

**MICROBES AS WEAPONS:  
IS THERE A LINE IN THE SAND?**

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# **A REMINDER ABOUT 'DUAL USE' TECHNOLOGY**

**PICTURE OF CAR**

**THE CIVILIAN PASSENGER SEDAN IS THE MOST  
EFFECTIVE WEAPON OF WAR IN IRAQ**

# WEAPON

1 : something (as a club, knife, or gun) used to injure, defeat, or destroy

2 : a means of contending against another

## WEAPON TYPES

KINETIC

RADIOLOGIC

NUCLEAR

CHEMICAL

ELECTRONIC

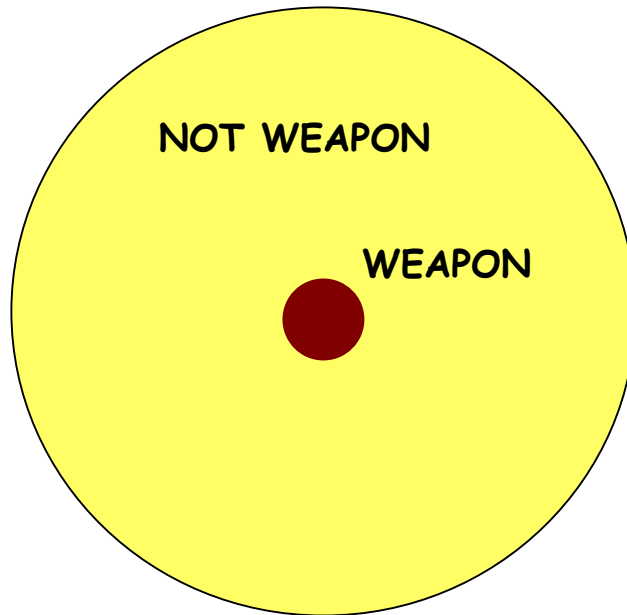
INFORMATIC

BIOLOGICAL

TYPES AND VARIETY  
LIMITED BY PHYSICAL LAWS

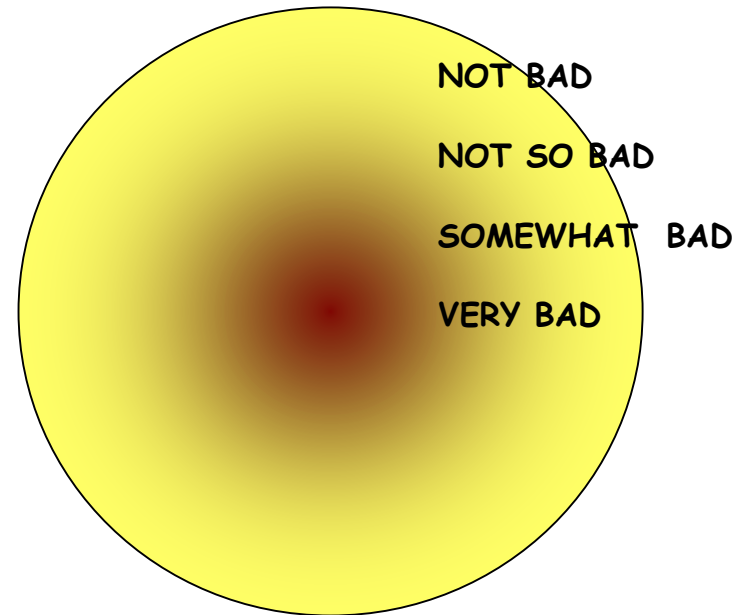
VARIETY IS ENORMOUS  
EFFICACY %f(host, microbe)  
NOT UNDERSTOOD

# VISIONS OF MICROBES AS WEAPONS



**TUNNEL  
VISION**

**OUTCOME: SELECT AGENT LIST**



**TUNNEL-MYOPIC  
VISION**

**MULTIPLE LISTS  
A, B, C CATEGORIES**

# IS THIS A WEAPON?



*Saccharomyces cerevisiae*

JOURNAL OF CLINICAL MICROBIOLOGY, June 2004, p. 2840–2842  
0095-1137/04/\$08.00+0 DOI: 10.1128/JCM.42.6.2840-2842.2004  
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Vol. 42, No. 6

