Cohort profile: a migratory cohort study of US Marines who train in Australia

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ABSTRACT

Purpose In 2012, US Marines and Sailors began annual deployments to Australia to participate in joint training exercises with the Australian Defence Force and other partners in the region. During their training, US service members are exposed to a variety of infectious disease threats not normally encountered by American citizens. This paper describes a cohort of US Marines and Sailors enrolled during five rotations to Australia between 2016 and 2020.

Participants Study participation is strictly voluntary. Group informational sessions are held prior to deployment to describe the study structure and goals, as well as the infectious disease threats that participants may encounter while in Australia. All participants provided written informed consent. Consented participants complete a pre-deployment questionnaire to collect data including basic demographic information, military occupational specialty, travel history, family history, basic health status and personal habits such as alcohol consumption. Blood is collected for serum, plasma and peripheral blood mononuclear cells (PBMC) processing. Data and specimen collection is repeated up to three times: before, during and after deployment.

Findings to date From the five rotations that comprised the 2016–2020 Marine Rotational Force-Darwin, we enrolled 1289 volunteers. Enrolments during this period were overwhelmingly white male under the age of 24 years. Most of the enrollees were junior enlisted and non-commissioned officers, with a smaller number of staff non-commissioned officers and commissioned officers, and minimal warrant officers. Over half of the enrollees had occupational specialty designations for infantry.

Future plans In the future, we will screen samples for serological evidence of infection with *Burkholderia pseudomallei*, *Coxiella burnetii*, Ross River virus, SARS-CoV-2 and other operationally relevant pathogens endemic in Australia. Antigenic stimulation assays will be performed on PBMCs collected from seropositive individuals to characterise the immune response to these infections in this healthy American population.

INTRODUCTION

In recent years, the US Department of Defense (DoD) has expanded strategic focus to new theatres of operation in Asia and the Global South, regions with a significant burden of severe endemic infectious diseases and a high risk of emerging infectious diseases. To counter that threat, DoD force health protection (FHP) programmes implement a suite of preventive measures designed to protect US service members deployed overseas. The DoD defines FHP as ‘a framework that describes procedures for assessing the types of hazards, the exposure and populations at risk, and the monitoring of the health of all personnel deployed’.¹ This implies that accurate characterisation and understanding of hazards provides the foundation for effective interventions. While integrated risk assessment strategies for hazardous chemicals and toxic materials have long been used to manage force health in the past, risk assessment strategies for biological threats are not consistently applied.

Biosurveillance is a key component of the military’s approach to prevent infections during deployments. For example, arthropod-borne diseases such as malaria, dengue, leishmaniasis and scrub typhus constitute a significant threat to military forces serving in endemic areas.²,³ Vector surveillance, identifying pathogens within their arthropod vector, can rapidly identify pathogenic threats endemic to a new theatre of operation.⁴ As a working example, vector surveillance is credited for characterising the threat of leishmaniasis to coalition forces in Iraq, where mitigation strategies dramatically decreased the incidence of leishmaniasis cases.⁵ Similar
surveillance programmes for zoonoses (Coxiella burnetii), water-borne infections (leptospirosis, schistosomiasis), and respiratory infections (influenza, tuberculosis and more recently COVID-19) have proven valuable for characterising the threat of diseases with military relevance.11

In 2012, US Marines began annual deployments to Darwin, Australia, located in the Top End region of Australia’s Northern Territory (figure 1). Deployed units participate in joint training exercises with the Australian Defence Force and other partners in the Indo-Pacific Region with the overall goal of improved interoperability. The Top End of the Northern Territory covers an area of approximately 500000 km², with a coastline of over 10000 km. The population is around 200000 people with around 150000 living in the coastal capital city of Darwin (12°S latitude) and its surrounds, which include the military bases housing Marine Rotational Force-Darwin (MRF-D). The climate of the Top End is described as the ‘wet–dry’ tropics, with a monsoonal wet season from November through April, followed by the dry season with minimal rain. Darwin is closer to many Southeast Asian cities than it is to Sydney and Melbourne in southern Australia. Townsville (19°S latitude) in north Queensland on the east coast of Australia also cycles between a wet and dry tropical climate.

