vary by region. For example, in Thailand, 97.7% of *B. pseudomallei* isolates express type A OPS, and in Australia, 85.3% express type A OPS, with 13.8% expressing type B OPS.[42] Studies characterizing the structures of these OPS antigens show that they appear to be unique to *Burkholderia* species,[43,44] a feature that is beneficial for the development of serodiagnostic assays. It should be noted,

FIGURE 3a. Hcp1-ELISA results representative of negative antibody titer



[a]Pooled sera from 9 individuals (negative controls).
Hcp1-ELISA, hemolysin co-regulated protein 1-based enzyme-linked immunosorbent assay.

FIGURE 3b. Hcp1-ELISA results representative of positive antibody titer



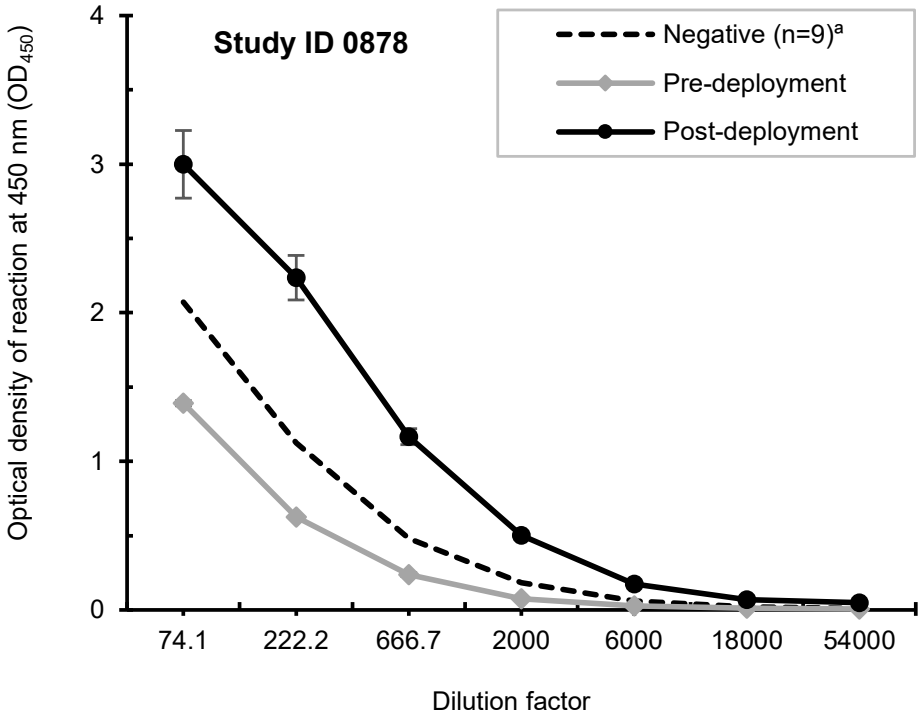
[a]Pooled sera from 9 individuals (negative controls).
Hcp1-ELISA, hemolysin co-regulated protein 1-based enzyme-linked immunosorbent assay.

however, that some non-pathogenic, environmental near-neighbor species express OPS moieties that are the same or similar to *B. pseudomallei* OPS (e.g., *B. thailandensis* type A and *B. humptydooensis* type B2) and could potentially yield false-positive results.[45] To help address this concern, additional target antigens (e.g., Hcp1) have been developed as serodiagnostic tools, and these have also been included in this study. More research is needed to determine whether or not serotype-specific OPS (A, B, or B2) assays may be useful for certain populations and environments under study.

Hcp1 expression is tightly regulated in *B. pseudomallei*. Several studies have shown that Hcp1 is highly expressed in vivo and following uptake by host cells but is undetectable when *B. pseudomallei* is cultured in vitro in rich media conditions.[46,47] Hcp1 is immunogenic and stimulates high antibody titers in melioidosis patients.[17] These features make Hcp1 a potentially attractive indicator of asymptomatic infection and clinical melioidosis because replication within the host is a requirement for anti-Hcp1 antibody production. Furthermore, because Hcp1 expressed by *B. pseudomallei* is structurally different from homologous proteins found in other *Burkholderia* species, an Hcp1-based assay may provide higher specificity by discriminating between exposure to *B. pseudomallei* and neighbor species (e.g., *B. thailandensis*).[18] Finally, use of Hcp1 should also allow for the identification of individuals exposed to *B. pseudomallei* strains expressing type B OPS that would be missed using the type A OPS-specific ELISA described above.

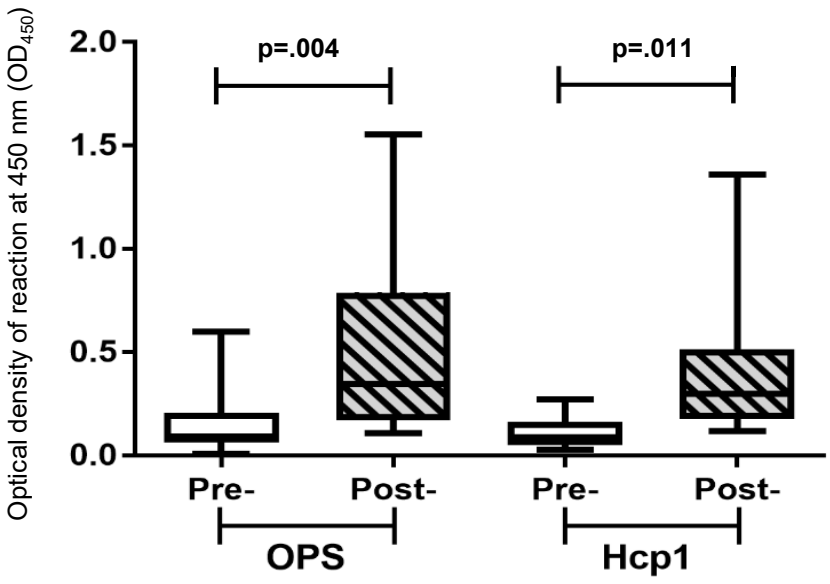
More research is needed to determine if individuals who seroconverted to OPS but remained negative by Hcp1 may represent seroconversion to near neighbors or transient exposure to *B. pseudomallei* with rapid clearance of infection. Determining whether serology assays using Hcp1 or other specific *B. pseudomallei* antigens will help differentiate between transient and persisting but still asymptomatic infection with *B. pseudomallei* will require further prospective studies. Such an assay would be very useful to future efforts to characterize the risks to MRF-D.

FIGURE 3c. Hcp1-ELISA results representative of seroconversion



[a]Pooled sera from 9 individuals (negative controls).
Hcp1-ELISA, hemolysin co-regulated protein 1-based enzyme-linked immunosorbent assay.

FIGURE 4a. Comparison of all OPS and Hcp1 seroconverters



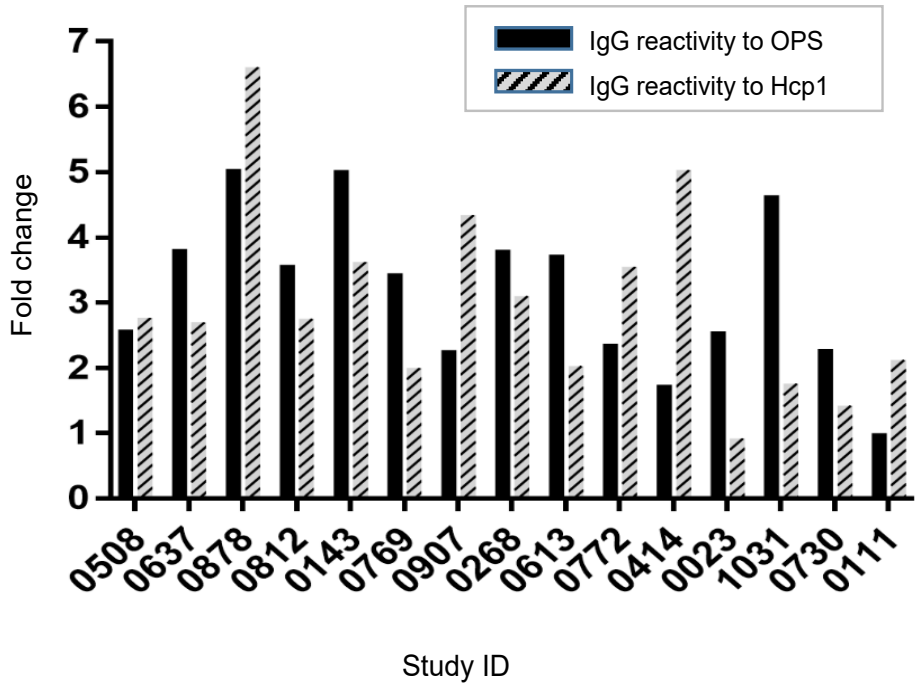
OPS, O-polysaccharide; Hcp1, hemolysin co-regulated protein 1.

Reconciling the low IHA results of the Marines when compared with the OPS- and Hcp1-ELISA results in this report is a critical issue for further study. IHA positivity on initial blood samples from patients presenting with acute melioidosis in Darwin has been as low as 56%.[12] This reflects the rapidity of disease onset after infection, and the large majority of surviving initially seronegative patients with melioidosis seroconvert on IHA after hospital admission.[48] However, up to 15% of culture-positive melioidosis patients may remain persistently negative by IHA.[11,12] Additionally, longitudinal analysis demonstrated that approximately 8% of IHA-positive melioidosis patients lose their positive titers over time.[12] Here, 4 Marines seroconverted to OPS and/or Hcp1 and demonstrated a weakly-increasing IHA titer (<1:20 pre-deployment to 1:20 post-deployment). It is unclear if these results truly represent infection with *B. pseudomallei* during deployment. Nine Marines who seroconverted by OPS and/or Hcp1 assays failed to produce any demonstrable reaction to the IHA. It is speculated that conflicting IHA and enzyme immunoassay results may represent low or decreasing IHA titers that have been identified by the more sensitive ELISA platform.[49]

Several cases of melioidosis have occurred in Australian military service members operating in the same training areas in northern Australia over the last 2 decades[7] (also unpublished data, Dr. Bart Currie, Royal Darwin Hospital), but no melioidosis serosurveys have been undertaken in the Australian military. The current serological survey of a cohort of archived samples from U.S. Marines who trained in Australia found possible evidence of *B. pseudomallei* infection in the form of antibodies directed against the in vivo expressed *B. pseudomallei* protein Hcp1. A serosurvey of a healthy cohort of active civilians in the same region of Australia showed high IHA levels (titers ≥1/80) in 11/354 individuals, some of whom had a specific but transient clinical illness thought to possibly represent melioidosis that resolved without therapy.[50]

Limitations during this study did not allow for definitive conclusions to

FIGURE 4b. Fold change in anti-OPS and anti-Hcp1 titers for seroconverters



OPS, O-polysaccharide; Hcp1, hemolysin co-regulated protein 1; IgG, immunoglobulin G.

be drawn in several areas. Specifically, the metadata lacked detailed information about locations in Australia, clinical information regarding symptoms experienced during training, and background data such as birthplace and personal travel to explain background levels of antibodies directed against *B. pseudomallei*. Importantly, the study sample was not a random sampling of a rotation. A prospective study would provide more detailed subject data and a representative cross section of a rotation to more fully characterize the risks of melioidosis to MRF-D.

It is now recognized that melioidosis is predominantly a disease of those with predisposing risk factors and that mortality is strongly correlated with the presence of risk factors such as diabetes.[4] Young, healthy adults, such as active duty U.S. Marines, lack these risk factors, and the vast majority of those infected with *B. pseudomallei* will have no clinical illness.

TABLE 2. Demographic and military characteristics of seroconverters

							Fold increase (pre- vs post-)	
Study ID	Rotation year	Sex	Race/ethnicity	Enlisted rank	MOS	No. days in Australia	OPS	Hcp1
MEL0637	2013	Male	Non-Hispanic white	Senior	Infantry	152	3.8	2.7
MEL0878	2014	Male	Non-Hispanic white	Junior	Infantry	212	5.1	6.6
MEL0812	2014	Male	Non-Hispanic white	Junior	Infantry	212	3.6	2.8
MEL0508	2012	Male	Non-Hispanic white	Junior	Infantry	120[a]	2.6	2.8
MEL0268	2014	Male	Non-Hispanic white	Junior	Infantry	213	3.8	3.1
MEL0143	2014	Male	Non-Hispanic white	Junior	Infantry	213	5.0	3.6
MEL0769	2014	Male	Unknown	Junior	Infantry	213	3.4	2.0
MEL0907	2013	Male	Hispanic	Junior	Administration	152	2.3	4.3
MEL0772	2014	Male	Non-Hispanic white	Junior	Infantry	212	2.4	3.6
MEL0613	2014	Male	Non-Hispanic white	Junior	Infantry	212	3.7	2.0
MEL1031	2014	Male	Hispanic	Junior	Precision equipment repair	213	4.6	NA[c]
MEL0730	2014	Male	Hispanic	Junior	Infantry	213	2.3	NA[c]
MEL0023	2012	Male	Non-Hispanic white	Junior	Infantry	120[a]	2.6	NA[c]
MEL0414	2014	Male	Non-Hispanic white	Junior	Communications	212	NA[b]	5.0
MEL0111	2012	Male	Non-Hispanic white	Junior	Intercept officer	121	NA[b]	2.1

[a]Approximately 2-month deployment in Thailand mid-Australia.
[b]Positive Hcp1 titer but negative OPS titer.
[c]Positive OPS titer but negative Hcp1 titer.
MOS, military occupational specialty; No., number; OPS, O-polysaccharide; Hcp1, hemolysin co-regulated protein 1.