## Use of Paraffin-Embedded Tissue for Identification of *Saccharomyces cerevisiae* in a Baker's Lung Nodule by Fungal PCR and Nucleotide Sequencing

Ping Ren,<sup>1</sup> Sundara Sridhar,<sup>2</sup> and Vishnu Chaturvedi<sup>1,3\*</sup>

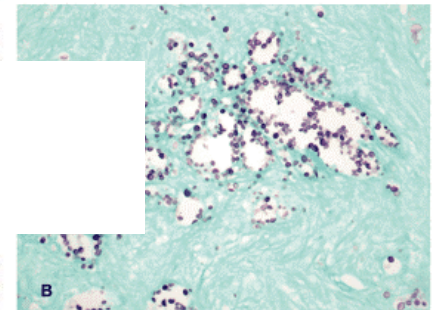
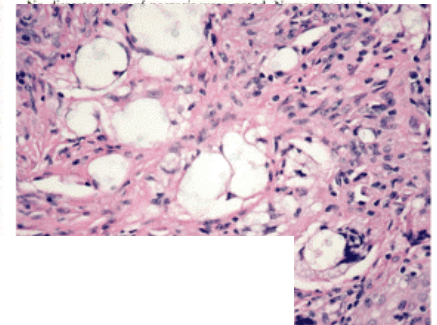
*Mycology Laboratory, Wadsworth Center, New York State Department of Health,<sup>1</sup> and Department of Biomedical Sciences,<sup>2</sup> SUNY School of Public Health, University at Albany, Albany, and Department of Pathology, Coney Island Hospital, Brooklyn,<sup>3</sup> New York*

Received 17 November 2003/Returned for modification 16 December 2003/Accepted 4 March 2004

**A 40-year-old healthy male employed in a bakery presented with a single lung nodule and underwent investigations to rule out pulmonary carcinoma. Biopsy was positive for yeast cells, which did not match common fungal pathogens. PCR assay of paraffin-embedded tissue and nucleotide sequencing with ribosomal ITS1-ITS2 universal primers revealed the presence of *Saccharomyces cerevisiae*.**

Identification of fungal pathogens in histological sections frequently requires application of specialized stains (6). Many pathogenic yeasts appear as budding, rounded cells without any characteristic tissue forms (9). This situation is alleviated in instances in which the incriminating fungus can be alleviated in culture. However, tissue specimens are not always available for culture. Recently, the application of PCR and nucleotide sequencing has been extended for identification of pathogenic fungi in histological sections. The paraffin-embedded tissue is used as a source of template DNA for a PCR assay with universal fungal ribosomal gene primers and/or a nested PCR assay with pathogen-specific primers, and the amplicons are then analyzed by restriction fragment length polymorphism and/or nucleotide sequencing for confirmation of fungal identity (2–5, 8, 11, 13). This approach is very promising in diagnostics, causal preservation, male the lung care were containing an unincriminated specimen of yeast and nucleotide sequencing.

A 40-year-old healthy male was referred to the surgeon at Coney Island Hospital for a lung nodule discovered during a routine chest X-ray done as part of an annual physical examination. The medical illness A 0.7-cm-dian lung parenchyma cated from the any calcificatic inflammatory m with a modera posed of an e



\* Corresponding author. Mailing address: Mycology Laboratory, Wadsworth Center, New York State Department of Health, SUNY School of Public Health, University at Albany, Albany, NY 12201-2002. Phone: (518) 453-6412. Fax: (518) 486-7811. E-mail: vishnu@wadsworth.org.