MRF-D began as a modest force of approximately 200 Marines and Sailors, but since has gradually increased to its target size of approximately 2500 US service members. The exact composition of each rotation differs each year. Two consistent features of each rotation is the involvement of a battalion-sized ground combat element (GCE) and an air combat element (ACE). Additional supporting units such as a logistics combat element and artillery units attach once in Australia. At this time, MRF-D arrives in April and departs in October. Therefore, to date, MRF-D has avoided the northern Australian wet season and there is very little rainfall exposure during their rotation. During the deployment, units are engaged in prolonged field exercises with regular and ongoing exposure to soil, dust, surface water and arthropods.

Australia has a variety of infectious disease threats rarely encountered by US expeditionary forces. For example, the tropical north of the Northern Territory is considered a hyper-endemic area for Burkholderia pseudomallei, the bacterium that causes the disease melioidosis. B. pseudomallei is a gram-negative soil saprophyte and is common in the soil of the northern Northern Territory and other tropical areas of Australia. The spectrum of melioidosis ranges from asymptomatic infection to severe sepsis, and modelling has predicted approximately 89000 deaths per year globally.12 Darwin has the highest reported incidence of melioidosis of any city globally, with annual rates up to 50 per 100000 population.13 B. pseudomallei also has a long history as a threat to military forces, including over 300 documented active cases in US forces during the Vietnam War. Furthermore, serological surveys of Vietnam War veterans suggest hundreds of thousands of additional asymptomatic B. pseudomallei infections with the potential for latency and reactivation even decades later.14–19 Underscoring the need for FHP surveillance of MRF-D, we recently demonstrated that US Marines are experiencing asymptomatic infection with B. pseudomallei in Australia by screening serum samples obtained from the DoD Serum Repository (DoDSR) for antibodies directed against B. pseudomallei antigens.20 We have also identified one potential case of acute melioidosis in a Marine returning from Australia as part of MRF-D.21

Here, we describe a migratory cohort enrolled in an observational study of US Marines and Sailors who deployed to Australia for a period of 6 months. To date, 1289 volunteers have consented to participate. Up to three times, pre-deployment, mid-deployment and post-deployment, participants have blood drawn and respond to questionnaires concerning personal history, deployment history and demographics. Recruitment and consent are permitted at any point before, during or after deployment. This study was originally conceived as an FHP study for a single deployment-related pathogen (B. pseudomallei) for the purposes of characterising the risk of melioidosis to MRF-D as well as identifying novel correlates of immunity to B. pseudomallei in a healthy American military population. However, the eligibility and consent structure of our study has allowed the focus to expand as needed, resulting in an agile platform for FHP disease surveillance in US Expeditionary Forces.

COHORT DESCRIPTION
Participants, recruitment and consent
Active duty Marines and Sailors who have deployed or were scheduled to deploy to Australia were eligible for recruitment.
Participation was strictly voluntary. Group informational sessions were held to describe the study structure and goals, as well as the infectious disease threats they may encounter while in Australia. Particular focus was placed on *B. pseudomallei* and melioidosis. Throughout the recruitment process, potential volunteers were repeatedly reminded that participation was voluntary. Active duty members of the study team did not wear uniforms and made no mention of rank in the presence of potential participants.

Participants provided written informed consent. As part of the consent process, participants could authorise their samples to be used for the *B. pseudomallei* study only, for other studies related to their deployment to Australia or for any future Institutional Review Board-approved studies.

**Questionnaire**

Consented participants were given a questionnaire to collect data including basic demographic information (sex, age, birthplace, race, ethnicity, rank), military occupational specialty (MOS), travel history (personal travel and prior deployments), family history (diabetes, chronic liver disease, kidney disease), basic health status (fever, cough, joint pain, headache, chest pain, skin disease), and personal habits such as alcohol consumption. Pre-deployment and post-deployment questionnaires can be found in the online supplemental material.

