

Presentation 5 – Lea Steele

# Infectious Diseases

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Research Advisory Committee on Gulf War Veterans' Illnesses

## Leishmaniasis in Veterans of Desert Storm & Iraqi Freedom



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Slide adapted from: Magill AJ. Leishmaniasis in Veterans of Desert Storm & Iraqi Freedom. Presentation at: Meeting of the Research Advisory Committee on Gulf War Veterans' Illnesses; February 23, 2004; Washington, DC.

### Leishmania Parasite Life Cycle

The diagram illustrates the life cycle of the Leishmania parasite. It features four panels: a sand fly on the left, a human skin lesion on the right, a microscopic view of promastigotes in a sand fly, and a microscopic view of amastigotes in a mammalian host. A circular arrow indicates the cycle between the sand fly and the mammalian host.

**Promastigotes in sand fly**

**Amastigotes in mammalian host**

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### Leishmania infection and 1990-91 Gulf War

- **What else did we see?**
- **Atypical “viscerotropic leishmaniasis”**
  - ***L. tropica* parasites**
  - **Desert rodent or human reservoir??**
  - **Sand fly vector?**
- **N = 12 cases, parasitologically confirmed**
- **N = ?? cases total**

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## What was unusual?

- **Did not expect to see VL in Saudi Arabia**
- **Atypical, non-specific clinical syndrome**
  - Not typical Visceral Leishmaniasis
  - Smear negative, culture positive
- **Isolation of *Leishmania* from bone marrow**
- **Characterization of isolates as *L. tropica***
- **Difficult diagnosis, insensitive tests**

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## NEJM. May 13, 1993

**Table 1. Clinical Presentation of Eight Male Patients with Visceral Leishmaniasis, at the Time of Confirmatory Culture.**

PATIENT No.	INCUBATION PERIOD (Mo)	SIGNS AND SYMPTOMS AT PRESENTATION	ABDOMINAL				PHYSICAL EXAMINATION
			FEVER	PAIN*	MALAISE*	FATIGUE*	
1	2	Adenopathy	Yes	++	+	++	Hepatomegaly, splenomegaly, adenopathy
2	1-4	Fever	Yes	+	++	+	Normal findings
3	2-8	Gastroenteritis	No	+++	+++	+	Splenomegaly
4	2-6	None	No	No	No	No	Normal findings
5	4-12	Chronic fatigue with hepatosplenomegaly	Yes	+	+	+++	Hepatomegaly, splenomegaly
6	7-14	Chronic fatigue with adenopathy	No	+	+	+++	Hepatomegaly, adenopathy
7	1-6	Mononucleosis	Yes	+/-	+++	+	Normal findings
8	3-12	Fever of unknown origin	Yes	+	++	++	Hepatomegaly, splenomegaly

\*One plus sign indicates that the patient reported the symptom when questioned by the examiner; two plus signs, that the patient himself reported the symptom without questioning; and three plus signs, that the symptom was the primary one. Patient 7, represented by the plus-minus sign, reported abdominal pain of brief duration associated with diarrhea.

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## Leishmania in 1st Gulf War



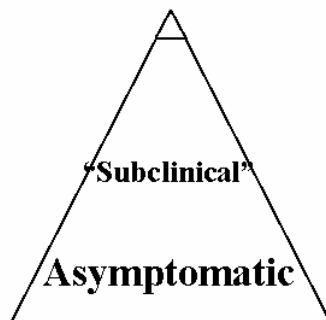
FIGURE 1. Map of the possible sites for collection of the Desert Storm participants. Open symbols = *Leishmania major*; solid symbols = *L. tropica*; □ = major cities. The numbers are Desert Storm identification numbers (see Tables 1 and 2).