# YOGURT – IS THERE A WEAPON HERE?



June 2001, Volume 21, Number 4, Pages 258-260

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Clinical Perinatal/Neonatal Case Presentation

## ***Lactobacillus acidophilus* Sepsis in a**

Charles Thompson MD<sup>1</sup>, Yvette S McCarter PhD<sup>2</sup>, Peter J Krause MD<sup>3</sup> and Victor C Herson

***L. acidophilus***  
**FOOD?**  
**MICROBE?**  
**COMMENSAL?**  
**OPPORTUNIST?**  
**PATHOGEN?**  
**WEAPON?**

# SELECT LIST ASSIGNMENT

## HISTORICAL USE: PRIOR USE BY MILITARY?

e.g. *Y. pestis*, *B. anthracis*

## HISTORY OF CAUSING PANDEMICS

e.g. Variola major

## 'JUDGEMENT' CALLS

e.g. Assessment of deliverability, weaponization potential, etc

### MANY ISSUES

1. UNSUITABLE FOR NEW AGENTS
2. MANY MICROBES EXCLUDED  
e.g. INFLUENZA VIRUS  
*NEISSERIA MENINGITIDIS*  
*GROUP A STREPTOCOCCUS*
3. NOT BASED ON MICROBIAL PATHOGENESIS
4. FIXED IN TIME
5. SPECIES BASED (NET IS TOO BROAD)
6. DOES IT MAKES US SAFER OR MORE VULNERABLE?

**WANTED: A SYSTEM TO DETERMINE THE WEAPON POTENTIAL OF A MICROBE GROUNDED ON THE PRINCIPLES OF MICROBIAL PATHOGENESIS**

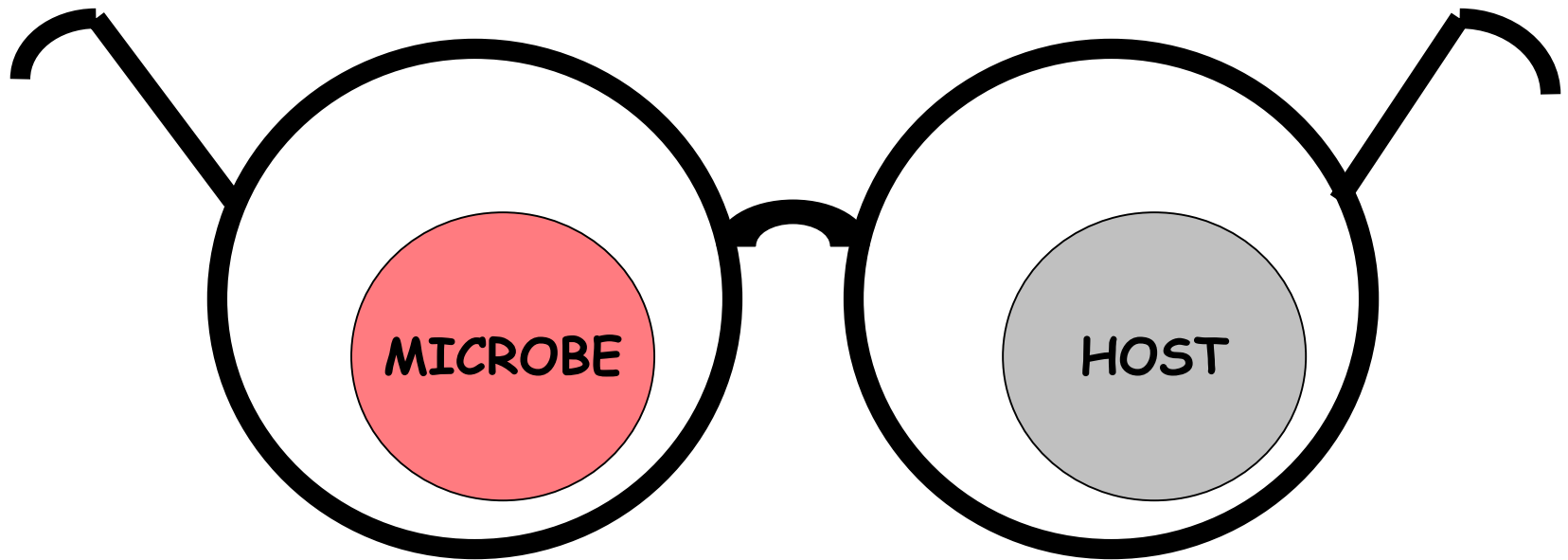
**ASSUMPTIONS:**

- 1. EACH MICROBES HAS SOME WEAPON POTENTIAL**
- 2. WEAPON POTENTIAL IS A FUNCTION OF VARIABLES THAT DETERMINE MICROBIAL PATHOGENESIS**
- 3. WEAPON POTENTIAL IS QUANTIFIABLE**