**Study schedule**

If the MRF-D training schedule allowed, the study team travelled to Australia at the approximate midpoint of the deployment. There, the study team followed up with enrolled participants for additional blood draws and questionnaires. Following redeployment to their home base, the study team again engaged the participants in order to obtain information and post-deployment blood samples. Mid-deployment and post-deployment questionnaires included the locations of specific training areas as well as any exposures they may have experienced (surface water, dust, soil) in Australia. The protocol was structured to allow for additional recruitment and consent before, during and after the rotation. Therefore, additional recruitment and consent occurred throughout the process. Individuals who enrolled after the study pre-deployment enrolment visit can provide consent for the study team to obtain pre-deployment serum from the DoDSR.20 22

**Sample collection and processing**

Blood was collected by trained phlebotomists and consisted of 10 mL of blood in a red cap serum separator tube and two 10 mL green top LiH plasma tubes. Samples collected within the contiguous USA were centrifuged onsite and shipped overnight to the Naval Medical Research Center in Frederick, Maryland. Samples collected in Australia were processed at the Menzies School of Health Research in Darwin. Serum and plasma were removed and stored in aliquots, while peripheral blood mononuclear cells (PBMC) were cryopreserved for future use.

Because some study participants enrolled while in Australia are augmentees from different home units, they were frequently unavailable for follow-up blood sampling. At any stage in the schedule, a volunteer’s official duties may prevent follow-up blood draws, as the training schedule always takes precedence over the study. For individuals who provided specific consent to do so, gaps in our sample collection were filled by the DoDSR.20 22

**Data**

Information from paper informed consent forms was entered into a REDCap Database and 100% verified by a second authorised user. Information from paper questionnaires was also entered into a REDCap Database. For quality assurance, 10% of questionnaires are verified by a second authorised user.

**Patient and public involvement**

There was no patient or public involvement in the development of this protocol.

**RESULTS**

From the five rotations that comprised the 2016–2020 MRF-D, we enrolled a total of 1289 volunteers (figure 2). Enrolments during this period were overwhelmingly male, with 1248 men and 41 women enrolled. Sixty-nine per cent (869 of 1289) of participants described themselves as white/Caucasian (figure 3A). Seventy-six per cent of the participants were under the age of 24 years, while the remainder of the cohort ranged from 25 to 49 years of age (figure 3B).

Ideally, encounters with study volunteers occur three times: prior to deployment at their home base, during deployment while in Australia and post-deployment after returning to their home base. However, due to the structure of our study and the rigours of operational training schedules, participation was variable. For rotation year 2016, only the post-deployment time point was
completed. All time points were completed for rotation years 2017 and 2018. During rotation year 2019 and 2020, no mid-deployment time point was able to be captured. Additionally, in 2019, a force enhancement battalion joined MRF-D in Darwin. These Marines were enrolled post-deployment (figure 2, 2019*). Initial consent was obtained at pre-deployment for 594 (46%) Marines and Sailors. Additional 173 (13%) volunteers consented at mid-deployment, and 522 (41%) joined our study at post-deployment (table 1A). Enrollee completion for all pre-deployment, mid-deployment and post-deployment visits, when applicable, was variable each rotation year due to this being a voluntary study. For rotation years where we were able to capture multiple events, most enrollees returned for follow-up study events (table 1B).

The study team had access to members of the GCE during all deployment rotations. During deployment year 2018, we also had access to the ACE. In 2018, 74 volunteers and in 2019, 38 volunteers were from ACE, with the remaining participants being from the GCE. The majority of volunteers were enlisted Marines (figure 4). In terms of MOS, over half of participants were members of the Infantry (table 2).