What remains uncertain is how many asymptomatic people with positive serology, presumably reflecting infection with *B. pseudomallei* at some time point, have not cleared their infection and have bacteria still present in undetermined latent foci.[4] Furthermore, of those with latent infection, it is unknown how many will subsequently have activation of infection resulting in clinical disease. The issue of whether asymptomatic infection with *B. pseudomallei* is truly occurring during deployment of U.S. Marines to Darwin is critical for future deployments of military and civilian personnel to melioidosis-endemic locations. Melioidosis should become an important aspect of the force health protection planning. Further prospective surveillance and protocols for clinically assessing Marines with potential seroconversion are required.

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Disclaimer: The study protocol was approved by the Naval Medical Research Center Institutional Review Board in compliance with all applicable Federal regulations governing the protection of human subjects. Several of the authors of this work are military service members or employees of the United States Government. This work was prepared as part of their official duties. Title 17 U.S.C. §105 provides that "Copyright protection under this title is not available for any work of the United States Government." Title 17 U.S.C. §101 defines a U.S. Government work as a work prepared by a military service member or employee of the U.S. Government as part of that person's official duties. The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, or the U.S. Government.

REFERENCES

1. Perumal Samy R, Stiles BG, Sethi G, Lim LHK. Melioidosis: clinical impact and public health threat in the tropics. *PLoS Negl Trop Dis.* 2017;11(5):e0004738.
2. Win ZZ, Phokrai P, Aung Z, et al. Use of rapid enzyme-linked immunosorbent assay for serological screening of melioidosis in Myanmar. *Am J Trop Med Hyg.* 2018;98(5):1300–1302.
3. Limmathurotsakul D, Golding N, Dance DA, et al. Predicted global distribution of *Burkholderia pseudomallei* and burden of melioidosis. *Nat Microbiol.* 2016;1(1):15008.
4. Currie BJ. Melioidosis: evolving concepts in epidemiology, pathogenesis, and treatment. *Semin Respir Crit Care Med.* 2015;36(1):111–125.
5. Limmathurotsakul D, Kanoksil M, Wuthiekanun V, et al. Activities of daily living associated with acquisition of melioidosis in Northeast Thailand: a matched case-control study. *PLoS Negl Trop Dis.* 2013;7(2):e2072.
6. Short BH. Melioidosis: an important emerging infectious disease—a military problem? *ADF Health.* 2002;3(1):13–21.
7. Currie BJ, Ward L, Cheng AC. The epidemiology and clinical spectrum of melioidosis: 540 cases from the 20 year Darwin prospective study. *PLoS Negl Trop Dis.* 2010;4(11):e900.
8. Alexander AD, Huxsoll DL, Warner AR, Jr., Shepler V, Dorsey A. Serological diagnosis of human melioidosis with indirect hemagglutination and complement fixation tests. *Appl Microbiol.* 1970;20(5):825–833.
9. Ashdown LR, Johnson RW, Koehler JM, Cooney CA. Enzyme-linked immunosorbent assay for the diagnosis of clinical and subclinical melioidosis. *J Infect Dis.* 1989;160(2):253–260.
10. Peacock SJ, Cheng AC, Currie BJ, Dance DA. The use of positive serological tests as evidence of exposure to *Burkholderia pseudomallei*. *Am J Trop Med Hyg.* 2011;84(6):1021–1022.
11. Harris PN, Ketheesan N, Owens L, Norton RE. Clinical features that affect indirect-hemagglutination-assay responses to *Burkholderia pseudomallei*. *Clin Vaccine Immunol.* 2009;16(6):924–930.
12. Cheng AC, O'Brien M, Freeman K, Lum G, Currie BJ. Indirect hemagglutination assay in patients with melioidosis in Northern Australia. *Am J Trop Med Hyg.* 2006;74(2):330–334.
13. Wuthiekanun V, Chierakul W, Langa S, et al. Development of antibodies to *Burkholderia pseudomallei* during childhood in melioidosis-endemic Northeast Thailand. *Am J Trop Med Hyg.* 2006;74(6):1074–1075.
14. Chantratita N, Wuthiekanun V, Thanwisai A, et al. Accuracy of enzyme-linked immunosorbent assay using crude and purified antigens for serodiagnosis of melioidosis. *Clin Vaccine Immunol.* 2007;14(1):110–113.
15. Limmathurotsakul D, Chantratita N, Teerawattanasook N, et al. Enzyme-linked immunosorbent assay for the diagnosis of melioidosis: better than we thought. *Clin Infect Dis.* 2011;52(8):1024–1028.
16. Suttisunhakul V, Wuthiekanun V, Brett PJ, et al. Development of rapid enzyme-linked immunosorbent assays for detection of antibodies to *Burkholderia pseudomallei*. *J Clin Microbiol.* 2016;54(5):1259–1268.
17. Chieng S, Mohamed R, Nathan S. Transcriptome analysis of *Burkholderia pseudomallei* T6SS identifies Hcp1 as a potential serodiagnostic marker. *Microb Pathog.* 2015;79:47–56.
18. Pumpuang A, Dunachie SJ, Phokrai P, et al. Comparison of O-polysaccharide and hemolysin co-regulated protein as target antigens for serodiagnosis of melioidosis. *PLoS Negl Trop Dis.* 2017;11(3):e0005499.
19. Rubin HL, Alexander AD, Yager RH. Melioidosis—a military medical problem? *Mil Med.* 1963;128:538–542.
20. Howe C, Sampath A, Spotnitz M. The pseudomallei group: a review. *J Infect Dis.* 1971;124(6):598–606.
21. Clayton AJ, Lisella RS, Martin DG. Melioidosis: a serological survey in military personnel. *Mil Med.* 1973;138(1):24–26.
22. Spotnitz M. Disease may be Vietnamese time bomb. *Med World News.* 1966;7:55.
23. Sanford JP, Moore WL, Jr. Recrudescent melioidosis: a Southeast Asian legacy. *The Am Rev Respir Dis.* 1971;104(3):452–453.
24. Mackowiak PA, Smith JW. Septicemic melioidosis. Occurrence following acute influenza A six years after exposure in Vietnam. *JAMA.* 1978;240(8):764–766.
25. Mays EE, Ricketts EA. Melioidosis: recrudescence associated with bronchogenic carcinoma twenty-six years following initial geographic exposure. *Chest.* 1975;68(2):261–263.
26. Johnson AB, Ali N. Reactivation of latent melioidosis. *Postgrad Med J.* 1990;66(779):732–733.
27. Koponen MA, Zlock D, Palmer DL, Merlin TL. Melioidosis. Forgotten, but not gone! *Arch Internal Med.* 1991;151(3):605–608.
28. Kronmann KC, Truett AA, Hale BR, Crum-Cianflone NF. Melioidosis after brief exposure: a serologic survey in US Marines. *Am J Trop Med Hyg.* 2009;80(2):182–184.
29. Rubertone MV, Brundage JF. The Defense Medical Surveillance System and the Department of Defense Serum Repository: glimpses of the future of public health surveillance. *Am J Public Health.* 2002;92(12):1900–1904.
30. Schully KL, Berjohn CM, Prouty AM, et al. Meli-

oidosis in lower provincial Cambodia: a case series from a prospective study of sepsis in Takeo Province. *PLoS Negl Trop Dis.* 2017;11(9):e0005923.
31. Ileri SZ. The indirect haemagglutination test in the diagnosis of melioidosis in goats. *Br Vet J.* 1965;121:164–170.
32. Boey KW, Chan KB, Ko YC, Goh LG, Lim GC. Work stress and psychological well-being among the nursing profession in Singapore. *Singapore Med J.* 1997;38(6):256–260.
33. Embi N, Suhaimi A, Mohamed R, Ismail G. Prevalence of antibodies to *Pseudomonas pseudomallei* exotoxin and whole cell antigens in military personnel in Sabah and Sarawak, Malaysia. *Microbiol Immunol.* 1992;36(8):899–904.
34. Thin RN. Melioidosis antibodies in Commonwealth soldiers. *Lancet.* 1976;1(7949):31–33.
35. Parameswaran U, Baird RW, Ward LM, Currie BJ. Melioidosis at Royal Darwin Hospital in the big 2009–2010 wet season: comparison with the preceding 20 years. *Med J Aust.* 2012;196(5):345–348.
36. Pang L, Harris PNA, Seiler RL, et al. Melioidosis, Singapore, 2003–2014. *Emerg Infect Dis.* 2018;24(1).
37. Stewart JD, Smith S, Binotto E, McBride WJ, Currie BJ, Hanson J. The epidemiology and clinical features of melioidosis in Far North Queensland: implications for patient management. *PLoS Negl Trop Dis.* 2017;11(3):e0005411.
38. Samuel M, Ti TY. Interventions for treating melioidosis. *Cochrane Database Syst Rev.* 2002(4):CD001263.
39. Parthasarathy N, Saksena R, Kovac P, et al. Application of carbohydrate microarray technology for the detection of *Burkholderia pseudomallei*, *Bacillus anthracis* and *Francisella tularensis* antibodies. *Carbohydr Res.* 2008;343(16):2783–2788.
40. Charuchaimontri C, Suputtamongkol Y, Nilakul C, et al. Antilipopolysaccharide II: an antibody protective against fatal melioidosis. *Clin Infect Dis.* 1999;29(4):813–818.
41. Anuntagool N, Wuthiekanun V, White NJ, et al. Lipopolysaccharide heterogeneity among *Burkholderia pseudomallei* from different geographic and clinical origins. *Am J Trop Med Hyg.* 2006;74(3):348–352.
42. Tuanyok A, Stone JK, Mayo M, et al. The genetic and molecular basis of O-antigenic diversity in *Burkholderia pseudomallei* lipopolysaccharide. *PLoS Negl Trop Dis.* 2012;6(1):e1453.
43. Heiss C, Burtnick MN, Roberts RA, Black I, Azadi P, Brett PJ. Revised structures for the predominant O-polysaccharides expressed by *Burkholderia pseudomallei* and *Burkholderia mallei*. *Carbohydr Res.* 2013;381:6–11.
44. Norris MH, Schweizer HP, Tuanyok A. Structural diversity of *Burkholderia pseudomallei* lipopolysaccharides affects innate immune signaling. *PLoS Negl Trop Dis.* 2017;11(4):e0005571.
45. Tuanyok A, Mayo M, Scholz H, et al. *Burkholderia humptydooensis* sp. nov., a new species related to *Burkholderia thailandensis* and the fifth member of the *Burkholderia pseudomallei* complex. *Appl Environ Microbiol.* 2017;83(5):e02802.
46. Lim YT, Jobichen C, Wong J, et al. Extended loop region of Hcp1 is critical for the assembly and function of type VI secretion system in *Burkholderia pseudomallei*. *Sci Rep*. 2015;5:8235.
47. Burtnick MN, Brett PJ, DeShazer D. Proteomic analysis of the *Burkholderia pseudomallei* type II secretome reveals hydrolytic enzymes, novel proteins, and the deubiquitinase TssM. *Infect Immun.* 2014;82(8):3214–3226.
48. Chang CD, Cheng KY, Jiang LX, et al. Evaluation of a prototype *Trypanosoma cruzi* antibody assay with recombinant antigens on a fully automated chemiluminescence analyzer for blood donor screening. *Transfusion.* 2006;46(10):1737–1744.
49. Cooper A, Williams NL, Morris JL, Norton RE, Ketheesan N, Schaeffer PM. ELISA and immunopolymerase chain reaction assays for the sensitive detection of melioidosis. *Diagn Microbiol Infect Dis.* 2013;75(2):135–138.
50. James GL, Delaney B, Ward L, Freeman K, Mayo M, Currie BJ. Surprisingly low seroprevalence of *Burkholderia pseudomallei* in exposed healthy adults in the Darwin region of tropical Australia where melioidosis is highly endemic. *Clin Vaccine Immunol.* 2013;20(5):759–760.

Zika Virus Surveillance in Active Duty U.S. Military and Dependents Through the Naval Infectious Diseases Diagnostic Laboratory

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The Naval Infectious Diseases Diagnostic Laboratory (NIDDL) serves as a reference clinical laboratory that supports Department of Defense (DoD) military treatment facilities worldwide in the detection and identification of high-risk and emerging infectious diseases. Since the emergence of Zika virus (ZIKV) in the Western Hemisphere in 2016, the NIDDL has been a central hub for ZIKV testing for DoD personnel and beneficiaries. Samples collected during patients' clinical evaluations were screened for evidence of possible exposure to ZIKV using molecular and serological methods. An in-house ZIKV plaque reduction neutralization test was used to confirm the presence of ZIKV immunoglobulin M antibody. Of 1,420 individuals tested, ZIKV infection was confirmed by quantitative real-time polymerase chain reaction (PCR) assay in 11 (0.8%); an additional 26 recent flaviviral infections (possibly ZIKV) were identified based on serology (1.8%). These findings contribute to the understanding of the burden of ZIKV infections among DoD personnel and beneficiaries and highlight the role of the NIDDL in clinical diagnosis during emerging infectious disease outbreaks.

WHAT ARE THE NEW FINDINGS?

In 2016–2017, the NIDDL tested samples from 1,420 individuals and confirmed 11 cases of ZIKV infection by PCR assay and 26 flavivirus infections (possibly ZIKV) by serology. This report highlights the role of the NIDDL in clinical diagnosis during emerging infectious disease outbreaks and in understanding the burden of ZIKV infections within the DoD.

WHAT IS THE IMPACT ON READINESS AND FORCE HEALTH PROTECTION?

The NIDDL provides valuable information to characterize the burden of emerging diseases in U.S. military personnel and DoD beneficiaries, to inform risk assessments, and to guide diagnostics and countermeasure development. Given the low percentage of symptomatic cases among those infected, future serological studies should be undertaken to better determine the actual infection rates among U.S. military personnel.

