Presentation 17 – Beatrice Golomb

June 2004 Review of Recent Gulf War Research

Beatrice A. Golomb, MD, PhD

R 1

Epidemiology

R 2

Smith 2004: Registries vs Hospitalizations

Question: Is Gulf War registry participation a marker of worse health -- by the objective outcome of hospitalizations

Subject:All US GWV in DoD registry (N=69189) vs all GW-era deployed nonparticipants (N=477,333). Excludes Reserve, National Guard.

Method: Cox proportional hazards on hospitalizations from 8-1-91 to 6-1-94 = day before initiation of DoD registry

Analysis: Cox regression. Saturated and manual backward stepwise.

Covariates: demographics; exposures*, deployment

Exposures: Khamisyah plume; Ax/BT; oil fire plume. (PB, pesticides not assessed.)

Smith TC et al 2004 J Occup Env Medicine 46

R 3

Smith 2004: Registries vs Hospitalizations

Results: Risk Factors for Hospitalization

Factor	RR (95%CI)
Registry participant	**1.44 (1.40-1.46)**
Female	1.55 (1.51-1.59)
Age >31	1.12 (1.10-1.15)
Army vs Navy/CoastGd	1.36 (1.33-1.39)
Enlisted (vs officer)	1.50 (1.46-1.55)
Prewar hospitalization	1.66 (1.62-1.70)
BT vaccine	1.43 (1.12-1.82)
Healthcare worker	1.27 (1.23-1.31)

NS: Ax vax 1.03, .92-1.14, rel to not and unknown)

NS: Khamisiyah 0.99 (0.96-1.01)

R 4

Hotopf 2004: Predicting Persistent Illness in GWV

Question: What predicts persistent illness in GWV?

Subjects: UK GWV sampled stratified on fatigue: N = 511; 484; 250 with Chalder fatigue > 9, 4-8, <4

Design: Retrospective cohort: assess how baseline RF relate to persistent health outcomes

Outcomes: 1. Chalder fatigue; 2. GHQ-12 psych distress; 3. SF-36 phys functioning. BUT did not give results for phys functioning!

Covariates/ Factors assessed

- Model 1: Sociodemog: age, sex, rank, service, marital
- Model 2: Model 1 + severity of sx at baseline
- Model 3: Model 2 + tot exposures (0-29), tot vaccines, believe have GWS, case in GHQ-12

Analysis: Regression; then stepwise regression; then posthoc by RF

Hotopf M et al 2004. Psychological Med 34: 747-54.

R 5

Hotopf 2004: Predicting Persistent Illness in GWV

RF for remaining a "case" for that outcome

(Prev published RFs for being a case @ stage 2)

RF	Fatigue	GHQPsych
Age (yr)	NS	1.04*
Male	NS	2.0* (Male!)
Single	NS	NS
Army v Navy	2.7**	NS
Case@ stage 1	1.23**	1.12*
GHQ case	1.6*	(1.12*)
Self-report GWS	2.0**	1.8*
Total exposures	NS	NS
Vaccine quintile	NS	NS

Hotopf M et al 2004. Psychological Med 34: 747-54.

R 6

Hotopf 2004: Predicting Persistent Illness in GWV

Post-hoc analysis: Individual exposures associated c persistence. Among 29 exposures (not cited here), adjusted for demographics and stage 1 score. {Note: Some variables' contribution will be through their impact on stage 1 score}

RF	Fatigue	GHQPsych
Smoke from oil fires	1.3*	1.4*
Burning rubbish/feces	1.6*	1.4*
Chem agt-resistant paint	1.4*	NS
See dead animals	1.3*	NS
See burnt/disfigured peopl	1.3*	NS
Wear NBC suits (x training)	1.4*	NS
Hear chemical alarms	1.5*	NS
Paints & solvents	NS	1.4*
Consuming local food	NS	1.4*

Hotopf M et al 2004. Psychological Med 34: 747-54.

R 7

Birth Outcomes

R 8

Araneta 2004: US women veterans conception & pregnancy outcomes

Question: Do pregnancy outcomes differ in GW vs nondeployed women, who became pregnant during or after the war?