For 76% of the cohort (983 of 1289), Australia was their first deployment; 61% (789 of 1289) had never previously travelled outside of the USA (deployments and personal travel included). Three hundred six of 1289 (24%) had one or more previous military deployments to land-based operations: 60% (184 of 306) to the Indo-Pacific (Indo-Pacific Command) region; 48% (145 of 306) to the Middle East (Central Command); 5% (16 of 306) to Africa (Africa Command); and 10% (30 of 306) to Cuba (Southern Command) (table 3A). Of the 22% of the cohort (279 of 1289) with personal travel outside of the continental USA, 29% had travelled to the Caribbean, 14% to Southeast Asia and 13% to Central America (table 3B). A total of 7% (90 of 1289) of participants were born outside of the USA (table 3C).

While deployed in Australia, participants’ training areas were concentrated in the Northern Territory and coast of Queensland as illustrated in figure 1, with many respondents reporting more than one training area. For the 75% of the cohort that listed training area responses (972 of 1289), the most reported training area with exposure to dust, soil, and/or surface water in the Northern Territory was Mt. Bundey at 74% (723), followed by 24% in Kangaroo Flats and 21% in general Darwin (table 4).

### Table 1

| (A) Consent | (B) Follow-up |
|-------------|---------------|
| Pre-deployment | Mid-deployment | Post-deployment | Mid-deployment | Post-deployment | Total |
| 2016 | ND-p | ND-m | 130 | ND-m | 130 | 130 |
| 2017 | 202 | 18 | 69 | 149 | 184 | 289 |
| 2018 | 134 | 155 | 17 | 247 | 211 | 306 |
| 2019* | ND-p | ND-m | 140 | ND-m | 199 | 250 |
| 2020 | 148 | ND-m | 10 | ND-m | 133 | 158 |

*Indicates force enhancement for 2019 MRF-D.
MRF-D, Marine Rotational Force-Darwin; ND-m, no enrolments, mid-deployment; ND-p, no enrolments, pre-deployment.
In Queensland, the most identified locations include 17% in Shoal Water Bay and 13% in Townsville.

**Strengths and limitations**

As demonstrated by the current SARS-CoV-2 pandemic, infectious diseases can weaken a military’s readiness as much as any weapon. FHP studies are essential to provide biothreat situational awareness to forces operating in Outside Continental United States (OCONUS) areas of responsibility. The DoDSR provides an invaluable resource for FHP studies but cannot serve as the sole source of biospecimens and information for deployment-related studies. Prospective studies, such as the one described here, have the potential to provide a depth and breadth of information that would not otherwise be available from archived specimens. For example, not all Marines in MRF-D deploy to Darwin. Each year, a significant number of MRF-D participants engage in training activities outside of Darwin for the entirety of their rotation. These areas differ from Darwin in many ways, including climate (figure 1) and potential biological exposures. Perhaps more importantly, some engage in shipboard activities and their time on land in Australia is limited. In each of these cases, a reported deployment history of ‘Australia’ would not provide sufficient granularity for useful data analyses. Only a prospective study can accurately determine specific training areas and duration of exposure in these areas. Our direct interaction with expeditionary forces and data collection practices allows us to accurately determine the threats of specific training areas and activities.