In 1947, Zika virus (ZIKV) was first discovered in the Zika Forest, Uganda, in the blood of a rhesus monkey.[1] The following year, ZIKV was isolated from *Aedes africanus* mosquitoes.[2,3] ZIKV is a member of the *Flavivirus* genus, which also includes dengue viruses, yellow fever virus, and West Nile virus. The first known human case of ZIKV infection was in 1953 in Nigeria.[4] Although serosurveys found anti-ZIKV antibodies in people throughout Africa, India, and Southeast Asia,[5] for more than 50 years after initial discovery, documented human cases of acute ZIKV infection were rare. Large numbers of human cases were not observed until 2007, when an outbreak occurred in Micronesia.[6] Other outbreaks in the Pacific Ocean region followed, starting in 2013 in French Polynesia,[7] with subsequent emergence in New Caledonia and American Samoa, among other locations.[8] ZIKV was unknown in the Western Hemisphere until 2015, when ZIKV emerged in Brazil and rapidly spread across the country.

ZIKV is transmitted to humans most commonly through the bites of infected *Aedes* mosquitoes, but instances of sexual transmission have also been reported. ZIKV usually causes a mild disease with symptoms lasting about 1 week that may include low-grade fever, rash, arthralgia, arthritis, myalgia, headache, conjunctivitis, and edema. Severe cases involving hospitalization are uncommon and deaths are rare.[9,10] In recent years, an increase in neurological complications has been associated with ZIKV infection.[3,11,12] Following the 2015 Zika outbreak in Brazil, there was a dramatic increase in the number of babies with microcephaly born to mothers who had experienced ZIKV infections.[3,11,12] On 1 February 2016, the World Health Organization declared the ZIKV outbreak a "Public Health Emergency of International Concern."[12] ZIKV became a growing concern to the U.S. military because of increased ZIKV transmission in the Western Hemisphere, an association between ZIKV infection and microcephaly and other birth defects, and a potential for sexual transmission.

In response, the Naval Infectious Diseases Diagnostic Laboratory (NIDDL),[13] with the assistance of the Centers for Disease Control and Prevention (CDC),[14] provided support to military treatment facilities (MTFs) in testing patients for ZIKV and other arboviruses (e.g., dengue virus [DENV] and chikungunya virus [CHIKV]) in returning military travelers and their beneficiaries. The NIDDL is certified by the Clinical Laboratory Improvement Amendments (CLIA) program, is

accredited by the College of American Pathologists (CAP), and serves as a clinical reference laboratory specializing in the detection and identification of high-risk and emerging infectious diseases for clinicians at U.S. military healthcare facilities. This report summarizes laboratory testing at the NIDDL over the course of the ZIKV epidemic (29 January 2016 through 31 December 2017) and provides context for the NIDDL's role in diagnostic testing for the Department of Defense (DoD) during emerging disease outbreaks.

METHODS

Population and inclusion criteria

An algorithm[14] for recommended ZIKV testing was provided to healthcare practitioners at MTFs.[13] The algorithm included criteria for testing of individuals with Zika-like illness and travel history to endemic regions, individuals with Zika-like illness and possible sexual exposure with or without travel history, asymptomatic pregnant women with possible ZIKV exposure, and neonates with or without microcephaly with potential ZIKV exposure. However, healthcare providers often sent samples from asymptomatic men and non-pregnant women who had traveled to ZIKV-affected areas and who were concerned about infection. Therefore, testing was performed on serum or urine collected from patients based on travel to ZIKV high-risk areas, sexual contact with a partner who had traveled to a ZIKV high-risk area (especially for pregnant women), or symptoms possibly indicative of ZIKV infection associated with travel. Seventy-four MTFs sent samples, most of which were from patients with either possible sexual or travel exposure. One hundred seventy-nine samples with either no reported exposure or with no data regarding exposure or symptoms were submitted to the NIDDL.

Specimens

Zika genomic material can be found in bodily fluids, such as semen, urine, breast milk, saliva, and amniotic fluid.[15] ZIKV persists longer in urine (up to approximately 14 days) than it does in blood.[16,17] Based on the CDC guidelines, the healthcare facilities were instructed to send both urine and serum for polymerase chain reaction (PCR) testing.[14] All samples (serum and/or urine) were collected as part of routine clinical care when a patient visited an MTF to see a healthcare practitioner for acute care, routine screenings, or for other concerns. Samples were sent to the NIDDL for reference laboratory support during the ZIKV epidemic. Data for most samples were collected from manual logs submitted with the samples or from the Composite Health Care System, a database used by the Military Health System (MHS) to document patients' health information and history, electronically order laboratory and radiology tests/services, retrieve test results, and order and prescribe medications. The submitting MTFs were asked to provide the following information: patient demographics, date of exposure, geographic region of exposure or region of travel for sexual partner, symptoms and time of onset, pregnancy status, and trimester of exposure. However, not all data fields were complete with each submission. All data used in preparing this report were de-identified following release of clinical results to the ordering physician.

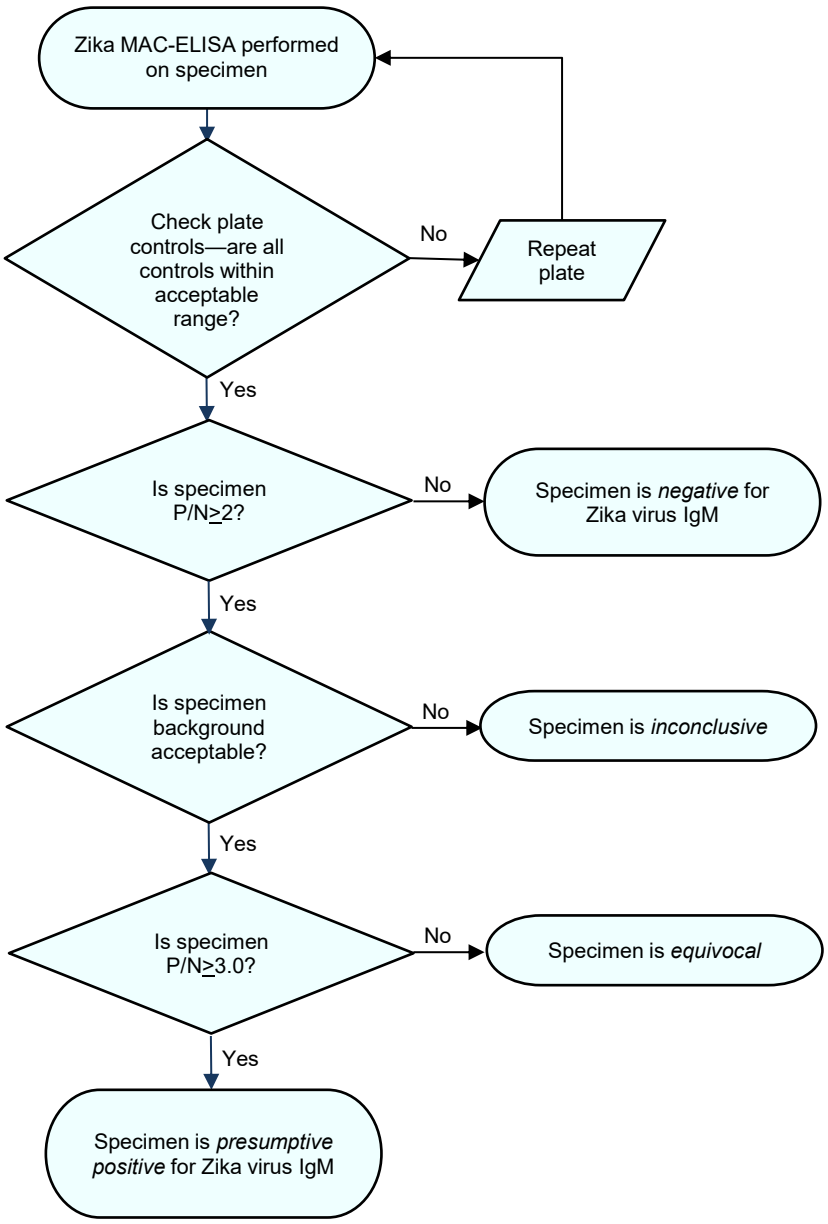
Laboratory testing

In the laboratory, total nucleic acid was extracted from samples using an automated system, the QIAGEN EZ1 Advanced XL with the EZ1 DSP Virus Kit (QIAGEN, Germantown, MD). Serum and urine collected from suspected ZIKV-infected patients were tested using the Trioplex quantitative real-time polymerase chain reaction (RT-PCR)[16] assay for multiplex detection of ZIKV, DENV, and CHIKV on the Applied Biosystems (ABI) 7500 Fast Dx Real-Time PCR Instrument using the SuperScript™ III Platinum™ One-Step qRT-PCR Kit (ThermoFisher Scientific, Waltham, MA).

In line with established standards, all sera were tested using the ZIKV immunoglobulin M (IgM) antibody capture enzyme-linked immunosorbent assay (ZIKV MAC-ELISA). The primers and probes for Trioplex qRT-PCR[16] and ZIKV MAC-ELISA antigens[18] were provided by the CDC arbovirus laboratory through the Laboratory Response Network (LRN). Plate controls were interpreted and results were reported as presumptive positive, equivocal, negative, or inconclusive (i.e., results uninterpretable because of high background optical density [OD]) **(Figure)**. The specimen P/N value, which is the ratio of the specimen mean OD value when reacted with ZIKV antigen (P) to the calibrator (negative control sera) mean OD value when reacted with ZIKV antigen (N), was calculated. A specimen P/N value <2 was interpreted as negative and no further testing or analysis was necessary. If the specimen P/N value was ≥2, then the background P/N value was assessed. The background P/N value is the ratio of the specimen mean OD value when reacted with ZIKV antigen (P) to the specimen mean OD value when reacted with Vero (negative) antigen (N). Both the specimen P/N value and the background P/N value must be ≥2 for the plate and specimens to be considered valid. The samples were deemed equivocal if the ratio was ≤2.0 P/N <3.0 or presumptive positive if the ratio was P/N ≥3.0. If the P/N value was ≥2 but the background value was <2, the specimen was deemed inconclusive. Inconclusive samples were re-tested and, if possible, another serum sample was requested from the submitting MTFs.

All inconclusive, equivocal, and presumptive positive specimens were referred to the in-house ZIKV plaque reduction neutralization test (PRNT).[19] A low passage of ZIKV strain PRVABC59 was used to detect neutralizing antibody to ZIKV. The ZIKV PRNT titer is the reciprocal of the last serum dilution to show 90% plaque reduction. The cut-off dilution for positivity for PRNT was set at 1:10 as recommended in the CDC guidance for Zika testing.[14] The PRNT is more specific than many other serological tests for the diagnosis of some viruses.[20] To ensure quality of all results, CDC provided and evaluated the proficiency testing as well as the protocols for all 3 assays.

FIGURE. Process flow chart of analysis of Zika virus MAC-ELISA data



MAC-ELISA, immunoglobulin M antibody enzyme-linked immunosorbent assay; P/N, specimen mean optical density value when reacted with ZIKV antigen (P) to the calibrator (negative control sera) mean optical density value when reacted with ZIKV antigen (N); IgM, immunoglobulin M.

Although the CDC laboratory criteria for diagnosis of ZIKV infection[21] includes ruling out DENV infection by PRNT, DENV PRNT was not established in the NIDDL and thus was not performed. We adopted a modified version of the CDC laboratory definition. Confirmed "recent ZIKV infection" was defined based on detection of viral ribonucleic acid (using the Trioplex qRT-PCR). "Recent Flavivirus infection, possible ZIKV" was defined as positive ZIKV IgM with positive ZIKV neutralizing antibody titers. In some cases, samples were sent to the CDC for DENV PRNT testing when requested by the clinician for clarification of results and patient management.

Analysis

Data were manually entered into a Microsoft Access database (2013, Microsoft Corporation, Redmond, WA). Data cleaning and extraction were performed using Microsoft Excel 2013 (2013, Microsoft Corporation, Redmond, WA) and R, version 3.2.1 (2015, R Foundation for Statistical Computing, Vienna, Austria). Data were analyzed using a combination of R, version 3.2.1, and Stata, version 13.0 (StataCorp LP, College Station, TX).

RESULTS

Between 29 January 2016 and 31 December 2017, samples from 1,420 individuals were received from DoD medical facilities around the world, including the U.S., Japan, Germany, Cuba, Guam, Spain, and Italy **(Table 1)**. Four hundred and twelve of these individuals had clinical symptoms consistent with ZIKV infection, 852 were asymptomatic but with epidemiologic linkage to ZIKV cases, and 179 had either no reported exposure to ZIKV or no data regarding exposure or symptoms. There were 1,361 samples that included information on sex, 1,330 that included information on age, and 1,241 that included information on whether the patient was symptomatic or asymptomatic. In light of concerns for impact on fetal development, the majority of samples tested were from females (961/1,361; 70.6%; **Table 2**). The median age was 30 years (0–80 years); the majority (1,161/1,330; 87.3%) were between the ages of 18 and 40 years **(data not shown)**.