Subjects: From records from 153 military hospitals: women pregnant from 8-90 to 5-92 who were in GW deployed units, comparing GW deployed to nondeployed from same units.

-1558 pregnancy related admissions include:

- 415 GW exposed pregnancies;
- 298 GWV postwar conceptions (difference btn reported date of return & date of birth > reported gestational age)
- 427 NDV conceptions (in deployed unit but state never deployed).

Design: Postal survey in 1997 & 1998. Deployment date by data for unit. Deployment status by self-report (whether deployed).

Outcomes: Stillbirth. Spont abortion. Ectopic preg.

Analysis: Multivariate adjusted regression

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Araneta 2004: US women veterans conception & pregnancy outcomes. Results

Stillbirths *lower* in deployed women unadjusted (2.3% vs 0.2%, 0.7%)

Adjusted Results*:	Spont ab	Ectopic preg
GW conception	1.45 (NS)	1.90 (NS)
GW postwar concepx	2.90 (1.86-4.53)	7.35 (2.97-18.2)

*Covariates: Age, race, educ, marital, rank, branch, parity, hx spont abortion, history of fetal los: demog from DMDC

*Compared to conceptions of nondeployed women from deployed units (ref group)

**Note: effects ~unchanged with adjustment*

Note: points out that misclassification may have been present for GW conception, since unit sent but some preg may have had deployment rescinded (deployment data by unit)

R 10

Araneta MR 2004. Ann Epidemiol 14: 109-116. DMDC = Defense Manpower Data Ctr

Doyle: UK Birth Outcomes

Question: Are Offspring of UK PGWV at incr risk of fetal death or malformation?

Ss: UK GWV vs "demographically similar" nondeployed era control group.

- 16442 male & 484 female UK PGWV (53% & 72% of questionnaires);
- 11,517 male & 377female era controls (42% & 60% of questionnaires).

Design: Retrospective cohort study, with repro hx by validated postal questionnaire btn 1998 and 2001.

Outcomes: Fetal death or malformation among 27959 pregnancies by men and 861 pregnancies by women conceived between GW1 and Nov 1997.

Death:

- Early miscarriage: < 12 weeks
- Late miscarriage: 12-23 weeks
- Stillbirth: ≥23 weeks
- Exclude: Ectopic pregnancies, terminations*

Malformation: diagnosed in utero, at birth, or any time after birth

- Coding by info from clinician wher available, otherwise by parent description
- Double count anomalies if in more than one group (just once if multiple, same group)
- Exclude: minor anomalies

R 11

Doyle: UK Birth Outcomes

Analysis: logistic regression, adjusted

Unit of analysis: pregnancy for a miscarriage; fetus/infant for stillbirth or malformation

Covariates adjusted:

- Year of pregnancy end; pregnancy order for that parent; age of mother; service; rank (All analyses)
- Previous fetal death; analysis of late miscarriage/stillbirth/congen malformation for multiplicity. (Fetal death analyses) (None changed estimate by > 1-2%).

Comparator groups:

- Nuclear Industry Family Study
- England & Wales Annual registered stillbirths by maternal age for

Doyle P 2004. Int J Epi 33: 74-6

R 12

Doyle: UK Birth Outcomes

	Fetal death	fetal death		Stillbirth
MEN	<12wk	12-23wk	all	
All pregnancies since PGW	1.5 (1.3,1.6)	1.2 (1.1,1.3)	1.4 (1.3,1.5)	0.9 (.7,1.3)
All 1st pregnancy since PGW	1.5 (1.4,1.7)	1.2 (1.0,1.3)	1.4 (1.3,1.5)	0.9 (.6,1.4)
1st preg p PGW, conc'd '90-'91	1.5 (1.2,1.9)	1.2 (0.9,1.7)	1.4 (1.2,1.7)	1.9 (.7,5.1)
WOMEN				
All pregnancies since PGW	1.0	0.8	1.0	2.0 (.3,15)
All 1st pregnancy since PGW	1.4 (0.8,2.5)	-0.7 (0.3,1.7)	1.2 (0.7,1.9)	∞