- Characterizations of *L. tropica* based on CAE of 21 enzymes
- 3 clusters of *L. tropica*
- *Am J Trop Med Hyg.* 1993. 49:357

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## Visceral Leishmaniasis Disease Spectrum

1-3% with overt VL



- "Subclinical" Syndromes
  - Chronic systemic illness
  - Acute febrile illness
- Risk factors for progression
  - Malnutrition
  - Immunosuppression (AIDS)
  - Genetic?
- Cause of death
  - Measles
  - Pneumonia
  - TB
  - dysentery

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## Can Cytokines Cause Disease?

- **Chronic disease**

- Fever, malaise, myalgias, arthralgias, fatigue, anorexia, nausea
- Inflammatory bowel disease, rheumatoid arthritis,
- $\text{TNF}\alpha$ ,  $\text{INF}\gamma$ , IL-2, IL-12, etc.

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## Persistent *Leishmania* Infection

- **Intracellular pathogen of the macrophage**
- **Lifelong, persistent infection**
- **Treat disease, never eradicate parasites**
- **Mycobacteria: TB, leprosy**
- **Bacteria: *Brucella***
- **Fungal: *Histoplasma***
- **Viral: *HIV***

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## Research on Infectious Diseases in Gulf War Veterans

Assessments of Infectious Diseases in CDC Study of Gulf veterans in PA Air National Guard  
(Fukuda et al, JAMA1998 280:981-8)

- 99 GW multisymptom illness cases veterans vs. 59 GW 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, Karimbad, Isfahan, shistosomiasis species



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## Research on Infectious Diseases in Gulf War Veterans

### 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 diff by case
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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## Research on Infectious Diseases in Gulf War Veterans

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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 mono 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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## Research on Infectious Diseases in Gulf War Veterans

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Cellular and Humoral Immune Abnormalities in Gulf War Veterans  
(Vojdani et al, Env Health Perspect 2004 112:840-846)

- 100 symptomatic Gulf veterans in clinical lab sample, compared to 50 asymptomatic nondeployed Army and 50 civilian controls (age/sex matched)
- Symptomatic Gulf veterans had significantly elevated mean antibody titers of:
  - > EBV IgM (VCA)
  - > CMV IgG
  - > HSV-1 IgG
  - > HSV-2 IgG
  - > HHV-6 IgG
  - > VZV IgG



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## Research on Infectious Diseases in Gulf War Veterans

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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, unpublished)

- 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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## Research on Infectious Diseases in Gulf War Veterans

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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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## Research on Infectious Diseases in Gulf War Veterans

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### Mycoplasma infection

- *Mycoplasma* organisms lack a cell wall, 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



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## Research on Infectious Diseases in Gulf War Veterans

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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; detection required specialized PCR methods
- He also reported these infections and multisymptom illness can be treated successfully with multiple extended courses of doxycycline, other antibiotics



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## Research on Infectious Diseases in Gulf War Veterans

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### Mycoplasma infection in Gulf veterans

- Nicolson et al: 45% of symptomatic Gulf veterans test positive for mycoplasma with forensic PCR testing, compared to 9% of controls
- Vojdani et al: 55 % of ill Gulf vets test positive for mycoplasma species (vs. 8 or 15% of healthy controls)  
*(also 49% of RA patients, 52% of CFS patients)*
- Donta et al: 40% of ill Gulf veterans tested positive for mycoplasma when screened for recruitment into VA's antibiotic treatment trial.



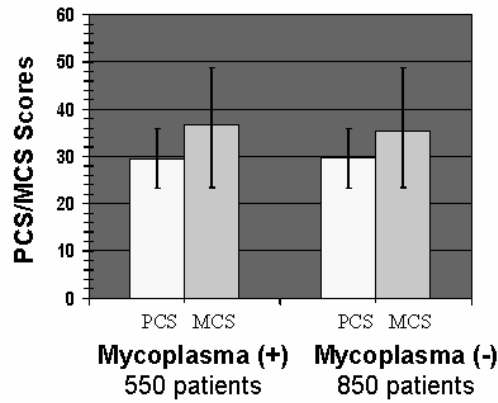
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# CSP#475 ANTIBIOTIC TREATMENT OF GULF WAR VETERANS' ILLNESSES

Slide adapted from: Donta S. Antibiotic treatment of Gulf War Veterans' Illnesses. Presentation at Meeting of the Research Advisory Committee on Gulf War Veterans' Illnesses; February 24, 2004; Washington, DC.