Of the samples that included information on symptom status of the individual, (n=1,241; 87.8%), 389 (31.3%) were categorized as having come from symptomatic patients and 852 (68.7%) were categorized as having come from asymptomatic patients **(data not shown)**. Of the samples identified as having come from symptomatic patients and that included information on travel to endemic regions (n=329), nearly all were associated with recent travel to endemic regions (n=314/329; 95.4%) or travel as well as potential sexual exposure (n=11/329; 3.3%). However, 2 (0.6%) of the samples were from newborns with reported ZIKV-infected mothers and 2 (0.6%) were from females without recent travel but

TABLE 1. Most common referring institutions

Referring institution	No. specimens submitted[a]	No. ZIKV RT-PCR positive samples
NMC San Diego, CA	395	4
Walter Reed NMMC, MD	274	1
Fort Belvoir, VA	181	2
NH Okinawa, Japan	73	1
NHC Annapolis, MD	9	1
NBHC Groton, CT	8	1
NH Lemoore, CA	7	1
Total	947	11

[a]An additional 18 MTFs submitted 353 specimens for testing, which were not positive.
No., number; ZIKV, Zika virus; RT-PCR, reverse transcription polymerase chain reaction; NMC, Naval Medical Center; NMMC, National Military Medical Center; NH, Naval Hospital; NHC, Naval Health Clinic; NBHC, Naval Branch Health Clinic.

TABLE 2. Participant demographics (n=1,420)

	Total	% of total
Sex[a]	1,361	
Male	400	29.4
Female	961	70.6
Age, years[b]	1,335	
Male (mean, range)	32.3 (0–80)	
Female (mean, range)	30.4 (0–76)	
Symptomatic	389	
Male	182	46.8
Female	184	47.3
No information on sex	23	5.9
Pregnant	413	
Symptomatic	23	
Travel exposure	15	65.2
Sexual contact	1	4.3
Travel exposure and/or sexual contact	4	17.4
Possible source of exposure not provided	3	13
Asymptomatic	390	
Travel exposure	239	61.2
Sexual contact	73	18.7
Travel exposure and/or sexual contact	28	7.2
Possible source of exposure not provided	50	12.8

[a]Missing data on sex for 59 individuals.
[b]Missing data on age for 85 individuals.

with sexual contact with partners recently returning from endemic regions **(data not shown)**. Of the samples from asymptomatic individuals that included information on travel (n=784), 615 (78.4%) were associated with recent travel to endemic regions, 120 (15.3%) were associated with sexual contact with a partner with recent travel history, 45 (5.7%) were associated with potential travel and sexual exposure, and 4 (0.5%) were associated with potential congenital exposure **(data not shown)**.

Samples were received from 413 pregnant women; 23 (5.6%) of the women were symptomatic and 390 (94.4%) were asymptomatic **(Table 2)**. Of the patients who were asymptomatic and pregnant, testing was indicated because of geographical exposure such as travel to or living in endemic regions (n=239; 70.3%), sexual contact with potential ZIKV-infected partners (n=73; 21.5%), or both (n=28; 8.2%).

Overall, individuals with reported potential travel exposure (n=985), including travel and sexual exposure, reported having visited or lived in Central America or the Caribbean (n=466; 47.3%), the Asia-Pacific region (n=246; 25.0%), South America (n=59; 6.0%), Africa (n=44; 4.5%), and/or potentially endemic regions of the U.S. (n=132; 13.4%); 57 individuals had no travel location provided **(data not shown)**.

A variety of samples (n=1,948) from the 1,420 patients were submitted for testing, including, but not limited to, serum (n=891); serum and urine (n=500); serum and whole blood (n=15); serum, whole blood, and urine (n=5); urine only (n=4); semen and urine (n=1); semen, urine, and sputum (n=1); and semen only (n=1) **(data not shown)**. For some patients, multiple samples were received, and for others, just 1 sample was received. Serum samples were tested using Zika MAC-ELISA, PRNT, and the CDC Trioplex qRT-PCR assay, whereas urine and semen samples were tested with the CDC Trioplex qRT-PCR assay only. Out of the 1,299 individuals tested using Trioplex qRT-PCR, there were 11 ZIKV-positive individuals (0.8%) (4 positive in serum, 6 in urine, and 1 in both sample types) and 8 (0.6%) DENV-positive individuals (3 positive in serum and 5 positive in both serum and urine) **(Table 3)**. None of the samples tested were positive for CHIKV **(Table 3)**. Among the 11 samples that were qRT-PCR-positive for ZIKV, 8 came from symptomatic patients, of whom 5 had travel histories to endemic locations in the Western Hemisphere (i.e., Puerto Rico, Mexico, or Bonaire) and 1 had been to the Philippines **(Table 4)**. Travel histories were not available for the other 2 symptomatic individuals with ZIKV-positive samples **(Table 4)**. The remaining 3 ZIKV-positive samples lacked information on symptom status and travel history.

Serum samples from 1,409 individuals were screened by Zika MAC-ELISA **(Table 3)**. Of these, 56 were classified as presumptive positive, 44 were equivocal, 1,278 were negative, and 31 were inconclusive. All positive, equivocal, or inconclusive samples were analyzed by Zika PRNT for confirmation. Among Zika IgM-positive samples, 52% (29/56) were PRNT positive; among equivocal or inconclusive only samples, 4% (3/75) were PRNT-positive. Excluding the serology results from qRT-PCR-confirmed ZIKV and DENV infections, serological testing resulted in 26 individuals classified as "Recent Flavivirus infection, possible ZIKV." Eleven of these individuals were symptomatic, 11 were asymptomatic, and 4 had no history provided. Therefore, combining molecular and serological testing, a total of 37 individuals had laboratory results consistent with recent ZIKV infection: 11 confirmed and 26 possible recent ZIKV infections **(Table 3)**.

Of the 413 pregnant women screened, 8 (1.9%) were considered to have recent flavivirus, possible ZIKV infections by Zika MAC-ELISA with PRNT confirmation. One was symptomatic. All 8 ZIKV-positive pregnant women reported recent travel to ZIKV-endemic areas, including Puerto Rico, the Philippines, and Brazil, and therefore mosquito transmission during travel rather than sexual contact was considered the likely route of exposure **(data not shown)**.

Additionally, 9 infants were tested for recent ZIKV infection, including 3 with reported microcephaly. None of the 3

TABLE 3. Laboratory results

Trioplex RT-PCR	n=1,299	%
ZIKV positive	11	0.8
DENV positive	8	0.6
CHIKV positive	0	.
Multiple	0	.
Zika MAC-ELISA	n=1,409	
Presumptive+ (≥3.0)	56	4.0
Equivocal (≤2.0–<3.0 and background ≥2.0)	44	3.1
Negative (<2.0)	1,278	90.7
Inconclusive (≥2.0 and background <2.0)	31	2.2
Zika PRNT	n=131	
Positive	32	24.4
Negative	99	75.6

RT-PCR, reverse transcription polymerase chain reaction; ZIKV, Zika virus; DENV, dengue virus; CHIKV, chikungunya virus; MAC-ELISA, immunoglobulin M antibody enzyme-linked immunosorbent assay; PRNT, plaque reduction neutralization test.

TABLE 4. ZIKV RT-PCR-positive individuals

Sex	Symptomatic?	Treatment location	Country of travel
M	Unknown	NMC San Diego, CA	Unknown
F	Yes	Fort Belvoir, VA	Puerto Rico
M	Unknown	NHC Annapolis, MD	Unknown
F	Yes	NMC San Diego, CA	Unknown
M	Unknown	NBHC Groton, CT	Unknown
F	Yes	NMC San Diego, CA	Puerto Rico
F	Yes	NH Lemoore, CA	Unknown
F	Yes	NMC San Diego, CA	Mexico
M	Yes	Fort Belvoir, VA	Puerto Rico
M	Yes	Walter Reed NMMC, MD	Bonaire
M	Yes	NH Okinawa, Japan	Phillippines

ZIKV, Zika virus; RT-PCR, reverse transcription polymerase chain reaction; NMC, Naval Medical Center; NHC, Naval Health Clinic; NBHC, Naval Branch Health Clinic; NH, Naval Hospital; NMMC, National Military Medical Center.

infants with reported microcephaly tested positive for ZIKV by any of the tests. One infant without reported microcephaly tested inconclusive by Zika MAC-ELISA but was positive by PRNT, most likely due to circulating maternal antibody to ZIKV; the infant's mother reported recent travel to Puerto Rico **(data not shown)** and also tested positive by Trioplex qRT-PCR, MAC-ELISA, and PRNT.

EDITORIAL COMMENT

Starting in January of 2016, the NIDDL provided support to MTFs by testing for ZIKV and other arboviruses (DENV and CHIKV) in returning military travelers and their beneficiaries. Following the realization that ZIKV can be transmitted sexually, the NIDDL began screening females of childbearing age and those reporting pregnancy after sexual contact with a member "exposed" or symptomatic following travel to a region with ZIKV risk.

The NIDDL is a CLIA-certified and CAP-accredited infectious disease laboratory that serves multiple roles within the DoD, from clinical diagnostic laboratory to reference laboratory for the detection and identification of high-risk emerging infectious diseases even during large outbreaks. In addition, the NIDDL maintains a repository of samples for assay development and quality assurance. The NIDDL partners with CDC and the LRN to support surveillance studies of emerging infections among DoD personnel.

ZIKV poses a challenge for laboratory confirmation. Infection is often asymptomatic, and even symptomatic cases are characterized by low-grade undifferentiated febrile illness that is difficult to distinguish from illness caused by other co-circulating pathogens, such as DENV, CHIKV, rickettsial pathogens, and malaria. Serological confirmation is particularly challenging because of cross-reaction with other flavivirus antibodies, particularly DENV. The NIDDL provides diagnostic confirmation, not only using molecular methods such as qRT-PCR, but also ELISA with follow-up diagnostic PRNT confirmation.

The NIDDL experienced several challenges during the current study. First, testing guidelines were disseminated to healthcare practitioners, but they were not always followed. For example, many samples were submitted from asymptomatic men and asymptomatic non-pregnant women without any travel history or other risk factors, including sexual contact with a symptomatic partner who had traveled to a high-risk location. In addition, there were occasional instances of broken containers or samples that leaked during shipment as well as the submission of incorrect orders. Finally, patient travel/sexual history was missing for different subsets of samples. Despite these challenges, the NIDDL successfully tested all of the received samples, reported the results to the requesting facility, and reported results to CDC through the LRN messenger system.

The current study identified 37 confirmed and possible ZIKV infections (2.6%), including 19 symptomatic infections. The ZIKV infections reported here represent a subset of all cases reported across the MHS. Poss and colleagues[22] reported 156 confirmed cases between January and November 2016, including 5 in pregnant beneficiaries. Those reported cases were nearly all symptomatic, whereas the current study detected a number of asymptomatic infections among individuals with travel exposure to endemic regions. Given that the majority of ZIKV infections are asymptomatic or at least subclinical,[23] incidence rates among military personnel

will be largely undercounted, as predominantly symptomatic cases will be reported. Additionally, 8 pregnant women tested positive for ZIKV (1.9% of all pregnant women tested), all by Zika MAC-ELISA and PRNT serology. Exposure through travel, rather than sexual exposure, was likely responsible for all of these infections.

DoD personnel represent a unique population in many ways since they are deployed globally, often in austere environments, and thus are exposed to locally circulating pathogens. The NIDDL provides valuable information to characterize the burden of emerging diseases in U.S. military personnel and DoD beneficiaries, inform risk assessments, and guide diagnostics and countermeasure development. Because of the sequelae of Zika, an urgent need exists for a vaccine. There are a variety of ZIKV vaccines currently in development that employ a range of technologies and approaches (e.g., inactivated ZIKV, protein nanoparticle, synthetic peptide, DNA-based, virus-like particle, thermostable mRNA-based, live modified vaccinia virus Ankara, recombinant protein vaccines, and self-amplifying mRNA platform).[15] However, the vaccine must elicit protective immunity regardless of mode of transmission, be free of neurological side effects, and protect healthy adults, young children, pregnant women, and unborn fetuses.[15] Assessment of clinical efficacy when the majority of ZIKV infections are asymptomatic poses an additional challenge.[15]

Future work will involve sequencing of ZIKV isolates and looking for linkages between them, as the women were infected in a variety of locations, including the Caribbean, South America, and Asia. The NIDDL will also develop and validate a DENV PRNT assay to assess cross-reactivity and verify the presence of ZIKV antibodies. Additional work is needed to determine if pathogenesis of the organism varies in relation to sexual transmission when compared to vector-borne transmission. Control of mosquito populations is imperative to prevent further spreading of the organism. Moreover, in light of the low percentage of symptomatic cases among those infected, future cross-sectional serological studies should be undertaken to better determine the actual infection rates among U.S. military personnel.