VS NIFS COMPARATOR GROUP

-GWV not different from comparator groups, BUT:

-NDV fetal loss low: 30%↓ early fetal death (p = 0.004); 40% ↓ late fetal death (p=0.001)

- No diff in stillbirths NDV or GWV (and no dif vs England & Wales stillbirths)

Doyle P 2004. Int J Epi 33: 74-6

R 13

Doyle: UK Birth Outcomes: Self-report

Malformation	Adjusted OR
MEN (selected, significant or strong)	
Any	1.5 (1.3-1.7)
CNS	1.4 (0.9-2.3)
Genital	1.8 (1.0-3.0)
Urinary	1.6 (1.1-2.3)
- Renal	1.6 (1.0-2.7)
- Urinary tract	1.6 (1.0-2.4)
Musculoskeletal	1.8 (1.3-2.4)
- Other musculoskeletal	1.4 (1.0-2.1)
Other nonchromosomal	1.7 (1.0-3.0)
- Non-specified nonc'somal	3.5 (1.5-8.4)
Cranial neural crest	1.3 (1.0-1.7)
Metabolic & Single Gene defect	2.0 (0.9., 4.8)
WOMEN (all were nonsignificant)	
Any	1.7 (0.7-3.9)
C'somal	3.1 (0.3, 29.9)

R 14

Doyle: UK Birth Outcomes: Clinically Confirmed

Clinically confirmed Male birth outcomes: N = 330 GWV/ 196 NDV

(vs Self-report, prior page

Malformation	Adjusted OR
Any	1.3 (1.0-1.5)
Urinary	1.6 (1.0-2.5)
Musculoskeletal	1.5 (1.0-2.2)
- Other musculoskeletal	2.0 (1.0-4.1)

Note: OR show trend or effect of increase in 23 conditions, trend to decrease in 8. This is significantly different than expected by chance

Doyle P 2004. Int J Epi 33: 74-6

R 15

Objective Markers in III PGWV

R 16

Hippocampal Cell Loss by proton MR Spect

Question: Is there evidence of hippocampal neuron loss in ill PGWV?

Comment: The HC (hippocampus) is important in acquiring new memories.

Previous work by Sapolsky has shown that HC neuron loss occurs when energy demand exceeds supply AND there is stress.

Subjects: 10 ill PGWV. 5 well PGWV. 6 Vietnam veterans.

Outcome: NAA/Creatine ratio in HC (thought to signify neuron loss). Choline:creatine ratio comparing ill PGWV to controls.

ALSO: compare NAA:creatine in younger, age < median 44.2, vs older ∀

Result:

- NAA/Creatine ratio is significantly lower in ill GWV than either whole control group (p < 0.0057) or well PGWV controls (p =0.04)

- Younger group (< median -- all GWV) had lower value than older group; though neuron loss normally expected with age.

Menon PM 2004. Brain Res 1009: 189-194.

R 17

Cellular and Humoral Immune Abnormalities

Question: Do symptomatic PGWV have immune abnormalities?

Subjects: Delivered by R. Haines of U S Army req testing of 10th Army Unit GWV with sx. No medical records or health histories were forwarded.

Cases: 100 "symptomatic" GWV (no case def -- but reportedly with "common symptoms (joint pain, fatigue, headache, memory or concentration difficulties, sleep disturbance, and rash);

Controls: 100, including 50 asymptomatic vaccinated nondeployed & 50 healthy asymptomatic subjects for annual checkup. "Matched" on age & sex

Outcomes: CBC, chem, lipids, LFTs, T3, T4, TSH, AHA, RF, total IgA/IgG/IgM. Lc subset enumeration. NK cell cytotox assay. Lc mitogen assay. Myelin basic protein antibody. Striated & smooth muscle antibody. Immune complexes (IgG, IgM, IgA). Antibodies to EBV, CMV, HSV-1, HSV-2, HHV-6, VZV by ELISA.