### Mycoplasma Status and PCS/MCS



Slide adapted from: Donta S. Antibiotic treatment of Gulf War Veterans' Illnesses. Presentation at: Meeting of the Research Advisory Committee on Gulf War Veterans' Illnesses; February 24, 2004; Washington, DC.

### Treatment Success (PCS $\geq$ 7 from Baseline) By Rating Period

Rating Period	Doxycycline % Success	Placebo % Success	P-Value
3 Month	21.5	9.9	.001
6 Month	19.7	13.6	.086
9 Month	17.6	14.4	.385
12 Month	18.1	17.3	.905
18 Month	18.2	13.5	.168

Slide adapted from: Donta S. Antibiotic treatment of Gulf War Veterans' Illnesses. Presentation at: Meeting of the Research Advisory Committee on Gulf War Veterans' Illnesses; February 24, 2004; Washington, DC.

## ABT: Antibiotic Treatment Trial

491 Gulf War veterans at 26 study sites; 12 mo. doxycycline

	% improved 7 pts. on SF-36	mean SF-36 scores baseline, 12 mos	% mycoplasma neg. @ 18 mos
Doxycycline	18.0 %	30.2 → 32.0	90 %
Placebo	17.3 %	30.1 → 30.9	87 %



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## CONCLUSIONS

- Study shows that Doxycycline is an ineffective treatment for GWVI.
- Study casts doubt on the relationship between a persistent mycoplasma infection and GWVI.
- Study documents that patients with GWVI are very ill.

Slide adapted from: Donta S. Antibiotic treatment of Gulf War Veterans' Illnesses. Presentation at Meeting of the Research Advisory Committee on Gulf War Veterans' Illnesses; February 24, 2004; Washington, DC.

**Successful Antibiotic Treatment  
Of The Gulf War Syndrome  
A Pilot, Randomized, Placebo  
Controlled, Blinded Trial**

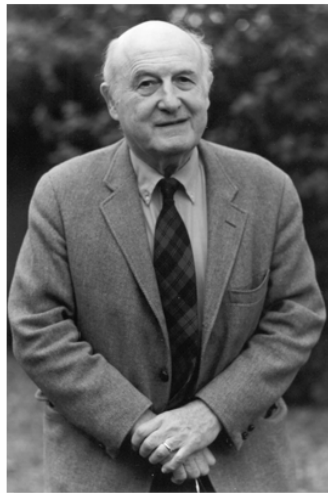
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**Successful Trial Of Urine Microscopy  
For Control Of Antibiotic Treatment  
Of Systemic Coccoal Disease**

Edward S. Hyman M.D, FACP  
William Weiss  
and Quentin B. Deming M.D.

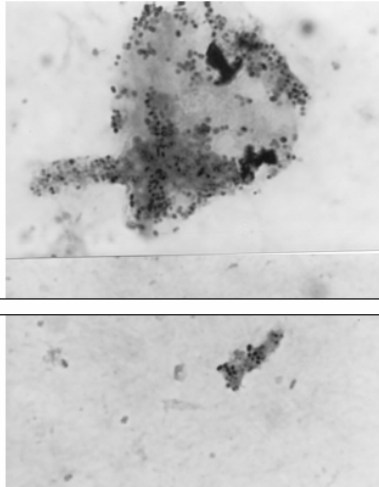
Slide adapted from: Deming QB and Weiss W. Successful Antibiotic Treatment of the Gulf War Syndrome: A Pilot, Randomized, Placebo Controlled, Blinded Trial. Presentation at: Meeting of the Research Advisory Committee on Gulf War Veterans' Illnesses; October 26, 2004; Washington, DC.