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REFERENCES

1. Dick GW, Kitchen SF, Haddow AJ. Zika virus. I. Isolations and serological specificity. *Trans R Soc Trop Med Hyg.* 1952;46(5):509–520.
2. Song BH, Yun SI, Woolley M, Lee YM. Zika virus: history, epidemiology, transmission, and clinical presentation. *J Neuroimmunol.* 2017;308:50–64.
3. Rasmussen SA, Jamieson DJ, Honein MA, Petersen LR. Zika virus and birth defects—reviewing the evidence for causality. *New Engl J Med.* 2016;374(20):1981–1987.
4. Macnamara FN. Zika virus: a report on three cases of human infection during an epidemic of jaundice in Nigeria. *Trans R Soc Trop Med Hyg.* 1954;48(2):139–145.
5. Lessler J, Chaisson LH, Kucirka LM, et al. Assessing the global threat from Zika virus. *Science.* 2016;353(6300):aaf8160.
6. Duffy MR, Chen TH, Hancock WT, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. *New Engl J Med.* 2009;360(24):2536–2543.
7. Cao-Lormeau VM, Roche C, Teissier A, et al. Zika virus, French Polynesia, South Pacific, 2013. *Emerg Infect Dis.* 2014;20(6):1085–1086.
8. Petersen LR, Jamieson DJ, Honein MA. Zika Virus. *New Engl J Med.* 2016;375(3):294–295.
9. Paixao ES, Barreto F, Teixeira Mda G, Costa Mda C, Rodrigues LC. History, epidemiology, and clinical manifestations of Zika: a systematic review. *Am J Public Health.* 2016;106(4):606–612.
10. Chang C, Ortiz K, Ansari A, Gershwin ME. The Zika outbreak of the 21st century. *J Autoimmun.* 2016;68:1–13.
11. Wikan N, Smith DR. Zika virus: history of a newly emerging arbovirus. *Lancet Infect Dis.* 2016;16(7):e119–e126.
12. World Health Organization. WHO Director-General summarizes the outcome of the Emergency Committee regarding clusters of microcephaly and Guillain-Barré syndrome. 1 February 1 2016. https://www.who.int/news-room/detail/01-02-2016-who-director-general-summarizes-the-outcome-of-the-emergency-committee-regarding-clusters-of-microcephaly-and-guillain-barr%C3%A9-syndrome. Accessed 31 January 2018.
13. Naval Infectious Disease Diagnostic Laboratory. Interim Zika Virus Testing Criteria. http://www.med.navy.mil/sites/nmrc/SiteAssets/Interim%20Zika%20Virus%20Testing%20Criteria.pdf. Accessed 9 April 2018.
14. Centers for Disease Control and Prevention. Updated Guidance for US Laboratories Testing for Zika Virus Infection. https://www.cdc.gov/zika/laboratories/lab-guidance.html. Accessed 13 May 2019.
15. Poland GA, Kennedy RB, Ovsyannikova IG, Palacios R, Ho PL, Kalil J. Development of vaccines against Zika virus. *Lancet Infect Dis.* 2018;18(7):e211–e219.
16. Centers for Disease Control. Trioplex Real-time RT-PCR Assay. 6 April 2017. https://www.cdc.gov/zika/pdfs/trioplex-real-time-rt-pcr-assay-instructions-for-use.pdf. Accessed 31 January 2018.
17. Gourinat AC, O'Connor O, Calvez E, Goarant C, Dupont-Rouzeyrol M. Detection of Zika virus in urine. *Emerg Infect Dis.* 2015;21(1):84–86.
18. Centers for Disease Control and Prevention. Zika MAC-ELISA. 3 May 2017. https://www.cdc.gov/zika/pdfs/zika-mac-elisa-instructions-for-use.pdf. Accessed 31 January 2018.
19. Rabe IB, Staples JE, Villanueva J, et al. Interim guidance for interpretation of Zika virus antibody test results. *MMWR Morb Mortal Wkly Rep.* 2016;65(21):543–546.
20. Shan C, Xie X, Ren P, et al. A rapid Zika diagnostic assay to measure neutralizing antibodies in patients. *EBioMedicine.* 2017;17:157–162.
21. Centers for Disease Control and Prevention. Zika Virus Disease and Zika Virus Infection, 2016 Case Definition, Approved June 2016. https://wwwn.cdc.gov/nndss/conditions/zika/case-definition/2016/06/. Accessed 9 April 2018.
22. Poss DE, Writer JV, Harris S. Zika virus infections in Military Health System beneficiaries since the introduction of the virus in the Western Hemisphere, 1 January 2016 through 30 November 2016. *MSMR.* 2016;23(12):7–11.
23. Shuaib W, Stanazai H, Abazid AG, Mattar AA. Re-emergence of Zika virus: a review on pathogenesis, clinical manifestations, diagnosis, treatment, and prevention. *Am J Med.* 2016;129(8): 879.

Brief Report

Department of Defense Midseason Estimates of Vaccine Effectiveness for the 2018–2019 Influenza Season

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Military populations have historically been at high risk for acute respiratory infections, particularly training and deployed populations, who have living conditions that are often crowded and may be austere.[1] Respiratory infections are responsible for over 300,000 medical encounters each year among U.S. active component service members, and the associated health care creates a substantial public health and economic burden on the Military Health System (MHS).[1,2] Respiratory infections also account for approximately one-third of convalescence in quarters determinations and as such are a significant contributor to lost duty days.[3] Viral respiratory pathogens are highly transmissible, and the specific types, trends, and risks often vary regionally and by setting.[1] These variations are important for a globally dispersed force, as they inform risk assessments and ensure that proper preventive measures are implemented. Thus, the Department of Defense (DoD) conducts surveillance for respiratory infections both within the force and in other global populations. The Armed Forces Health Surveillance Branch's (AFHSB) Global Emerging Infections Surveillance (GEIS) section supports a global surveillance program, executed primarily by DoD service laboratories, at approximately 400 locations in over 30 countries. Respiratory infection surveillance data are regularly shared with the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC). Because of frequent genetic mutations and the associated pandemic potential, influenza is of particular interest to the DoD and is a major focus of these surveillance efforts. Because influenza vaccination is the primary preventive countermeasure, the seasonal influenza vaccine's effectiveness is also closely monitored. Estimates of vaccine effectiveness (VE) are calculated twice annually: during the middle and at the end of the influenza season.

METHODS

Three sites produced VE estimates for the DoD at midseason. The U.S. Air Force School of Aerospace Medicine/AFHSB-Air Force (USAFSAM/AFHSB-AF) satellite VE estimate was produced from sentinel site surveillance within non-active component MHS beneficiaries (retirees and family members) receiving care at military treatment facilities (MTFs). The Naval Health Research Center (NHRC) VE estimate was derived from sentinel site influenza surveillance within civilian populations at clinics near the U.S.–Mexico border and among MHS beneficiaries (service members, retirees, and family members) receiving care at MTFs. The AFHSB's Epidemiology and Analysis (E&A) section VE estimate was derived from electronic health record (EHR) data from active component service members receiving care at MTFs.

For the 2018–2019 midseason, all 3 VE estimates were calculated using a test-negative case-control study design; crude and adjusted VE estimates, along with 95% confidence intervals (CIs), were calculated as (1 - odds ratio) x 100% and were obtained from multivariable logistic regression models. VE results were considered statistically significant if 95% CIs around VE estimates did not include zero.

USAFSAM/AFHSB-AF satellite's analysis adjusted for age group, time of specimen collection, region, and sex. NHRC's analysis adjusted for age group. AFHSB E&A's analysis adjusted for age group, month of diagnosis, 5-year vaccination status as a dichotomous variable, and sex. Analyses were performed for influenza types and subtypes as available. Cases were laboratory confirmed as influenza positive, and controls were influenza test negative. At NHRC and USAFSAM/AFHSB-AF satellite, influenza positives were confirmed through reverse transcription polymerase chain reaction (RT-PCR) and/or viral culture. AFHSB also used these methods for confirmation and included positive rapid tests, but individuals with only a negative rapid test, without another confirmatory test result were excluded from calculation of VE. USAFSAM/AFHSB-AF satellite verified vaccination status through EHR and self-report data, E&A verified vaccination status through EHR data, and NHRC used self-reported vaccination data. Nearly all vaccinated active duty and beneficiary patients received the inactivated influenza vaccine.

RESULTS

Non-active component MHS beneficiary data were collected from 9 December 2018 through 16 February 2019. The analysis was restricted to this time period to provide a more accurate VE estimate, as earlier months of the influenza season are control-heavy. By the end of the surveillance period, 48% of 645 cases and 64% of 1,446 controls had been vaccinated **(Table)**. Non-active component MHS beneficiary cases tended to be younger than controls. U.S.–Mexico border population civilian and MHS beneficiary data were collected from 30 September 2018

through 15 February 2019, during which time 13% of 251 cases and 27% of 1,185 controls were vaccinated. Border population and MHS beneficiary cases tended to be younger than controls. Active component service member data were collected from 1 December 2018 through 16 February 2019, and 92% of 1,594 cases and 91% of 2,548 controls were vaccinated. In the active component service member group, controls tended to be younger than cases.

As shown in the **Table** and **Figure**, adjusted VE for all influenza types for non-active component MHS beneficiaries was 47% (95% CI: 35–57), indicating moderate protection against influenza infection. For active component service members, adjusted VE for all influenza types was low, at 13% (95% CI: -11–32). For all influenza A, adjusted VE for non-active component MHS beneficiaries was 48% (95% CI: 36–58), VE for U.S.–Mexico border population civilians and MHS beneficiaries was 58% (95% CI: 38–72), and VE for active component service members was 12% (95% CI: -13–31). For influenza A(H1N1), adjusted VE for non-active component MHS beneficiaries was 57% (95% CI: 44–68), VE for U.S.–Mexico border population civilians and MHS beneficiaries was 65% (95% CI: 46–77), and VE for active component service members was 34% (95% CI: -19–64). Influenza A(H3N2) was not detected in high enough proportions in most populations to calculate VE, but for non-active component MHS beneficiaries, adjusted VE was 36% (95% CI: 14–53), indicating low-to-moderate protection. Similarly, influenza B was not detected in high enough proportions in most populations early in the 2018–2019 season to calculate VE; however, for active component service members, adjusted VE was 25% (95% CI: -8–48), indicating low protection.

EDITORIAL COMMENT

The DoD laboratories and partners conducting respiratory infection surveillance provide a valuable global perspective and capability. Monitoring global trends, particularly for influenza, provides

TABLE. DoD midseason influenza VE estimates, 2018–2019

Influenza type/subtype	Population	Vaccination status	Cases n (%)	Controls n (%)	Crude VE (%)	Crude VE 95% CI	Adjusted VE (%)	Adjusted VE 95% CI
Non-active component MHS beneficiaries (USAFSAM/AFHSB-AF satellite)[a]								
Overall	All	Vaccinated	312 (15)	929 (44)	48	37–57	47	35–57
		Unvaccinated	333 (16)	517 (25)				
	2–17 yrs	Vaccinated	199 (19)	398 (37)	43	27–56	45	29–58
		Unvaccinated	223 (21)	253 (24)				
	18+ yrs	Vaccinated	113 (11)	531 (52)	49	31–62	48	28–63
		Unvaccinated	110 (11)	264 (26)				
Influenza A (any subtype)	All	Vaccinated	307 (15)	929 (45)	48	37–57	48	36–58
		Unvaccinated	327 (16)	517 (25)				
	2–17 yrs	Vaccinated	198 (19)	398 (37)	43	26–55	44	27–57
		Unvaccinated	219 (21)	253 (24)				
	18+ yrs	Vaccinated	109 (11)	531 (52)	50	32–63	50	30–64
		Unvaccinated	108 (11)	264 (26)				
A(H1N1)	All	Vaccinated	122 (7)	929 (53)	60	48–69	57	44–68
		Unvaccinated	170 (10)	517 (30)				
	2–17 yrs	Vaccinated	69 (8)	398 (47)	63	49–74	66	52–76
		Unvaccinated	120 (14)	253 (30)				
	18+ yrs	Vaccinated	53 (6)	531 (59)	47	20–65	39	5–61
		Unvaccinated	50 (6)	264 (29)				
A(H3N2)	All	Vaccinated	148 (9)	929 (54)	36	17–51	36	14–53
		Unvaccinated	129 (7)	517 (30)				
	2–17 yrs	Vaccinated	116 (14)	398 (46)	18	-13–40	20	-15–44
		Unvaccinated	90 (11)	253 (30)				
	18+ yrs	Vaccinated	32 (4)	531 (61)	59	33–75	67	44–80
		Unvaccinated	39 (5)	264 (30)				
U.S.-Mexico border population & MHS beneficiaries (NHRC)[b]								
Influenza A (any subtype)	All	Vaccinated	33 (9)	325 (91)	60	41–73	58	38–72
		Unvaccinated	218 (20)	860 (80)				
	0–17 yrs	Vaccinated	17 (11)	142 (89)	57	27–75	--	--
		Unvaccinated	150 (22)	537 (78)				
	18–64 yrs	Vaccinated	15 (8)	169 (92)	58	23–77	--	--
		Unvaccinated	59 (17)	280 (83)				
	65+ yrs	Vaccinated	1 (7)	14 (93)	66	-194–96	--	--
		Unvaccinated	9 (17)	43 (83)				
A(H1N1)	All	Vaccinated	26 (7)	325 (93)	66	48–78	65	46–77
		Unvaccinated	204 (19)	860 (81)				
	0–17 yrs	Vaccinated	14 (9)	142 (91)	61	31–78	--	--
		Unvaccinated	137 (20)	539 (80.0)				
	18–64 yrs	Vaccinated	11 (6)	169 (94)	69	38–84	--	--
		Unvaccinated	58 (17)	280 (83)				
	65+ yrs	Vaccinated	1 (7)	14 (93)	66	-197–96	--	--
		Unvaccinated	9 (17)	43 (83)				
Active component service members (AFHSB E&A)[c]								
Overall	All	Vaccinated	1466 (92)	2323 (91)	-11	-39–12	13	-11–32
		Unvaccinated	128 (8)	225 (9)				
Influenza A (any subtype)	All	Vaccinated	1388 (92)	2323 (91)	-13	-42–10	12	-13–31
		Unvaccinated	119 (8)	225 (9)				
A(H1N1)	All	Vaccinated	108 (89)	2323 (91)	25	-32–58	34	-19–64
		Unvaccinated	14 (11)	225 (9)				
Influenza B	All	Vaccinated	81 (89)	2323 (91)	12	-25–37	25	-8–48
		Unvaccinated	10 (11)	225 (9)				