**REQUIREMENT: A THEORY OF MICROBIAL PATHOGENESIS THAT TAKES INTO ACCOUNT THE CONTRIBUTION OF THE MICROBE AND THE HOST.**



# FOR TUNNEL AND TUNNEL-MYOPIA VISUAL DISTURBANCES...

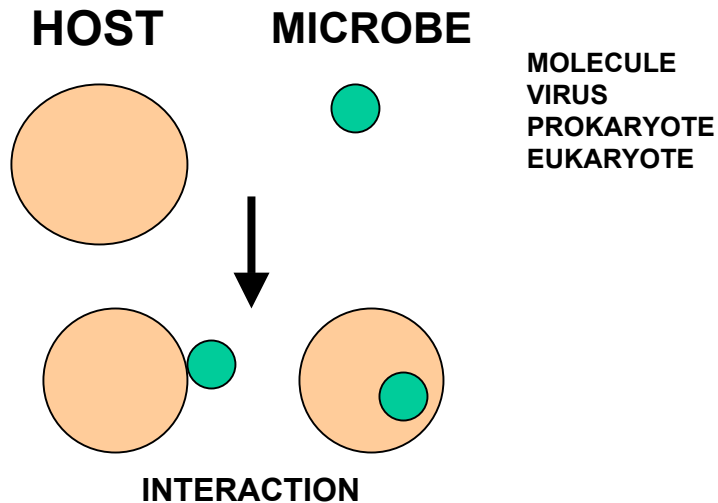


**PRESCRIPTION: DAMAGE-RESPONSE FRAMEWORK  
(AND ITS IMPLICATIONS)**

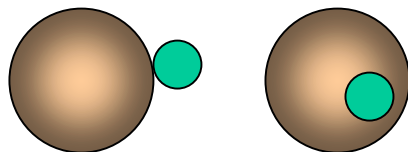
# DAMAGE-RESPONSE FRAMEWORK

## BASIC TENETS (OBVIOUS AND INCONTROVERTIBLE)

### 1. TWO ENTITIES



### 2. RELEVANT OUTCOME = HOST DAMAGE



### 3. DAMAGE CAN COME FROM HOST, MICROBE OR BOTH

# DAMAGE-RESPONSE FRAMEWORK

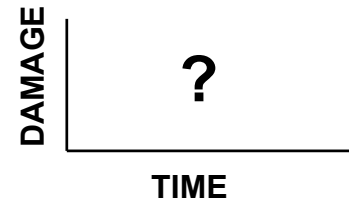
## TYPE OF HOST-MICROBE INTERACTION

**DAMAGE = f(HOST RESPONSE)**

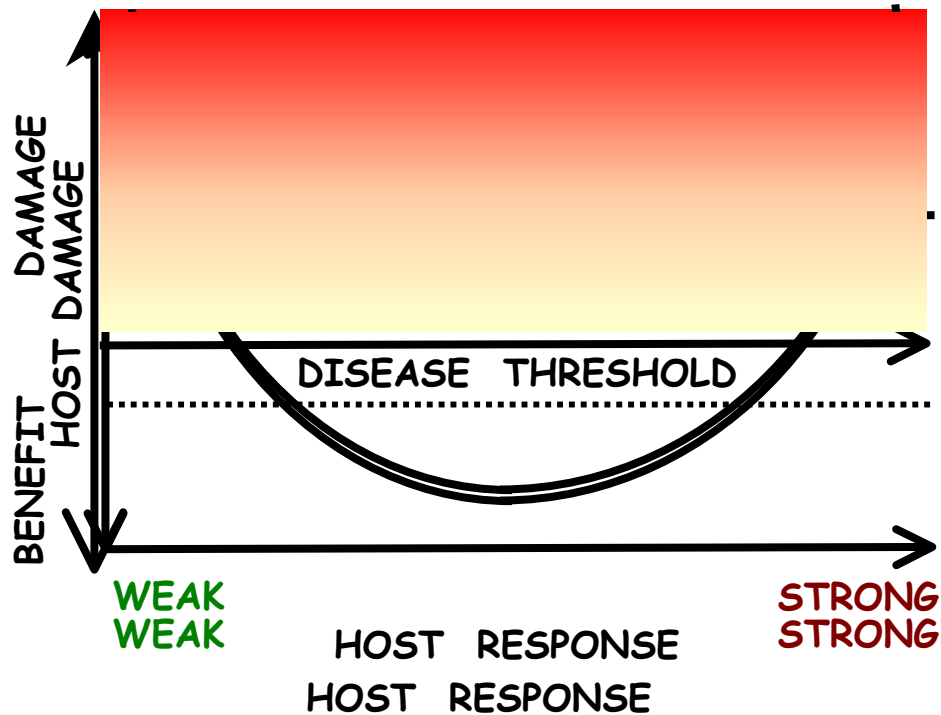


## STATE OF HOST-MICROBE INTERACTION

**DAMAGE = f(TIME)**



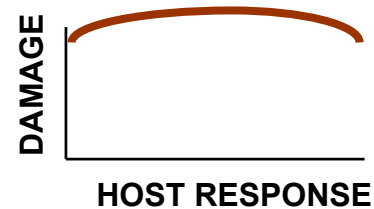
# BASIC RELATIONSHIP FOR 'DAMAGE-RESPONSE FRAMEWORK'



# BIOWEAPONS: THE VIEW FROM THE 'DAMAGE-RESPONSE FRAMEWORK'

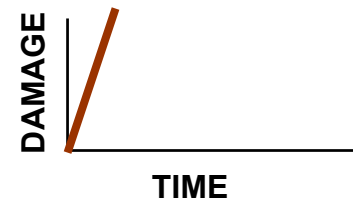
## TYPE OF HOST-MICROBE INTERACTION

$$\text{DAMAGE} = f(\text{HOST RESPONSE})$$



## STATE OF HOST-MICROBE INTERACTION

$$\text{DAMAGE} = f(\text{TIME})$$



BIOLOGICAL WEAPON =  $\uparrow$  DAMAGE  $\downarrow$  TIME'