**Edward S. Hyman M.D, FACP**



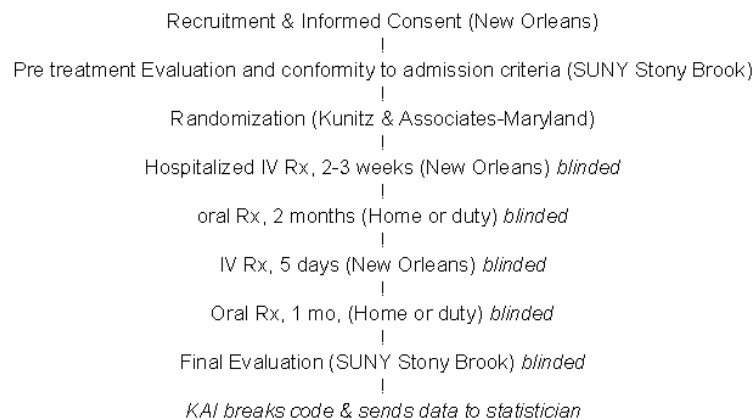
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## Clusters G+ cocci urine



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## Flow Diagram of Protocol



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## Study Cohorts

Evaluable cohort (n=36)

Intent-to-Treat cohort (n=38)

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## Baseline Urine Variables

	<u>Placebo</u>	<u>Treatment</u>	<u>Probability</u>
Protein, %< 2mg/dl	52.9	33.3	0.32
Gram + cocci	29.4	27.8	1.00
Abnormal cocci+	64.7	44.4	0.31
Exploded cocci	82.4	72.2	0.69
Gram - Rods	11.8	11.1	1.0

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## Outcome Variables at Baseline

OUTCOME VARIABLE	PLACEBO	TREATMENT	TOTAL N
Fisk, mean score (ms)	15.1	14.9	36
Fatigue Assessment Index (ms)	5.9	5.9	36
Neuropsych impairment index, median score	-0.72*	-0.60	35
Sleep Quality, median score	3.5	3.7	28
Headache, % patients with	88.9	83.3	36
Median number/month	13	18.5	36
Diarrhea, % $\geq$ 1/day	37.5	25.0	28
Severity score $\geq$ 3	55.6	33.3	36
Pain, McGill, median score	6.3	6.0	36
Dolorimeter, median score	0.5	1.5	34
Quality of Life, median score	20.0	22.5	36

\*one outlier excluded

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## Efficacy Evaluation Primary Variables

### FATIGUE

#### Modified Fatigue Impact Scale (Fisk)

Baseline No statistically significant difference  
 Final (4 months) p=0.0047  
 Final from Baseline p=0.0074

#### Fatigue Assessment Inventory

Baseline No statistically significant difference  
 Final (4 months) p=0.0005  
 Final from Baseline p=0.0002