Our study began as an observational study for *B. pseudomallei* infection. However, our protocol’s structure presents a model for an agile platform that can be leveraged for a variety of FHP surveillance studies, an especially important feature for threat-rich environments such as the Top End of Australia. In addition to melioidosis, vector-borne viruses (arboviruses) such as Ross River virus are common in tropical Australia. Ross River virus is the most common of the alphaviruses in Australia, occurring almost exclusively in that country. With a 5-year mean of 4758.6 cases per year in Australia, Ross River virus may have the most outbreak potential among US Marines. Q fever is another important endemic disease, and was first described in Australia in 1937. It is caused by *C. burnetii*, a gram-negative, obligate intracellular bacterium, which oscillates between an infectious small-cell variant and a replicative large-cell variant. Q fever lacks unique clinical signs or symptoms but may present with a wide spectrum of acute and chronic manifestations. It is endemic to many of the areas where US forces deploy, and seroprevalence studies clearly demonstrate deployment-related seroconversions in US and UK military personnel in Iraq and Afghanistan. Outbreaks in US service members underpin the need for Q fever to become an important consideration for FHP of US military personnel. Specimens from those who provide specific written consent are also used for Ross River virus, Q fever and SARS-CoV-2 FHP surveillance.
Our ability to enrol at any point during the MRF-D rotation is a considerable strength of our study. For example, enrolment at various stages provides us with access to MRF-D augments who were not present at pre-deployment, potentially eliminating bias that would result from enrolling exclusively from the main body battalion at pre-deployment. However, the variable participation at each time point that results represents a limitation as well. There are sizeable differences among rotations represented in our cohort. Specifically, the 2016 MRF-D was enrolled approximately 9 months after their return from Darwin. In 2017 and 2018, we completed pre-deployment, mid-deployment and post-deployment activities. In 2019 and 2020, the study team was not able to join MRF-D in Australia but completed pre-deployment and post-deployment activities with the main body of both rotations. The 2019 rotation also included a force enhancement battalion who joined the main body in Darwin. These Marines were enrolled post-deployment. Still, our ability to obtain missing serum samples from the DoDSR has resulted in very few gaps in our sample repository.

COVID-19 had a significant impact on the 2020 MRF-D. They usually arrive in Australia en masse in April, resulting in a consistent time in country. In previous rotation years, the median time in Australia was 182 days (range 29–213).

### Table 3  Travel outside of the continental USA. (A) Prior deployments: 306 enrollees responded with prior deployments; % is n/306. (B) Personal travel: 279 enrollees responded with Outside Continental United States (OCONUS) personal travel; % is n/279. (C) Birthplace: 90 participants were born outside of the USA; % is n/90

| N  | %  | Primary locations                                      |
|----|----|-------------------------------------------------------|
|    |    | Australia; Okinawa, Japan; Philippines; South Korea; Thailand |
|    |    | Afghanistan; Iraq; Kuwait                             |
|    |    | Cuba                                                  |
|    |    | Spain                                                 |
|    |    | Africa; Djibouti                                      |

### Table 4  Australia training areas where respondents reported being exposed to dust, soil, and/or surface water (% represented as number of responses out of total enrollees who reported at least one training area, N/972)

| %  |
|----|
| Mt. Bundey | 723 | 74 |
| Kangaroo Flats | 229 | 24 |
| Darwin | 202 | 21 |
| Shoal Water Bay | 169 | 17 |
| Townsville | 124 | 13 |
| Bradshaw | 48 | 5 |
| Tully | 44 | 5 |

AFRICOM, Africa Command; CENTCOM, Central Command; EUCOM, European Command; INDOPACOM, Indo-Pacific Command; SOUTHCOM, Southern Command.
Then, following a 2-week quarantine period, they rotated into Darwin in waves of 200 approximately every 2 weeks. On each wave’s arrival, they entered a second 2-week quarantine. The entire battalion was present and cleared from quarantine in early August. As a result, not only did the 2020 rotation have a shorter overall time in Australia than previous rotations, but there were also four possible durations of time spent in Australia depending on which flight wave personnel arrived on. This will affect person-time calculations. Finally, the 2020 Marines remained in Darwin for the duration of their training. No MRF-D Marines participated in jungle warfare training, Rim of the Pacific Exercise (RIMPAC), or any other operation outside of the Northern Territory.

In conclusion, our study provides a comprehensive framework for infectious disease threat assessment of immunologically naïve US expeditionary forces who deploy to Darwin, Australia as part of MRF-D. The infectious disease threats that exist in the Northern Territory are well known. However, the potential impact on expeditionary forces operating there remains undetermined. This cohort will provide crucial information and allow for the prioritization of risks and the development of risk management strategies for expeditionary forces operating in Australia. Furthermore, in addition to risk assessment, analyses of the memory immune response elicited by seropositive individuals will provide insight into a protective immune response required for future medical countermeasure development.

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