# A WEAPON POTENTIAL RELATIONSHIP

$$\text{WEAPON POTENTIAL} = \left[ \begin{array}{c} \text{BASIC MICROBIAL} \\ \text{PATHOGENESIS} \\ \text{PARAMETER} \end{array} \right] \left[ \begin{array}{c} \text{TECHNOLOGICAL} \\ \text{CAPACITY OF} \\ \text{AGGRESSOR} \end{array} \right] \left[ \begin{array}{c} \text{HUMAN} \\ \text{NATURE} \\ \text{(PANIC...)} \end{array} \right]$$

**f(VIRULENCE)**

**AMPLIFICATION FACTORS**

$$\text{WEAPON POTENTIAL} = \left[ \begin{array}{c} \text{BASIC MICROBIAL} \\ \text{PATHOGENESIS} \\ \text{PARAMETER} \end{array} \right] \left[ \begin{array}{c} \text{DELIVERABILITY} \\ \text{'D'} \end{array} \right] \left[ \begin{array}{c} \text{TERROR} \\ \text{'X'} \end{array} \right]$$

$$\text{WEAPON POTENTIAL} = \left[ \begin{array}{c} \uparrow \text{DAMAGE} \\ \downarrow \text{TIME} \end{array} \right] \left[ \begin{array}{c} \text{D} = 1.0 \end{array} \right] \left[ \begin{array}{c} \text{X} = 1.0 \end{array} \right]$$

# VIRULENCE

**DEFINED AS THE RELATIVE CAPACITY OF A MICROBE TO CAUSE DAMAGE IN A HOST** [Casadevall & Pirofski, Infect.Immun 1999; Casadevall & Pirofski, Nature Microbiol. Rev. 2003]

**A NECESSARY FOR BUT NOT SUFFICIENT CONDITION FOR ASSESSING WEAPON POTENTIAL**

**FOR CALCULATING WEAPON POTENTIAL NEED A QUANTITATIVE DEFINITION FOR VIRULENCE**

$$V_{\text{WEAPON POTENTIAL}} = \frac{\text{FRACTION SYMPTOMATIC}}{\text{INOCULUM}}$$