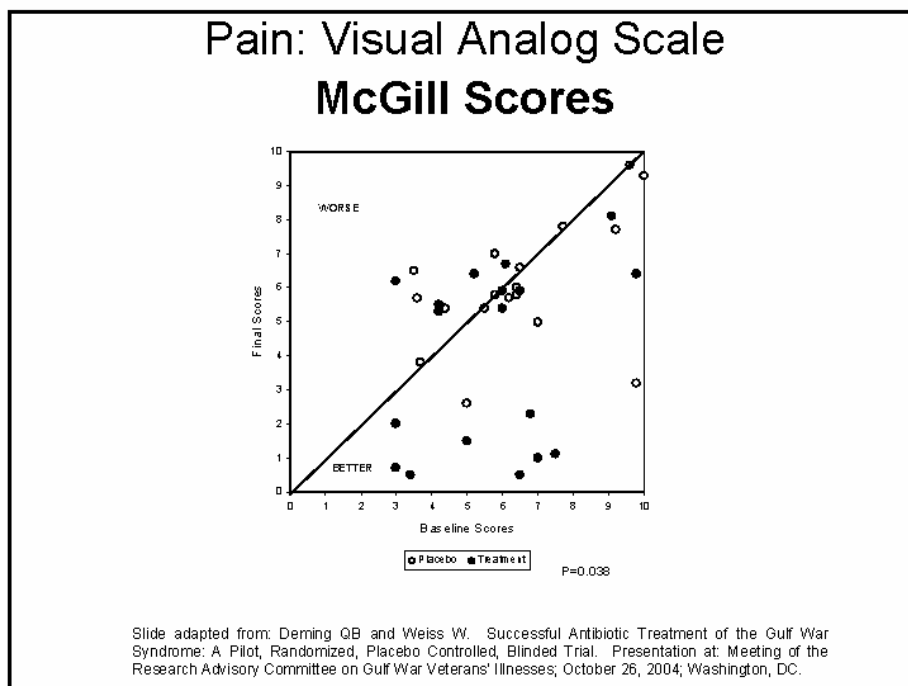
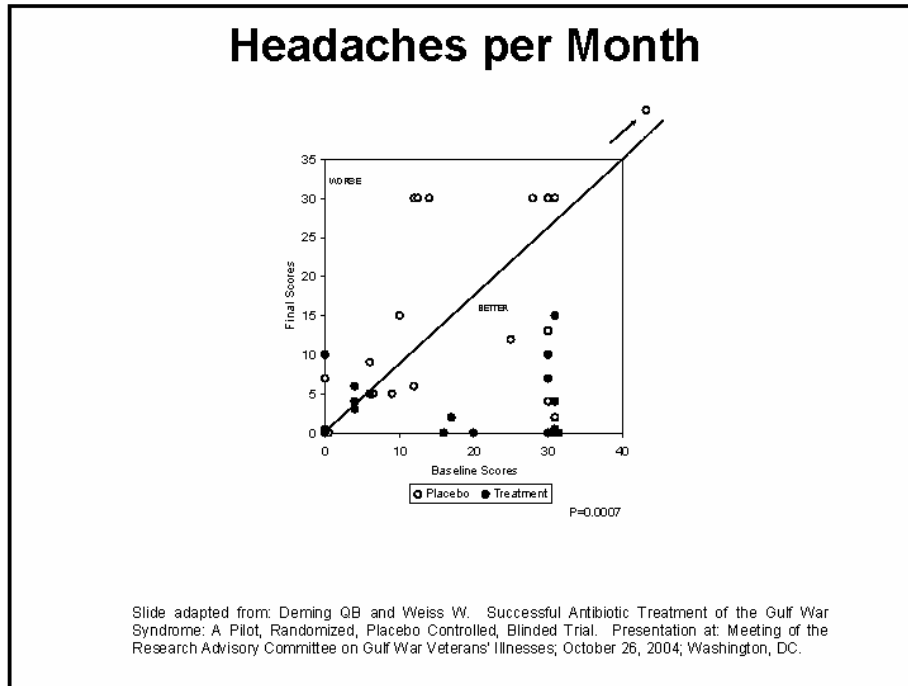
Combined Wilcoxon rank sum test p=0.0007

### NEUROPSYCHOLOGICAL IMPAIRMENT INDEX

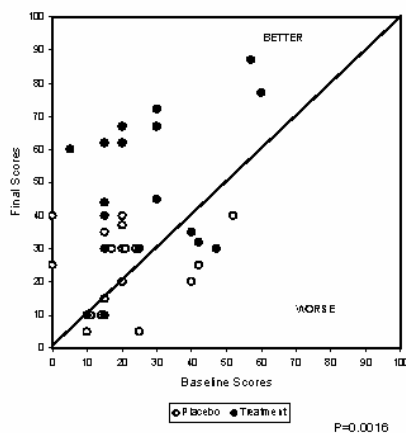
Baseline No statistically significant difference  
 Final (4 months) No statistically significant difference

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## Quality of Life Scores



Slide adapted from: Deming QB and Weiss W. Successful Antibiotic Treatment of the Gulf War Syndrome: A Pilot, Randomized, Placebo Controlled, Blinded Trial. Presentation at: Meeting of the Research Advisory Committee on Gulf War Veterans' Illnesses; October 26, 2004; Washington, DC.