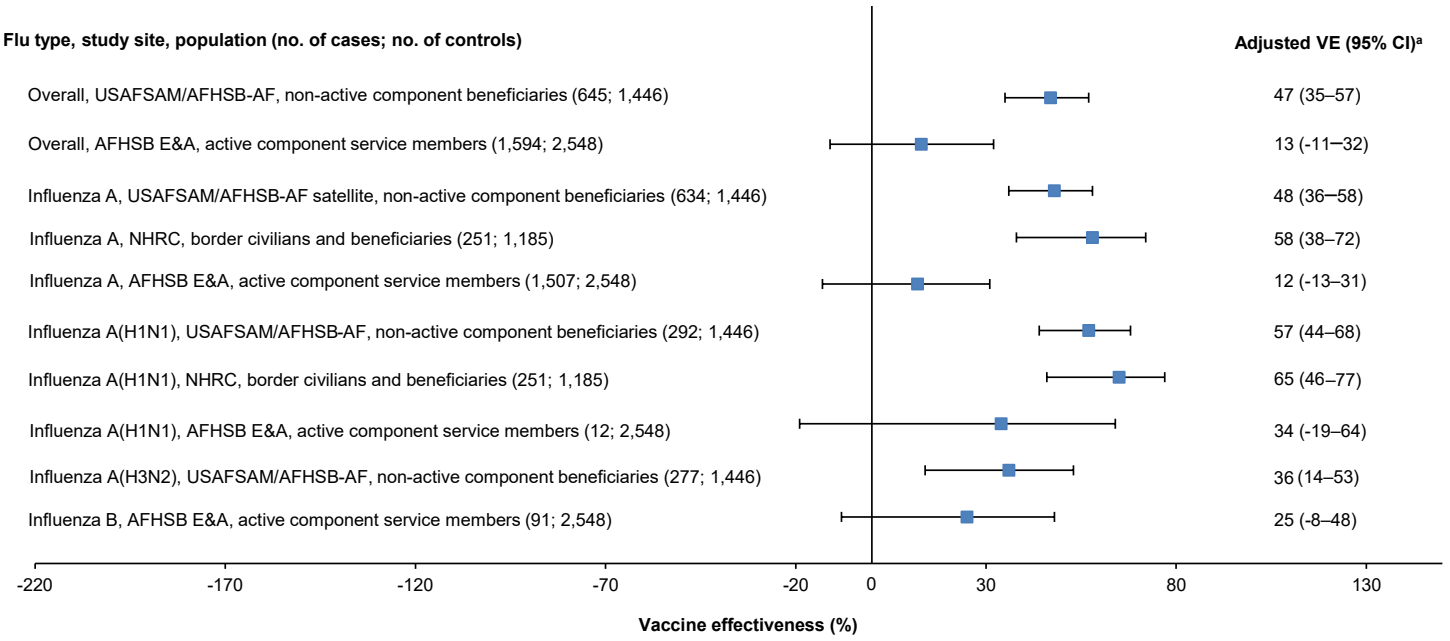
[a]VE adjusted for age group, time of specimen collection, region, and sex.

[b]VE adjusted for age group.

[c]VE adjusted for age group, month of diagnosis, 5-year vaccination status, and sex.

DoD, Department of Defense; VE, vaccine effectiveness; CI, confidence interval; MHS, Military Health System; USAFSAM/AFHSB-AF, U.S. Air Force School of Aerospace Medicine/Armed Forces Health Surveillance Branch-Air Force; NHRC, Naval Health Research Center; E&A, Epidemiology and Analysis section.

FIGURE. DoD midseason influenza VE, 2018–2019



[a]USAFSAM/AFHSB-AF satellite adjusted for age group, time of specimen collection, region, and sex; NHRC adjusted for age group; AFHSB E&A adjusted for age group, month of diagnosis, 5-year vaccination status, and sex.

DoD, Department of Defense; VE, vaccine effectiveness; no., number; CI, confidence interval; USAFSAM/AFHSB-AF, U.S. Air Force School of Aerospace Medicine/Armed Forces Health Surveillance Branch-AF; E&A, Epidemiology and Analysis; NHRC, Naval Health Research Center.

situational awareness for DoD leaders and informs current and future operation risk assessments and recommendations for preventive measures. This surveillance also facilitates sample sharing and further collaboration with WHO and CDC.

In general, for civilian populations, influenza vaccination provided moderate protection against infection, and DoD-generated VE estimates of non-service member beneficiaries and select civilian populations were similar to CDC estimates for the same time frame. CDC reported that adjusted VE for all influenza types was 47%, adjusted VE for influenza A(H1N1) was 46%, and adjusted VE for influenza A(H3N2) was 44%.[4] In CDC and DoD analyses, protection was greater for influenza A(H1N1) than influenza A(H3N2). However, for active component service members, adjusted VE estimates were much lower, though not statistically significant. This difference may be partially attributable to the requirement for annual influenza vaccination and the resulting high proportion of vaccination in this population. The effect is demonstrated by the case and control populations having nearly identical vaccination rates. The high vaccination rate makes it difficult to design a strong epidemiological study of VE in this population. Other factors, such as the requirement for service members to receive the vaccination annually, which may have biological effects such as attenuated immune response due to repeated exposures, may also impact the VE estimates. The timing of vaccination could also impact the VE estimates since service members typically receive the vaccine early in the influenza season or just before it starts. These factors should also be considered as potential contributors to the low VE estimates for the active component service members.

One important limitation of this study is potential non-differential misclassification of vaccination status due to poor recall on the self-reported questionnaire or documentation errors in the EHR. Also, the analyses did not assess vaccine impact on less severe cases of influenza since the VE estimates only include medically attended patients, and the populations studied are younger than the U.S. general population, which may reduce generalizability. More work, potentially using new methodologies, is needed to accurately estimate the vaccine's effect on reducing the influenza burden in active component service members and to determine the impact of repeat vaccinations on immune response to the vaccine or subsequent influenza exposures. Additional data and analyses in these areas would fill knowledge gaps and inform a more robust military influenza vaccination policy.

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The study protocol was approved by the Naval Health Research Center Institutional Review Board in compliance with all applicable Federal regulations governing the protection of human subjects. Research data were derived from an approved Naval Health Research Center Institutional Review Board protocol number NHRC.2007.0024.

REFERENCES

1. Sanchez JL, Cooper MJ, Myers CA, et al. Respiratory infections in the U.S. military: recent experience and control. *Clin Microbiol Rev*. 2015;28(3):743–800.
2. Armed Forces Health Surveillance Branch. Absolute and relative morbidity burdens attributable to various illnesses and injuries, active component, U.S. Armed Forces, 2018. *MSMR*. 2019;26(5):2–10.
3. Armed Forces Health Surveillance Branch. Ambulatory visits, active component, U.S. Armed Forces, 2018. *MSMR*. 2019;26(5):19–25.
4. Doyle JD, Chung JR, Kim SS, et al. Interim estimates of 2018–19 seasonal influenza vaccine effectiveness–United States, February 2019. *MMWR Morb Mortal Wkly Rep*. 2019;68(6)135–139.

Infectious Mononucleosis, Active Component, U.S. Armed Forces, 2002–2018

Shauna Stahlman, PhD, MPH; Valerie F. Williams, MA, MS; Saixia Ying, PhD

Infectious mononucleosis (IM) is an acute infectious illness characterized by swollen lymph nodes, fever, pharyngitis, fatigue, and head and body aches. This report describes the incidence rates, trends, and demographic correlates of IM among active component service members during 2002–2018. During the surveillance period, there were 23,780 incident cases of IM, resulting in an overall incidence rate of 104.2 cases per 100,000 person-years (p-yrs). The incidence of IM diagnoses was highest among the youngest age groups and decreased with increasing age. The rate of incident IM diagnoses was markedly higher among non-Hispanic white service members (123.4 per 100,000 p-yrs) compared to those in other race/ethnicity groups. The incidence of IM diagnoses among recruits (364.9 per 100,000 p-yrs) was 3.4 times that among other enlisted personnel (106.0 per 100,000 p-yrs) and 5.6 times that among officers (64.7 per 100,000 p-yrs). The incidence of IM diagnoses remained relatively stable during the surveillance period, at about 100 per 100,000 p-yrs. IM is not considered to be a serious illness; however, it can seriously impact availability for duty during the acute phase.

WHAT ARE THE NEW FINDINGS?

An average of 1,398 service members per year were diagnosed with IM during 2002–2018. Incidence rates were highest among the youngest age groups, recruit trainees, females, non-Hispanic whites, and healthcare workers. The crude overall incidence of IM diagnoses was 104.2 per 1,000 p-yrs; annual rates remained relatively stable over the 17-year surveillance period.

WHAT IS THE IMPACT ON READINESS AND FORCE HEALTH PROTECTION?

Symptomatic cases of IM can result in 2 weeks or more of limited duty, resulting in 2,797 weeks or more of lost duty time per year. Recruit trainees with IM may need to be recycled. Cases with possible splenic enlargement may be cautioned or prevented from strenuous physical activities for up to 4 weeks after onset of IM.

Infectious mononucleosis (IM) is an acute infectious illness characterized by swollen lymph nodes, fever, pharyngitis, fatigue, and head and body aches. Less common but more severe manifestations may include swelling of the liver or spleen.[1] It is estimated that at least 90% of cases of IM are caused by Epstein-Barr virus (EBV), but mononucleosis-like illnesses can be caused by other pathogens (e.g., cytomegalovirus, human herpes virus 6, human immunodeficiency virus type 1, *Toxoplasma gondii*).[2,3] Acute symptoms usually present within 4 to 6 weeks after infection with EBV and generally resolve within 2 to 4 weeks; however, fatigue and poor functional status may persist for months.[1] Rare acute complications include splenic rupture, hepatitis, and respiratory tract or nasopharyngeal obstruction (e.g., tonsillar enlargement).[4] Prospective studies suggest that 9–12% of adults with IM go on to meet the criteria for chronic fatigue syndrome 6 months following IM onset; however, no definitive causal relationship between IM and chronic fatigue syndrome has been determined.[5–8]

Viruses that cause IM are most commonly transmitted through saliva but can also be transmitted through other bodily fluids, including blood and semen.[1] EBV may be shed in salivary secretions at high levels for a prolonged period following clinical recovery, and the virus may be intermittently shed at lower levels in the oropharynx for decades.[9–11] Most people infected with IM will experience symptoms only once. However, reactivation of EBV may occur later in life in those who are immunocompromised (e.g., those who have had an organ transplant, those who are using immunosuppressive medication, or those with acquired immune deficiency syndrome).[12] Infection with EBV is very common worldwide, and it is estimated that 90% of adults are antibody positive before age 30.[2] Risk of acquiring IM is highest in populations of young adults who function or live in close proximity to one another, such as students or military service members.[13]

In the U.S., it is estimated that almost 90% of 18–19-year-olds test positive for EBV antibody.[11] In addition, studies have shown that the incidence of IM can range between 11 and 48 cases per 1,000 persons per year among students and military service members.[14] A previous *MSMR* analysis found an incidence of IM of 98.9 per 100,000 person-years (p-yrs) among active component service members.[15] The incidence of IM was higher among females, whites, younger individuals, and those in the Air Force and Navy, compared to their respective counterparts.[15]

Because IM is common among young adults living in close proximity and because it has a relatively long duration of symptoms, IM has the potential to reduce military operational readiness by contributing to lost or limited duty time. The purpose of this report is to describe the

overall incidence rates, trends, morbidity and healthcare burden, and demographic correlates of IM among active component service members during 2002–2018.

METHODS

The surveillance period was 1 January 2002 through 31 December 2018. The surveillance population included all active component service members who served in the Army, Air Force, Navy, or Marine Corps at any time during the surveillance period. All data used for analyses were abstracted from records routinely maintained in the Defense Medical Surveillance System (DMSS) for health surveillance purposes.

For the incidence analysis, an incident case of IM was defined by having a qualifying diagnosis (International Classification of Diseases, 9th edition [ICD-9]: 075; International Classification of Diseases, 10th edition [ICD-10]: B27*) in the first diagnostic position of an inpatient or outpatient medical encounter. An individual was counted as an incident case only once per lifetime. Prevalent cases (i.e., incident cases that occurred before the start of the surveillance period) were removed from the incidence analysis, and person-time was censored at the time of the incident diagnosis. If a service member had both an outpatient and an inpatient case-defining medical encounter on the same day, the inpatient diagnosis was prioritized over the outpatient diagnosis. Crude incidence rates of IM were calculated as incident IM diagnoses per 100,000 p-yrs.

Adjusted incidence rates of IM were calculated by certain military demographic characteristics (i.e., service branch, occupation, and rank/grade) using multivariable Poisson regression models. One model was run for each military demographic characteristic and adjusted for age, sex, and race/ethnicity. Adjusted incidence rates were calculated per 100,000 p-yrs.

To assess the prevalent burden of IM in the Military Health System (MHS), the total numbers of medical encounters and hospital bed days occurring during the surveillance period with a diagnosis of IM in the primary diagnostic position were counted using standard *MSMR* burden methodology as were the numbers of individuals affected.[16] These burden counts included encounters for both prevalent and incident cases of IM.

RESULTS

During the 17-year surveillance period, there were 23,780 incident cases of IM, resulting in a crude (unadjusted) overall incidence rate of 104.2 cases per 100,000 p-yrs **(Table 1)**. The vast majority (96.4%) of incident cases were diagnosed in outpatient settings. The crude annual rate of incident IM diagnoses fluctuated from a low of 86.5 per 100,000 p-yrs in 2014 to a high of 126.0 per 100,000 p-yrs in 2009 **(Figure 1)**. However, the incidence of IM diagnoses at the beginning of the surveillance period in 2002 (114.0 per 100,000 p-yrs) was very similar to the incidence at the end of the surveillance period in 2018 (113.7 per 100,000 p-yrs).

Overall incidence rates of IM diagnoses were highest among the youngest age groups and decreased with increasing age **(Table 1)**. Among female service members, the overall incidence of IM diagnoses among women less than 20 years old (531.7 per 100,000 p-yrs) was 17.1 times that among women in the oldest age group (40 years or older; 31.1 per 100,000 p-yrs). Similarly, among male service members, the overall rate of incident IM diagnoses among those less than 20 years old (294.7 per 100,000 p-yrs) was 18.5 times that of men aged 40 years or older (15.9 per 100,000 p-yrs). Overall, the incidence of IM diagnoses was 61.3% higher in females compared to males (153.9 per 100,000 p-yrs vs. 95.4 per 100,000 p-yrs, respectively). In addition, the overall rate of incident IM diagnoses was markedly higher among non-Hispanic white service members (123.4 per 100,000 p-yrs) compared to those in other race/ethnicity groups.