# WEAPON POTENTIAL

DEPENDS ON VIRULENCE BUT INFLUENCED BY  
COMMUNICABILITY ( $1 < C < 100$ )  
STABILITY ( $0 < S < 1.0$ )  
TIME (IN DAYS)

$$WP = \frac{V_{WP} CS}{T} = \frac{F_{SI} CS}{IT}$$

WP = WEAPON POTENTIAL

C = COMMUNICABILITY

S = STABILITY

T = TIME

I = INNOCULUM ( $LD_{50}$ ,  $LD_{10}$ ...)

BASIC RELATIONSHIP CAN BE MODIFIED BY TERROR  
POTENTIAL (X) AND DELIVERABILITY (D) PARAMETERS



## MAXIMUM WEAPON POTENTIAL

### SET:

COMMUNICABILITY ( $1 < C < 100$ ) = 100

STABILITY ( $0 < S < 1.0$ ) = 1.0

TIME (IN DAYS) = 1.0

FRACTION SYMPTOMATIC = 1.0

INOCULUM = 1.0

$$WP = \frac{V_{WP} CS}{T} = \frac{F_{SI} CS}{IT}$$

$$WP_{MAX} = (1.0)(100)(1.0)/(1.0)(1.0) = 100$$

# SAMPLE CALCULATION FOR *B. ANTHRACIS*

## FOR THE FRACTION SYMPTOMATIC ( $F_{SI}$ )

SVERDLOVSK ESTIMATE: 500 CASES AMONG 59,000 POTENTIALLY EXPOSED

= 0.008

BRENTWOOD MAIL FACILITY ESTIMATE: 2 CASES AMONG 2446 POTENTIALLY EXPOSED

= 0.0008

## FOR THE INOCULUM – EXTRAPOLATIONS FOR MONKEYS

$LD_{50}$  = 8000 SPORES

$LD_{10}$  = 50 SPORES

$LD_1$  = 1 SPORE

COMMUNICABILITY = NONE ( $C = 1.0$ )

STABILITY = 1.0 (EXTREMELY HARDY)

TIME TO DISEASE = 14.2 d (Sverdlovsk data)

$$WP = (0.008)(1/1.0)(1.0)(1.0)(1/14.2) = 5.6 \times 10^{-4}$$

# WP OF SEVERAL MICROBES

MICROBE	CLASS	V WP		C	S	T	WP
		FRACTION SYMPTOMATIC	INOCULUM				
<i>B.anthraxis</i>	A	0.008	1	1.0	1.0	14.2	5.6 x 10 <sup>-4</sup>
VARIOLA	A	0.76	100	90	0.25	10	1.7 x 10 <sup>-2</sup>
HIV	NOT IN LIST	0.99	1000	5	0.25	2920	4.2 x 10 <sup>-7</sup>
HIV	NOT IN LIST	0.99	1000	5	0.25	1	1.2 x 10 <sup>-3</sup>
<i>C. ALBICANS</i>	NOT IN LIST	0.29	7.9 x 10 <sup>8</sup>	5	0.75	5	2.7 x 10 <sup>-10</sup>
THEORETICAL MAXIMUM	?	1	1	100	1	1	100

IF TIME TAKEN INTO ACCOUNT:

VARIOLA > *B. anthracis* > HIV >> *C. albicans*

IF TIME IS NOT A CONSIDERATION

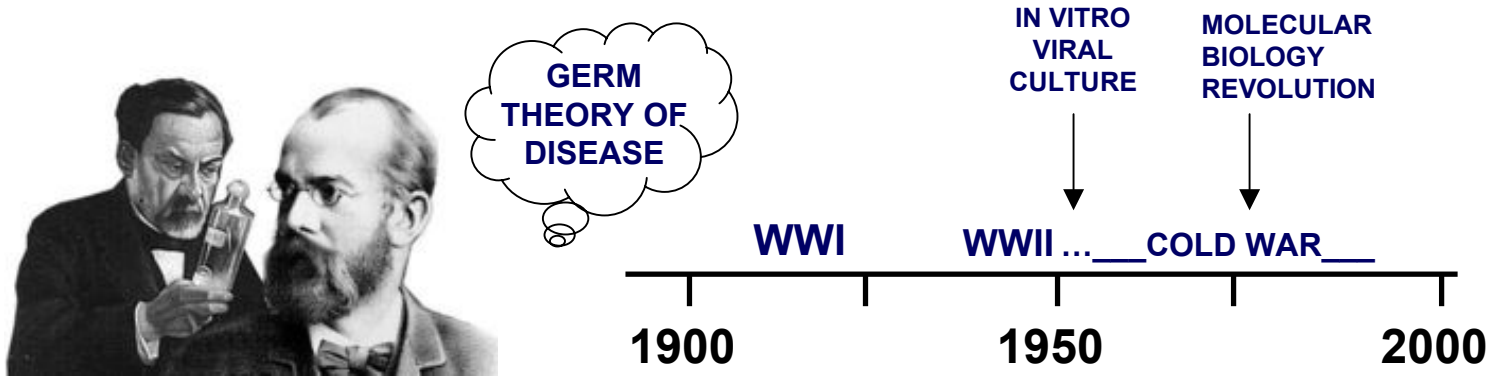
VARIOLA > HIV > *B. anthracis* >> *C. albicans*

# APPLICATIONS

## ESTIMATE WP OF NEW MICROBES...CONSIDER SARS

MICROBE	CLASS	V WP		C	S	T	WP
		FRACTION SYMPTOMATIC	INOCULUM				
<i>B.anthraxis</i>	A	0.008	1	1.0	1.0	14.2	5.6 x 10 <sup>-4</sup>
SARS VIRUS	NOT IN LIST	0.18	1000?	50	0.25	5.9	3.5 X 10 <sup>-4</sup>
VARIOLA	A	0.76	100	90	0.25	10	1.7 x 10 <sup>-2</sup>

# DELIVERABILITY AND IMMUNITY CHANGE WEAPON POTENTIAL OF MICROBE OVER TIME



PASTEUR & KOCH c1890

CLASS A AGENT	1890	1945	2004	2020
<i>Bacillus anthracis</i>	NO	YES	YES	?
<i>Yersinia pestis</i>	YES	YES	YES	?
Variola major	YES	NO	YES	?
<i>Francisella</i> spp.	NO	NO	YES	?
Hemorrhagic fever viruses	NO	NO	YES	?
<i>Coxiella</i> spp.	NO	YES	YES	?
POLIO VIRUS	NO	YES	NO	YES?*
MEASLES VIRUS	NO	YES	NO	YES?*

\*ASSUMING GLOBAL ERADICATION AND DISCONTINUATION OF VACCINATION

# CLOSING PERSONAL THOUGHTS

**ALL PATHOGENIC MICROBES ARE POTENTIAL WEAPONS**

**WP – A FUNCTION OF SUSCEPTIBILITY & INNOCULA  
DECISION OR WHERE TO DRAW THE LINE IS ‘POLITICAL’**

**PLACING OF MICROBES INTO THE VARIOUS ‘LISTS’ MAY ITSELF  
BE ACT OF ‘DUAL USE’: PROTECT AND/OR HARM HUMANITY?**

**THOUGHT EXPERIMENT: WOULD SARS HAVE BEEN  
CONTAINED IN <6 MONTHS IF REGULATIONS ON SHIPPING  
AGENTS, SELECT AGENT CLASSIFICATION, ETC BEEN IN PLACE  
FOR HUMAN CORONAVIRUSES OR NEW VIRAL ISOLATES?**

**WP OF A MICROBE CHANGES WITH TIME**

**PUBLIC HEALTH SUCCESSES CREATE WEAPONS (eg smallpox)  
ARE MEALES AND POLIO VIRUSES WEAPONS OF TOMORROW?**

**THE LINE IN THE SAND CANNOT BE FIXED FOR THE  
SANDS SHIFT WITH TIME...NEED SMARTER SYSTEMS IN PLACE**