## Conclusions

- A randomized, placebo-controlled, blinded, pilot study has shown that an antibiotic regimen, controlled by monitoring excretion of Gram positive cocci, is effective in ameliorating a syndrome which affects thousands of Gulf War veterans and for which no treatment has previously been proven effective.
- The validity and effectiveness of the urine microscopy method for diagnosis and for control of treatment has been confirmed.
- The hypothesis that Gulf War Syndrome is bacterial in origin, though not proven, is supported.

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## Louisiana study: Summary of Results

	Change from Baseline		p value
	Placebo	Treated	
Fatigue Assessment Inventory Score	+ 3%	+ 36%	<0.001
Fisk Fatigue Impact Scale (pts)	- 1.5	- 6.0	0.007
Headaches per month	- .5	- 16	<0.001
SF-36 score	+ 7	+ 22	0.002
Neuropsych Impairment Index	no change		ns
Sleep quality index score (pts)	+ .2	+ .8	0.06
Diarrhea severity score > 3	- 28%	- 22%	ns
Pain: McGill visual analog	- 0.5	- 0.6	ns



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## Infectious Diseases in Gulf War Veterans: What Do We Know?

### Leishmania

- "New" presentation of viscerotropic leishmaniasis clinically identified in small number of ill Gulf War veterans; number of undetected cases not known
- Leishmaniasis can be associated with chronic multisymptom illness; no reliable test available
- Pilot study of ELISA test identified leishmaniasis in ~9% of random sample of PGW vets
  - > 2<sup>nd</sup> pilot: potentially higher in symptomatic than nonsymptomatic veterans



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## **Infectious Diseases in Gulf War Veterans: What Do We Know?**

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### **Mycoplasma**

- **Consistently identified in ~40 % of symptomatic Gulf War veterans (reported in healthy controls @ 8-15%)**
- **Not clear whether generally associated with Gulf War deployment or nonspecific illness/debilitation**



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## **Infectious Diseases in Gulf War Veterans: What Do We Know?**

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### **Other**

- **Sand fly fever id'd in 9% CMI cases vs. 2% controls in CDC study**
- **Conflicting results re: herpesviruses**
- **Little other information re: persistent infections in ill Gulf War veterans**
- **VA ABT suggested 12 mo. doxycycline therapy ineffective; questions re: lab results and study success**
- **LA study of high-dose, complex antibiotic treatment appears to indicate substantial benefit**



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## Infectious Diseases in Gulf War Veterans: What Do We Know?

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### Antibiotic Treatment

- Nicholson case series suggested benefit of 6-wk cycles of antibiotic therapy
- VA ABT suggested 12 mo. doxycycline therapy ineffective; questions re: lab results and study success
- LA study of high-dose, complex antibiotic treatment appears to indicate substantial benefit



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## Infectious Diseases in Gulf War Veterans: Remaining Questions

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- Are any infections associated with GWI?
  - *As a primary cause?*
  - *As cofactors, linked to perpetuation/exacerbation of symptoms?*
  - *As opportunistic infections, resulting from general debilitation?*
- Evaluation and reliable detection of putative infectious agents in Gulf War veterans



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## Infectious Diseases in Gulf War Veterans: Remaining Questions

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- Does antibiotic treatment improve GWI symptoms?
  - *Potentially by eliminating specific types of infection or general burden of infection?*
  - *Potentially through mechanisms unrelated to antimicrobial action?*



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## Discussion of Recommendations

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### Animal Studies



### Human/Epidemiologic Studies

- **Comprehensive evaluation of multiple infectious organisms in GWI cases vs. controls**
  - *L. Tropica*
  - *Mycoplasma* species
  - **Other?** (e.g. sand fly fever, brucella. "gram negative cocci")



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