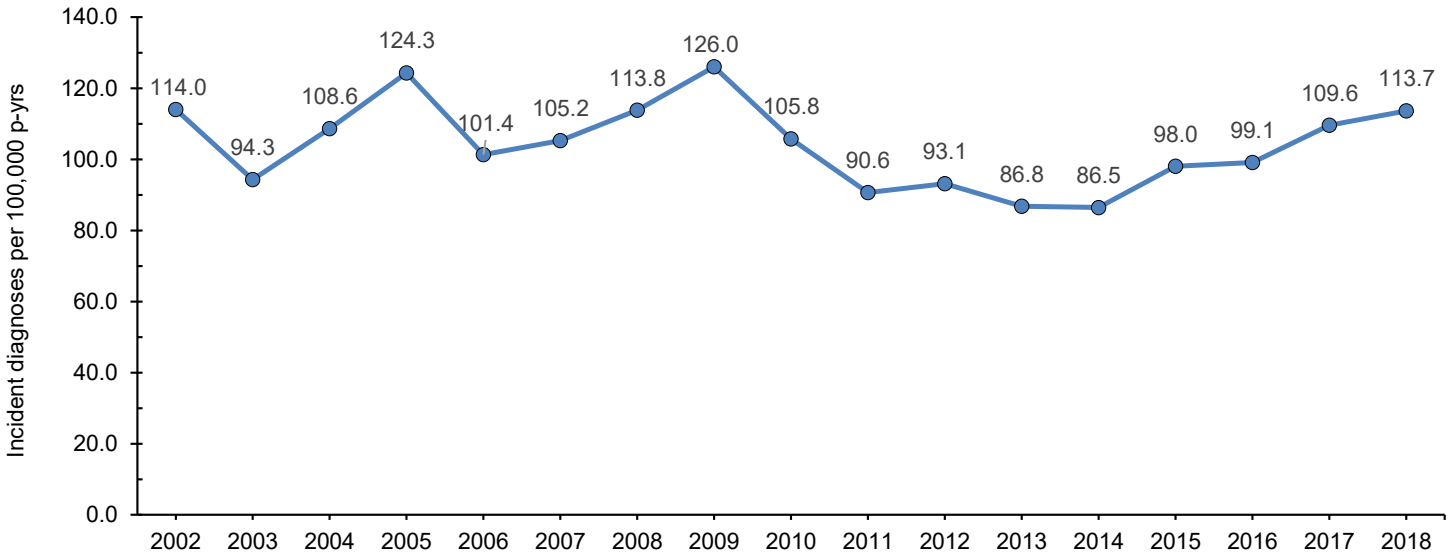
The crude overall incidence rates of IM diagnoses were generally similar across the service branches; however, the rate was lowest in the Army (95.0 per 100,000 p-yrs) and highest in the Navy (113.9 per

TABLE 1. Incident diagnoses and incidence rates[a] of infectious mononucleosis by demographic and military characteristics, active component, U.S. Armed Forces, 2002–2018

	Total 2002–2018	
	No.	Rate[a]
Total	23,780	104.2
Inpatient	850	3.7
Outpatient	22,930	100.4
Sex		
Male	18,548	95.4
Female	5,232	153.9
Age group (years)		
<20	5,114	335.1
20–24	12,149	163.4
25–29	3,968	75.9
30–34	1,369	39.4
35–39	749	27.3
40+	431	17.8
Sex and age group (years)		
Male		
<20	3,732	294.7
20–24	9,707	154.8
25–29	3,097	70.2
30–34	1,089	36.5
35–39	587	24.5
40+	336	15.9
Female		
<20	1,382	531.7
20–24	2,442	210.0
25–29	871	106.1
30–34	280	56.2
35–39	162	46.1
40+	95	31.1
Race/ethnicity		
Non-Hispanic white	17,103	123.4
Non-Hispanic black	2,599	67.8
Hispanic	2,140	77.0
Asian/Pacific Islander	380	44.2
Other/unknown	1,558	103.7
Service		
Army	8,075	95.0
Navy	6,387	113.9
Air Force	5,931	105.9
Marine Corps	3,387	108.6
Military occupation		
Combat-specific[b]	3,050	94.9
Motor transport	678	99.2
Pilot/air crew	567	65.2
Repair/engineer	6,489	96.4
Communications/ intelligence	4,683	91.7
Healthcare	2,266	115.4
Other/unknown	6,047	142.1
Rank/grade		
Recruit	1,661	364.9
Enlisted	19,612	106.0
Officer	2,507	64.7

[a]Rate per 100,000 person-years.
[b]Infantry/artillery/combat engineering/armor.
No., number.

FIGURE 1. Crude annual rates of incident IM diagnoses, active component, U.S. Armed Forces, 2002–2018



IM, infectious mononucleosis; p-yrs, person-years.

100,000 p-yrs). Service members working in health care had the highest overall rate (115.4 per 100,000 p-yrs) among the specific occupational categories. The high rate for those in the non-specific category of "other/unknown" was largely due to the fact that 37% (n=2,238) of the cases were among recruit trainees. The overall incidence of IM diagnoses among recruits (364.9 per 100,000 p-yrs) was 3.4 times that among other enlisted personnel (106.0 per 100,000 p-yrs) and 5.6 times that among officers (64.7 per 100,000 p-yrs). Incident IM diagnoses peaked among recruits in 2007 (747.0 per 100,000 p-yrs) and 2009 (925.6 per 100,000 p-yrs) **(data not shown)**.

After adjusting for age, sex, and race/ethnicity, the incidence rate of IM diagnoses was highest among service members in the Navy (64.7 per 100,000 p-yrs) and lowest among those in the Marine Corps (43.7 per 100,000 p-yrs) **(Table 2)**. Across military occupations, the adjusted overall rate was highest among healthcare workers (75.4 per 100,000 p-yrs) and lowest among those in combat-specific occupations. Finally, the adjusted overall rate of incident IM diagnoses among recruits was 86.1 per 100,000 p-yrs, which was 1.6 times that among other enlisted personnel and 1.3 times that of officers **(Table 2)**.

By location

During the 17-year surveillance period, the medical treatment facilities at 10 installations diagnosed at least 400 incident cases of IM each; when combined, these installations diagnosed more than one-quarter (27.3%) of all cases **(Table 3)**. Of these 10 installations, 2 provide support to recruit/basic combat training centers (Naval Branch Health Clinic [NBHC] Great Lakes, IL; Fort Benning, GA) and 4 support large combat troop populations (Camp Lejeune, NC; Fort Bragg, NC; Fort Hood, TX; Camp Pendleton, CA).

NBHC Great Lakes contributed the most incident cases of IM during the years 2002–2006, with the greatest number of cases documented in 2005 (n=246) **(data not shown)**. In 2007 (n=188), 2009 (n=138), and 2010 (n=76), Fort Benning diagnosed the greatest number of incident cases. Joint Base Lewis–McChord contributed the greatest number of cases in 2008 (n=76). In 2011 (n=41), 2012 (n=80), and 2013 (n=80), Camp Lejeune had the greatest number of cases. Naval Medical Center Portsmouth recorded the most incident cases during the years 2014–2018, with the greatest number occurring in 2017 (n=148) **(data not shown)**.

TABLE 2. Adjusted[a] overall incidence rates of infectious mononucleosis diagnoses by selected military characteristics, active component, U.S. Armed Forces, 2002–2018

	Total 2002–2018
	Rate[b]
Service	
Army	53.4
Navy	64.7
Air Force	59.7
Marine Corps	43.7
Military occupation	
Combat-specific[c]	46.4
Motor transport	49.0
Pilot/aircrew	60.2
Repair/engineer	52.1
Communications/intelligence	54.2
Healthcare	75.4
Other/unknown	62.3
Rank/grade	
Recruit	86.1
Enlisted	54.1
Officer	66.1

[a]Adjusted for age, sex, and race/ethnicity.
[b]Rate per 100,000 person-years.
[c]Infantry/artillery/combat engineering/armor.

TABLE 3. Incident infectious mononucleosis diagnoses by location (for the 10 installations with the most cases during the period), active component, U.S. Armed Forces, 2002–2018

Location of diagnosis	No.	% of total
NBHC Great Lakes, IL	1,019	4.3
Fort Benning, GA	1,015	4.3
NMC Portsmouth, VA	833	3.5
Camp LeJeune, NC	653	2.7
Fort Bragg, NC	555	2.3
NMC San Diego, CA	552	2.3
NH Pensacola, FL	537	2.3
Fort Hood, TX	467	2.0
Camp Pendleton, CA	436	1.8
Joint Base Lewis–McChord, WA	419	1.8
All other locations	17,294	72.7
Total	23,780	100.0

No., number; NBHC, Naval Branch Health Clinic; NMC, Naval Medical Center; NH, Naval Hospital.

Burden

During the surveillance period, there were 44,606 total medical encounters and 4,189 hospital bed days for IM among 23,861 individuals **(Figure 2)**. The annual number of encounters per person fluctuated between 1.6 and 2.0 throughout the 17-year period (mean=1.8). There were peaks in the total numbers of IM-related medical encounters in 2005 (3,206 encounters) and 2009 (3,474 encounters). A peak in the number of hospital bed days occurred in 2003, at 351 days.

EDITORIAL COMMENT

This report demonstrates that the crude overall incidence rate of IM diagnoses among active component service members has remained stable over many years, at about 100 per 100,000 p-yrs. Compared to their respective counterparts, younger service members and, in particular, recruits had the highest overall rates of incident IM diagnoses as did females and non-Hispanic whites.

Using data and associated serum samples from the U.S. National Health and Nutrition Examination Survey from 2003 through 2010, Balfour and colleagues showed that the overall age-adjusted EBV antibody prevalence was estimated to be considerably higher among non-Hispanic blacks (88%) compared to non-Hispanic whites (64%).[11] Similar results were reported from a study using data from children and adolescents who provided serum samples during the course of care at outpatient clinics in Minnesota. It is speculated that a combination of genetic susceptibility, family practices, and/or shared environment could play a role in acquisition of primary EBV infection before adolescence, which may account for this difference in antibody prevalence.[11,17] This finding could also explain why the incidence of IM diagnoses in the current analysis was higher among non-Hispanic whites, as they are less likely to have been exposed to EBV before joining military service.

In the U.S. military, IM has the potential to reduce military operational readiness by contributing to lost or limited duty time. If each patient with an incident diagnosis of IM is unable to perform their duties for 2 weeks following infection, in an average year, cases of IM could result in an estimated 2,797 weeks of lost duty time each year. The recruit training environment likely contributes to increased risk of IM transmission because of close and crowded living conditions as well as physical and psychological stresses that could temporarily depress immune system function.[18] Recruits who acquire IM during boot camp are at risk of being "recycled"—or having to repeat weeks of training with a new unit in an earlier cycle of training.

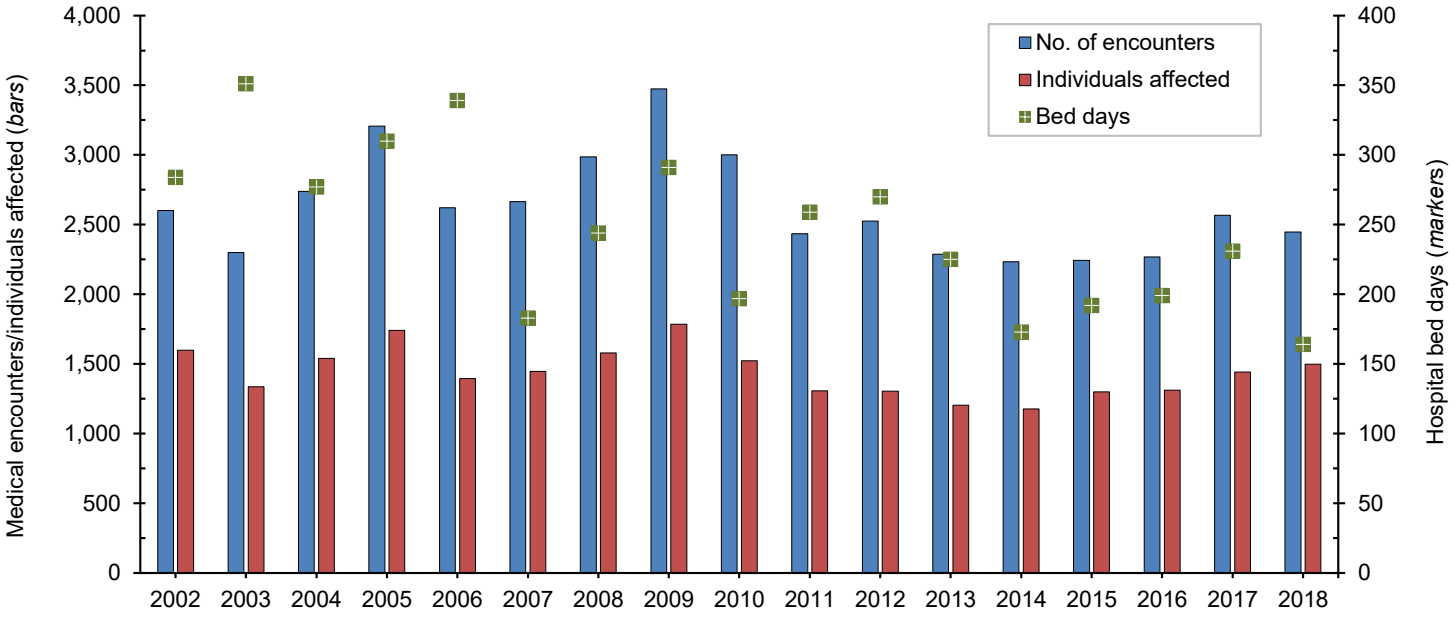
Currently, there is no vaccine available to prevent EBV infection; transmission can be avoided by not kissing infected individuals and by not sharing food, eating utensils, or drinking glasses with such individuals. Symptomatic treatment for IM consists mainly of supportive care and includes drinking fluids to stay hydrated, getting adequate rest and nutrition, and taking antipyretics and anti-inflammatory medications to reduce fever, throat discomfort, and malaise.[1] In addition, there has been some evidence to suggest that vitamin D may reduce the risk of developing acute mononucleosis by helping to suppress viruses and by controlling the inflammatory response.[19,20]

