

Presentation 14 – Lea Steele

Research Advisory Committee on Gulf War Veterans' Illnesses
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**Overview of Research on Infectious Diseases
in Gulf War Veterans**

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Research on Infectious Diseases in Gulf War Veterans

- Overview: Assessment of Infectious Diseases in Gulf Vets
- Leishmaniasis
- Mycoplasma

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- Infectious Disease Concerns during the Gulf War
 - Routine, familiar infections (URI, GI, skin, etc)
 - Regional organisms to which allied troops might not have immunity (esp. *shigellosis*, *malaria*, *sand fly fever*, *cutaneous Leishmaniasis*)
 - Food, water contamination
 - Vaccine contamination?
 - Use of biological weapons?

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Assessments of Infectious Diseases in CDC Study of Gulf veterans in PA Air National Guard (Fukuda et al, JAMA 1998; 280:981-8)

- 99 multisymptom illness case Gulf veterans vs. 59 controls
- Evaluated: Stool specimens for multiple organisms
Serologic (antibody) testing for multiple organisms
- Stool specimens: no salmonella, shigella, campylobacter, yersinia, e.coli, microsporidia, cryptosporidium, cyclospora
- Serology: no antibodies to West Nile, Toscana, Karimabad, Isfahan, shistosomiasis species

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Stool Specimen Testing

Blastocystis hominis 7% cases, 12% controls
Giardia 1% of mild cases, 2% of controls
Enteroviruses 9% of mild cases, 10% of controls

Serologic Testing

Yellow fever 83% positive (due to vaccine), no difference by case status
Botulinum toxin 6% positive, no difference by case status
Anthrax PA 9% positive, no difference by case status
Leishmania 5% positive; no difference by case status
Toxoplasma gondii 19% positive, no difference by case status
Dengue fever 10% positive, no difference by case status
Sand fly fever 9% cases, 2% controls

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Human Herpesviruses (Wallace et al, Clin Diag Lab Imm 1999 6:216-223)

- 46 Gulf veterans who met criteria for chronic fatigue syndrome vs. 32 in good health
- Evaluated: Antibody titers to HHV6 and EBV
PCR for HHV6, HHV7, EBV, CMV in periph mononuclear cells
- Found no differences by serology or PCR between sick and healthy veterans
- Gulf veterans, overall, had lower prevalence of herpes virus DNA than civilians

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Dr. Edward Hyman: "Antibacterial Treatment Method Based on Excretion of Dead and Dying Spherical Bacteria"

- Study done 1997-1999; info from DOD summary report
- 36 symptomatic veterans with coccal bacteria in urine randomized to treatment and placebo groups
- Treated with IV antibiotics according to level of excreted cocci
- Project report indicates "Results show a highly significant benefit in relieving fatigue and headache and in improving 'quality of life'. The results in improving pain approached but did not reach a $p=0.05$."

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Leishmania

- Several species endemic to the Middle East, had been a problem for foreign troops in World War II
- *L. tropica* usually causes cutaneous infection, but was associated with viscerotropic leishmaniasis in at least 12 Gulf veterans
- Systemic infection can be associated with chronic fatigue, abdominal pain and diarrhea, other symptoms
- Actual number of cases unknown: no sensitive screening test available
- Preliminary prevalence study done by Portland VAMC

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Prevalence of *Leishmania tropica* in a random sample of 200 Gulf War veterans (D Bourdette, M Riscoe, R Houghton, S Reed et al)

- First 200 subjects in population-based study tested for reactivity to *L. tropica* recombinant protein using an ELISA test.
- Samples considered positive if values > 3 SDs above the mean value in a population of healthy, nonveteran controls
- Positive serology found in 18 (9%) veterans; none had evidence of clinically active leishmaniasis

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Leishmania tropica and GWI case/control status