Analysis: t-test of difference in mean. % outside expected range in cases vs controls

Vojdani & Thrasher. Environ Health Perspec 112: 840-846.

R 18

Cellular and Humoral Immune Abnormalities

Results	Patients vs controls	Comparison	p
CD3 T-cells	↑ct: 30% vs 5% had >79%	↑% outside expected range	< 0.05
CD-19 B cells	↑ct: 16 vs 11	% B cells	<0.001
CD4:CD8 ratio	↑ct: ratio 2.2 vs 1.3	% outside expected range	< 0.001
NK lytic activity	↓ct: 25 vs 37 lytic units	% with < 20 lytic units	< 0.01
Lc Mitogen stim*	PHA: GW > ctrl	stim index < 75% expected	< 0.01
		stim index > 125% expected	< 0.05
	PWM:	stim index < 75%	< 0.01
		stim index > 125% expected	NS
Autoantibodies	↑ct: 46 vs 28 mean igM	Mean ELISA units, IgM	< 0.001
		% with > 50 ELISA units	< 0.001
Abs to muscle	↑ct: 43 vs 16, IgG titers	IgG abs to smooth & striated mus	< 0.001
Immune complexes	↑ct: 51vs 35mEq/ml	Mean	< 0.001
		% with > 50mEq/L	< 0.01
Antibodies to viruses	Higher for each	Mean ELISA units (IgM, IgG)	< 0.001

∴ *Sometimes mean not different, but distribution is -- with more out of range at both ends

PHA = phytohemagglutinin. PWM = pokeweed mitogen.

Vojdani & Thrasher. Environ Health Perspec 112: 840-846.

R 19

More immune changes: Cytokine profiles

Question: Are there changes in cytokine, esp Th1/Th2 balance

Ss: UK symptomatic sGWV (40); well wGWV (80); sympt. Bosnia (20); sympt. Era (39)

Determination of "symptomatic" status: SF-36 phys fc < 72.2 (bottom decile)

Design: nested case-control study (drawn from UK sample)

Outcome: Cytokine profiles.

Results: Mixed Th0 pattern of immune system activation

Marker in sGWV	vs well GWV	vs sBEV*
Nonstimulated:		
IL4 ↑ (Th2)	< 0.05	<0.001
IL2 ↑ (Th1)	< 0.01	<0.001
IFN-gamma ↑ (Th1)	NS	< 0.05
Stimulated:		
IL10 ↑ (Tr)	< 0.0001	= 0.05

After control for age, gender, vaccination srtatus, antidepressant use, depressed mood, hx of atopic illness (some of which are outcomes & not *necessarily* appropriate to adjust), retain assoc of IL-2 and IFN-gamma, but not IL-4

*BEV = Bosnia & Era veterans

Skowera A et al 2004. J Clin Immunol 224: 66-73

R 20

More immune changes: Cytokine profiles

			Mean difference (95% CI)	
Index	SGWV	wGWV	Age/Gender	Age, Gender, Vacc, BDI, antidepressnt, hx atopy
Nonstimulated				
IL-4 (Th2)	2.98	2.4	0.04	0.33
IL-10 (Tr)	1.70	1.63	0.6	0.5
IFN-gamma (Th1)	1.85	1.39	0.03*	0.01*
IL-2 (Th1)	1.59	1.11	0.008*	0.001*
Stimulated (Polyclonally activated)				
IL-4 (Th2)	4.33	3.93	0.3	0.4
IL-10 (Tr)	5.08	3.20	<0.001*	<0.001*
IFN-gamma (Th1)	12.0	11.6	0.6	0.14
IL-2 (Th1)	18.4	16.4	0.4	0.2

∴ There are immune differences. Some show only under challenge conditions.
Skowera A et al 2004. J Clin Immunol 224: 66-73.

R 21

Squalene Antibodies

Question: Are there squalene abs in human serum and plasma?

Method: Adapt method to measure squalene abs from animals to humans. Test IgG and IgM.