A recent systematic review found insufficient evidence for the use of corticosteroid therapy for symptom control in IM and that data on long-term efficacy and side effects were limited.[21] Corticosteroid therapy for routine cases of IM is not recommended because of concerns regarding immunosuppression during a clinical illness with a virus (EBV) that has been linked to a variety of malignancies (e.g., Burkitt's lymphoma, Hodgkin's lymphoma, and nasopharyngeal carcinoma).[22,23] However, corticosteroid use is warranted in the management of IM patients with impending airway obstruction.[24] Studies of the treatment of acute IM with the antiviral agents (i.e., acyclovir, valomaciclovir, and valacyclovir) have shown short-term suppression of oral viral shedding, but significant clinical benefit has not been demonstrated.[25–28] Moreover, the use of these medications in the treatment of acute IM raises concerns regarding the potential for adverse events and antiviral resistance.[28]

Because more than 50% of patients with IM develop enlarged spleens within the first 2 weeks of symptom onset, a primary concern is avoiding activities that may lead to splenic rupture.[29] Athletes with IM who are planning to resume contact sports are generally advised to gradually restart training 3 weeks after symptom onset. For strenuous contact sports or activities associated with increased intra-abdominal pressure (e.g., weightlifting), the general recommendation is to wait to resume such activities for a minimum of 4 weeks after symptom onset.[30,31] A recent systematic review advocated individualized return-to-play recommendations for athletes with IM because of the variable disease course and lack of evidence-based guidelines.

There are several limitations that should be considered when interpreting the results of this analysis. The incidence of IM may be underestimated to the extent that affected individuals do not seek care and do not receive a diagnosis for IM, and asymptomatic cases are unlikely to be captured in this analysis. Alternatively, the incidence may be overestimated to the extent

FIGURE 2. Numbers of medical encounters for IM and unique individuals affected, active component, U.S. Armed Forces, 2002–2018



IM, infectious mononucleosis; No., number.

that a presumptive diagnosis was made without laboratory confirmation. Finally, the new electronic health record for the MHS, MHS GENESIS, was implemented at several military treatment facilities during 2017. Medical data from sites that are using MHS GENESIS are not available in the DMSS. These sites include Naval Hospital Oak Harbor, Naval Hospital Bremerton, Air Force Medical Services Fairchild, and Madigan Army Medical Center. Therefore, medical encounters for individuals seeking care at any of these facilities during 2017–2018 were not included in this analysis.

IM is not considered to be a serious illness; however, it can impact duty requirements during the acute phase. Military service members and recruit trainees in particular will continue to present with IM. Military commanders should be aware of the risks of infection as well as how to limit transmission within their units.

REFERENCES

1. Epstein-Barr virus and infectious mononucleosis. Centers for Disease Control and Prevention website. https://www.cdc.gov/epstein-barr/about-mono.html. Accessed 11 May 2019.
2. Dunmire SK, Hogquist KA, Balfour HH. Infectious mononucleosis. *Curr Top Microbiol Immunol.* 2015;390:211–240.
3. Hurt C, Tammaro D. Diagnostic evaluation of mononucleosis-like illnesses. *Am J Med.* 2007;120(10): e911e.1–911e.8.
4. Fugl A, Andersen CL. Epstein-Barr virus and its association with disease–a review of relevance to general practice. *BMC Fam Pract*. 2019;20(1):62.
5. Buchwald DS, Rea TD, Katon WJ, Russo JE, Ashley RL. Acute infectious mononucleosis: characteristics of patients who report failure to recover. *Am J Med.* 2000;109:531–537.
6. Hickie I, Davenport T, Wakefield D, et al. Post-infective and chronic fatigue syndromes precipitated by viral and non-viral pathogens: prospective cohort study. *BMJ*. 2006;333(7568):575–580.
7. Katz BZ, Shiraishi Y, Mears CJ, Binns HJ, Taylor R. Chronic fatigue syndrome after infectious mononucleosis in adolescents. *Pediatrics.* 2009;124(1):189–193.
8. White PD, Thomas JM, Amess J, et al. Incidence, risk and prognosis of acute and chronic fatigue syndromes and psychiatric disorders after glandular fever. *Br J Psychiatry*. 1998;173:475–481.
9. Vetsika EK, Callan M. Infectious mononucleosis and Epstein-Barr virus. *Expert Rev Mol Med*. 2004;6(23):1–16.
10. Fafi-Kremer S, Morand P, Brion JP, et al. Long-term shedding of infectious Epstein-Barr virus after infectious mononucleosis. *J Infect Dis.* 2005;191(6):985–989.
11. Balfour HH Jr, Odumade OA, Schmeling DO, et al. Behavioral, virologic, and immunologic factors associated with acquisition and severity of primary Epstein-Barr virus infection in university students. *J Infect Dis*. 2013;207(1):80–88.
12. Lennon P, Crotty M, and Fenton JE. Infectious mononucleosis. *BMJ.* 2015;350:h1825.
13. Williams-Harmon YJ, Jason LA, Katz BZ. Incidence of infectious mononucleosis in universities and U.S. military settings. *J Diagn Tech Biomed Anal*. 2016;5(1).
14. Candy B, Chalder T, Cleare AJ, Wessely S, White PD, Hotopf M. Recovery from infectious mononucleosis: a case for more than symptomatic therapy? A systematic review. *Br J Gen Pract*. 2002;52(483):844-851.
15. Army Medical Surveillance Activity. Infectious mononucleosis among active duty US servicemembers, 1998–1999. *MSMR*. 2001;7(2):2–8.
16. Armed Forces Health Surveillance Branch. Absolute and relative morbidity burdens attributable to various illnesses and injuries, active component, U.S. Armed Forces, 2018. *MSMR*. 2019;26(5):2–10.
17. Condon LM, Cederburg LE, Rabinovitch MD, et al. Age-specific prevalence of Epstein-Barr virus infection among Minnesota children: effects of race/ethnicity and family environment. *Clin Infect Dis*. 2014;59(4):501–508.
18. Gleeson M. Immune function in sport and exercise. *J Appl Physiol*. 2007;103(2):693–699.
19. Maloney SR, Almarines D, Goolkasian P. Vitamin D levels and monospot tests in military personnel with acute pharyngitis: a retrospective chart review. *PLoS One*. 2014;9(7):e101180.
20. Maghzi H, Ataei B, Khorvash F, Yaran M, Maghzi A. Association between acute infectious mononucleosis and vitamin D deficiency. *Viral Immunol.* 2016;29(7):398–400.
21. Rezk E, Nofal YH, Hamzeh A, Aboujaib MF, AlKheder MA, Al Hammad MF. Steroids for symptom control in infectious mononucleosis. *Cochrane Database Syst Rev.* 2015;(11):CD004402.

22. Hjalgrim H, Askling J, Rostgaard K, et al. Characteristics of Hodgkin's lymphoma after infectious mononucleosis. *N Engl J Med*. 2003;349(14):1324–1332.
23. Cohen JI. Epstein-Barr virus vaccines [published correction appears in *Clin Transl Immunol*. 2015;4(4)e36]. *Clin Transl Immunol*. 2015;4(1):e32.
24. Kakani S. Airway compromise in infectious mononucleosis: a case report. *Cases J.* 2009;2:6736.
25. van der Horst C, Joncas J, Ahronheim G, et al. Lack of effect of peroral acyclovir for the treatment of acute infectious mononucleosis. *J Infect Dis.* 1991;164(4):788–792.
26. Tynell E, Aurelius E, Brandell A, et al. Acyclovir and prednisolone treatment of acute infectious mononucleosis: a multicenter, double-blind, placebo-controlled study. *J Infect Dis*. 1996;174(2):324–331.
27. Torre D, Tambini R. Acyclovir for treatment of infectious mononucleosis: a meta-analysis. *Scand J Infect Dis*. 1999;31(6):543–547.
28. De Paor M, O'Brien K, Fahey T, Smith SM. Antiviral agents for infectious mononucleosis (glandular fever). *Cochrane Database Syst Rev*. 2016;12:CD011487.
29. Hosey RG, Kriss V, Uhl TL, DiFiori J, Hecht S, Wen DY. Ultrasonographic evaluation of splenic enlargement in athletes with acute infectious mononucleosis. *Br J Sports Med*. 2008;42(12):974–977.
30. Auwaerter PG. Infectious mononucleosis: return to play. *Clin Sports Med.* 2004;23(3):485–487.
31. Becker JA, Smith JA. Return to play after infectious mononucleosis. *Sports Health.* 2014;6(3):232–238.

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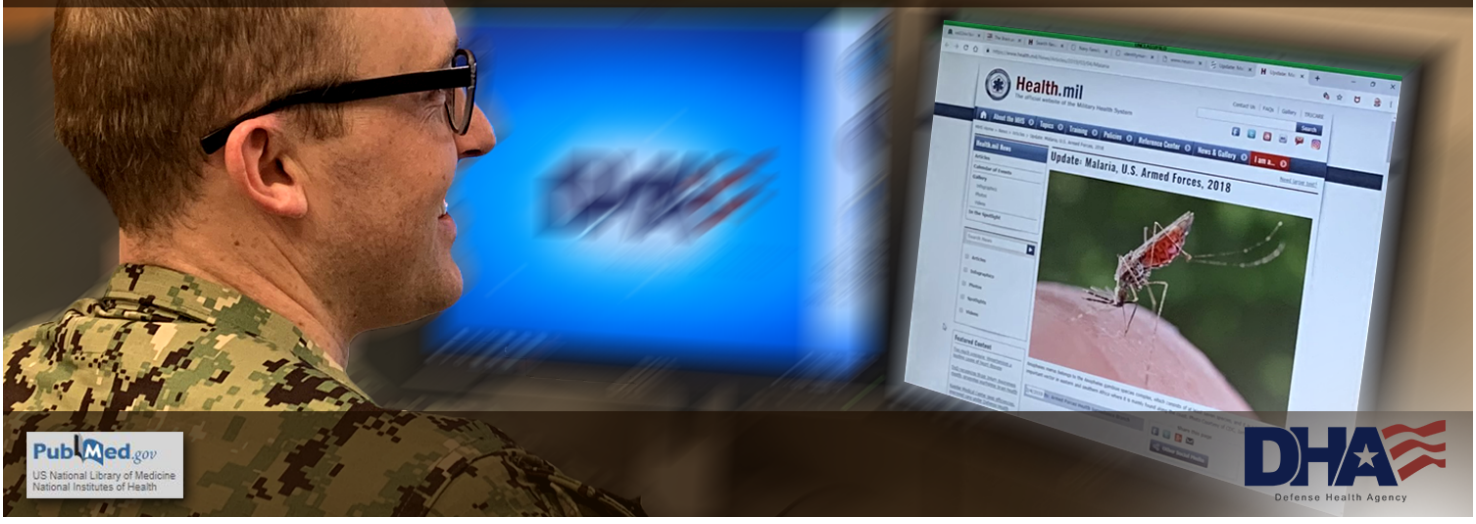
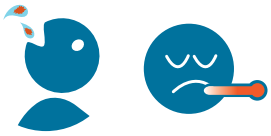


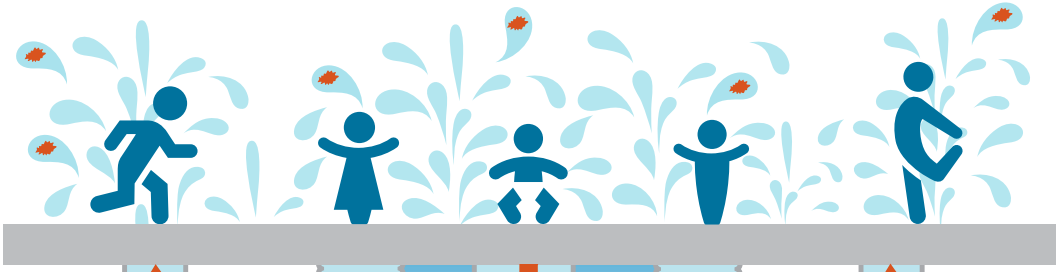
MAKE A HEALTHY SPLASH!

Swallowing water in interactive fountains, splash pads, and spray parks might make you sick.

Germs can get into the water through poop coming out or washing off our bodies.



Swallowing water containing germs can make you sick with diarrhea.



- Water in interactive fountains is typically recycled and might contain germs.
- Water treatments like chlorine don't kill germs instantly.
- Pee in water weakens the germ-killing power of chlorine.

Keep pee and poop OUT of the water.

DO:

- Stay out of the water if you have diarrhea.
- Take bathroom breaks every 60 minutes.

- Check diapers every 30-60 minutes and change diapers away from the water.

DON'T:

DON'T drink the water.

DON'T sit on the water jets.

U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

SPLASH HEALTHY!
www.cdc.gov/healthyswimming

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