- 110 Gulf veteran GWI cases; 57 controls
(cases: 1 or more of musculoskeletal pain, cognitive problems, gastrointestinal problems, skin lesions, fatigue)
- Antibody positive: 10% cases, 4% controls (exact p value = 0.149)
- Remaining subjects not assessed, findings not followed-up
- Sensitivity/specificity of test not known

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Mycoplasma infection

- *Mycoplasma* are small bacteria that lack a cell wall, but are capable of independent self-replication.
- *Mycoplasma* species are associated with human diseases affecting a variety of organ systems (e.g., *m. pneumoniae*, *m. genitalium*, *m. hominis*). They can be present without causing illness or can cause chronic infections, and can be particularly aggressive in immunocompromised patients
- *Mycoplasma fermentans* has been isolated from human saliva, urogenital tract, respiratory tract, bone, joints

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Mycoplasma infection in Gulf veterans

- Dr. Garth Nicolson first reported high infection rate by *mycoplasma fermentans* in ill Gulf veterans and family members
- Intracellular infection not detected with conventional serology, required specialized PCR methods
- He also reported these infections and multisymptom illness can be treated successfully with multiple extended courses of doxycycline, other antibiotics
- Source of mycoplasma infection? Bioweapons? Vaccine contaminant? Other?

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Mycoplasma infection in Gulf veterans

- Hart et al, US Army Research Institute of Infectious Diseases tested 4 lots of AVA from ODS/DS, found none contained mycoplasma DNA; mycoplasma did not survive in “spiked” samples of AVA
- Gray et al reported similar rate of symptomatic and healthy Gulf veterans test positive for mycoplasma using serologic tests, and similar rates of pre-war to post-war conversion.

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Mycoplasma infection in Gulf veterans

- Nicolson et al reported that 45% of symptomatic Gulf veterans test positive for mycoplasma with forensic PCR testing, compared to 3% of controls
- Vojdani et al reported that 55% of ill Gulf vets test positive for mycoplasma species, compared to 15% of controls (also 49% of RA patients, 52% of CFS patients)
- Donta et al reported that 40% of ill Gulf veterans tested positive for mycoplasma when screened for recruitment into VA's antibiotic treatment trial.

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Mycoplasma infection in Gulf veterans: Major Questions

- Is mycoplasma infection associated with GWI symptoms?
 - A primary cause?
 - A cofactor?
 - An opportunistic infection, may/may not be associated with chronic symps
- Does antibiotic treatment improve GWI symptoms?
 - By eliminating mycoplasma infection?
 - By eliminating other infection(s)?
 - Through mechanisms unrelated to antimicrobial action

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Is Mycoplasma Associated with Gulf War illnesses?

- Several studies have found that ill Gulf veterans have a higher rate of mycoplasma infection than healthy controls
- Many questions remain
 - Problems with testing reliability and validity (sample problems, reagent differences, many years after infection, no “gold standard”)
 - Prevalence of mycoplasma in healthy Gulf War veterans?
 - Is mycoplasma causally related to GWI? Is it an opportunistic infection?
 - Is there a chronic infection present? Chronic “after-effects” of acute infection?

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Does Antibiotic Treatment Improve GWI Symptoms?

- Summary presentation of ABT trial results generally indicated that 12 months of doxycycline was not associated with improved functional status.
- Unanswered questions remain
 - Study design:
 - Treatment protocol same as that reported to be effective by Nicolson?
 - Case definition for GWI suitable for testing hypothesis?
 - Is the primary outcome (7 pt. improvement on PCS of SF36) optimal for assessing treatment effects?
 - Confidence in laboratory identification of infected veterans?
 - Study results

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Infectious Disease Questions Remain

- Leishmaniasis: Detection? Prevalence of infection? Chronic sequelae of infection?
- Mycoplasma: Increased prevalence associated with illness?
- Antibiotic treatment effective in improving GWI symptoms?
- Problems due to other types of infections that have not been studied?
- Possible role for infections as a "result" of GWI, e.g., opportunistic infections, chronic immunological effects of aberrant response to infection?