Subjects: 3 human cohorts

A. 40: USAMRIID retirees: mean age 68 (most vaccinated)

B. 372: Frederick, MD: "Normal popn mean age 67 (most not vaccinated)

C. 299: Ft. Knox KY: Camp Memorial Blood Center, US Army Medical Departkment Activities (no addl info available)

USAMRIID = US Armacy Medical Research Institute of Infectious Diseases
Matyas G R et al 2004 J Immunological Methods 286:47-67

R 22

Squalene Antibodies

			Squalene Abs	
Cohort	AVA?	Age	IgG	IgM
US AMRIID	Most	68 (58-82)	7.5%	38%
Frederick	most not	67 (54-97)	15%	32%
Camp Memorial	presume not	Unknown	0%	19%
Difference			p<0.0001	p=0.0002

USAMRIID = US Armacy Medical Research Institute of Infectious Diseases
Matyas G R et al 2004 J Immunological Methods 286:47-67

R 23

Squalene Antibodies

Conclusion: Squalene abs are not rare; & not assoc with AVA (by this assay)

Support for safety of squalene adjuvants:

SQE-containing emulsion as part of flu vaccine licensed in Italy "has been given without adverse effects to hundreds of thousands of people"

"Numerous other clinical trials for influenza [and other] have used SQE emulsions. Vaccine reactions were typically mild. However, some moderate to severe reactions, which can be attributed to other adjuvants in the formulations, were reported."

Problem:

-Without active surveillance it was also thought AVA didn't have problems

-Attribution to other adjuvants is problematic; oil based adjuvants are unapproved in the US for a reason

-Did not assess relation of squalene abs to health

R 24

Squalene Antibodies

Issues:

- Cross reactivity of the assay?

-Age-relevant rates? Low rate in blood samples suggests may be low in younger age; relation to health not known BUT can't r/o contribution to health problems in the older population

-AVA recipients will have gotten AVA prior to 1990 inoculation program -- so comparability to age matched controls is of uncertain relevance

Matyas GR et al 2004 J Immunological Methods 286:47-67

R 25

Squalene Antibodies

Remaining Concerns:

It remains unconfirmed & unrepudiated whether squalene abs are more common in AVA recipients; or in ill PGWV.

It is unknown if there is a relation of squalene abs to health outcomes in the group studied

These authors own data from animals show that chol-abs have health consequences: *a fortiori* this could be expected for squalene abs

Additional study remains needed.

Matyas GR et al 2004 J Immunological Methods 286:47-67

R 26

Immune Effects of AChEi and chemical mixtures: Animal Studies

R 27

PB alters immune function in mice

- Subjects: Adult 7-8wk female B6C3F1 mice: 6-7 per group
- PB dosing/route/duration: 0, 1, 5, 10 or 20mg/kg/d po for 14 days 0 = distilled water control. (GW dose ~1.3mg/kg for 70kg man) Dose did not cause acute symptoms. The 1mg/kg/d PB dose did not alter BChE or AChE activity in mice
- Time to assessment:After the 14 ds (persistence not assessed)
- Outcomes: lymphoproliferation NK cell activity, SRBC-specific antibody plaque forming cell response, thymus and spleen weight and cellularity, thymic and splenic CD4:CD8 Lc populations
- No effect: spleen/thymus weight; spleen cellularity; Lc prolif resps; NK cell activity, wbc count, differential, RBC indices
- Hi dose effect: ↓ thymus cellularity, ↓ CD4:CD8, ↓ CD4-/CD8- cell types (20mg/kg/d; and in last case 10mg/kg/d)
- Lo dose effect: ↓ 1° IgM ab response to T-cell dependent antigen (includes 1mg/kg/d dose) (Humoral immunity)
- Peden-Adams MM et al 2004. Immunopharmacology and Immunotoxicology; 26:1-15. Pyridostigmine Bromide (PYR) alters

R 28

PB ↓ IgM abs in rats (humoral immunity)

- Conclusion: "It is clear that PYR suppresses humoral immunity... at treatment levels comparable to doses reported for military personnel"
- Comment: cite convergent evidence: also ↓ humoral immunity (SRBC-specific IgM production) by some other carbamates (ethylcarbamate; carbofuran), but reportedly not another (methyl carbamate).
- Some variability issues
- Aldicarb also ↓ ab production by one study, not another
- Depends on route of exposure? -- inhale carbaryl -> Response; oral/dermal -> no rsponse.
- Question: if there is short-term suppression, is there long-term upregulation (or suppression, or nothing) of ab production?

• Peden-Adams MM et al 2004. Immunopharmacology and Immunotoxicology 26: 1-15. Pyridostigmine bromide (PYR) alters immune function in B6C3F1 mice

R 29

Single or repeated low-level sarin on immune functions of inbred BALB/c mice)

•Subjects: Balb/C mice

•Exposure: sarin @ asymptomatic dose, 0.8µg/L in inhalation chamber, till 20-30% AChE inhibition. Vs air

•Timing of assessment: 1 week later

•Outcomes: Many in lungs, blood, spleen

•Findings: Indep of # exposures: ↓c CD3 cells in lungs. ↑ NK cell activity; ↓ lympoprolif & ↓ abil of paritoneal and alveolar macrophages to engulf microbes regardless of mitogen (former) or sarin concentration or # exposures.

•Depend on # exposures: N-oxide produx by peritoneal macrophages: ↓ after 1 exposure; vs ↑ after repeated low level exposures.

•Conclusion: Low level asympt sarin produces sustained changes in immune fcn even if single dose. Repeated doses may produce different effects

•Kanna J et al 2004. Basic and Clinical Pharmacol 94: 139-143. The influence of single or repeated low-level sarin exposure on immune functions of inbred BALB/c mice

R 30

Pesticide mixtures potentiate toxicity in murine thymocytes

Subjects: Mouse thymocytes (in vitro) from 8-12 week male C57BL/6 mice
Exposures: Lindane (L); malathion (M); permethrin (P) (separately or in mixtures of two)
Outcome:
-Apoptosis & necrotic cell death with 7-AAD staining

Result: More than additive:	**%apopt**	**%late apo/necro**
-L, M, both	**10,13,30**	**6,9,30**
-L, P, both	**8,13,17**	**9,16,36**

-P < 0.05 mixture vs sum of individual, all 4 cells

Olgun S et al 2004 Toxicology 196:181-195. Pesticide mixtures potentiate the toxicity in murine thymocytes

R 31

Question from Yesterday: Visceral Pain

R 32

Enhanced sensitivity to pain

Subjects: 12 GWV with abd pain & diarrhea s/p neg workup developed during PGW. 7 civilian & 5 veteran controls.

Exposure: a) rectal distension (35 & 55mm) & b) hot water R foot & hand (35° & 47°C x30sec)

Outcome: visual analog scale pain intensity & unpleasantness, 2 trials each

Finding: $p < 0.001$ higher rating of pain intensity and pain unpleasantness for both exposures

Conclusion: visceral hypersensitivity in PGWV with abd pain/diarrhea sim to that shown with irritable bowel. Also: cutaneous hypersensitivity "and higher levels of anxiety and somatic focus accounting for these differences in pain reporting" (no, attending them!)

Dunphy RC et al 2003, Pain 102: 79-85.

R 33

Backup Slide: Source of Information for Smith et al

R 34

Smith 2004: Registries vs Hospitalizations

Demographics: by Defense Manpower Data Center: gender, marital, age, race/ethnicity, home state, prewar hospitalization index from 7-31-89 to 8-1-90(the yr B4 war)

Exposures:

- Khamisiyah deployment (updated model);

- Oil well fire smoke from US Army Center for Health Promotion and Preventive Medicine with Natl Oceanic and Atmospheric Administration/Air Resources Lab estimate of 2hr unit exposures (meteorological and dispersion models overlayed onto troop location)

- Documented BT or anthrax vaccine

Deployment/Military: by Defense Manpower Data Center: Milit service branch, DoD occup specialty (of 10 major groups), milit pay grade, date of separation from milit, GW deployment hx, dates of entry and exit into theater

R 35