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

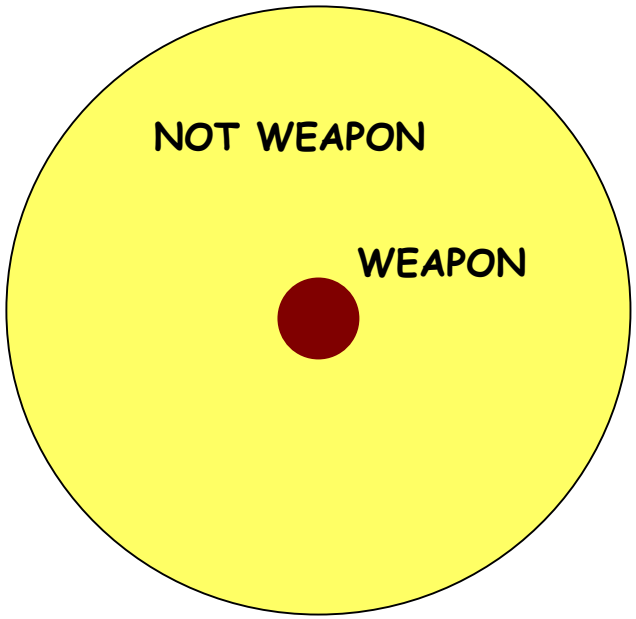
WEAPON TYPES

KINETIC
RADIOLOGIC
NUCLEAR
CHEMICAL
ELECTRONIC
INFORMATIC
BIOLOGICAL

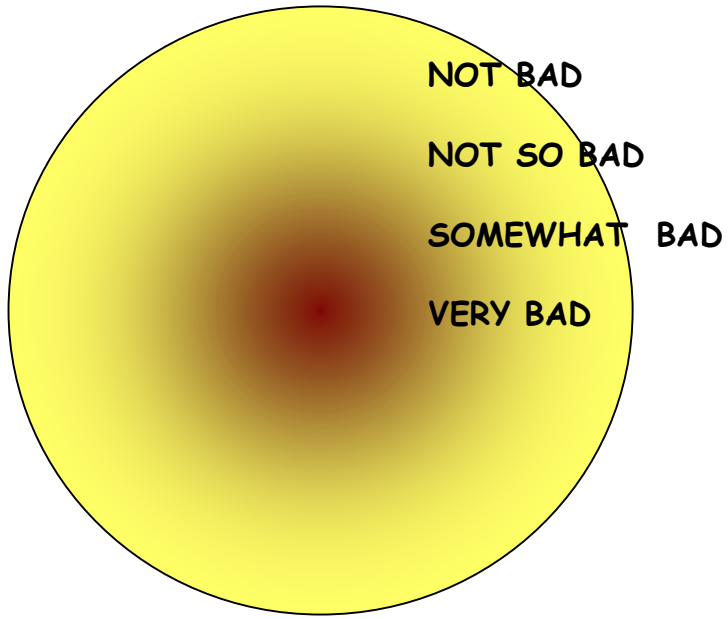
TYPES AND VARIETY
LIMITED BY PHYSICAL LAWS

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

VISIONS OF MICROBES AS WEAPONS



TUNNEL VISION



TUNNEL-MYOPIC VISION

OUTCOME: SELECT AGENT LIST

MULTIPLE LISTS
A, B, C CATEGORIES

IS THIS A WEAPON?

Saccharomyces cerevisiae

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0095-1137/04/$08.00+0 DOI: 10.1128/JCM.42.6.2840–2842.2004

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

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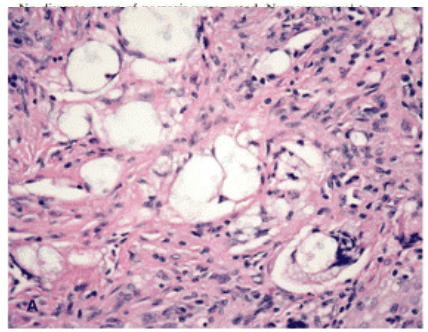
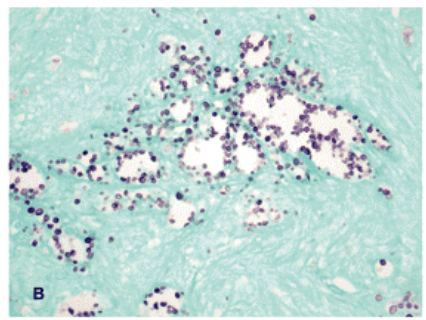
A 40-year-old healthy male employed in a bakery presented with a single lung nodule and underwent investigations to rule out pulmonary carcinoma. Biopsy was positive for yeast cells, which did not match common fungal pathogens. PCR assay of paraffin-embedded tissue and nucleotide sequencing with ribosomal *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 isolated 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, as it could lead to conclusive identification of the causal pathogen independently of histological or culture observations. We describe a case of a lung nodule in a healthy male that proved to be histologically negative for suspected lung carcinoma and instead revealed budding yeast cells, which were confirmed as *Saccharomyces cerevisiae* by PCR 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 patient was a nonsmoker with no history of any medical illness. A wedge resection of the lung was performed. A 0.7-cm-diameter solid grey-tan nodule was present in the lung parenchyma. The edges of the lesion were sharply demarcated from the surrounding normal lung parenchyma without any calcification. Histopathologic examination revealed an inflammatory mass composed of a background of fibrotic tissue with a moderately dense population of inflammatory cells composed of an equal admixture of histiocytes and lymphocytes.





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YOGURT – IS THERE A WEAPON HERE?

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

Clinical Perinatal/Neonatal Case Presentation

Lactobacillus acidophilus Sepsis in a

Charles Thompson MD[1], Yvette S McCarter PhD[2], Peter J Krause MD[3] 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:

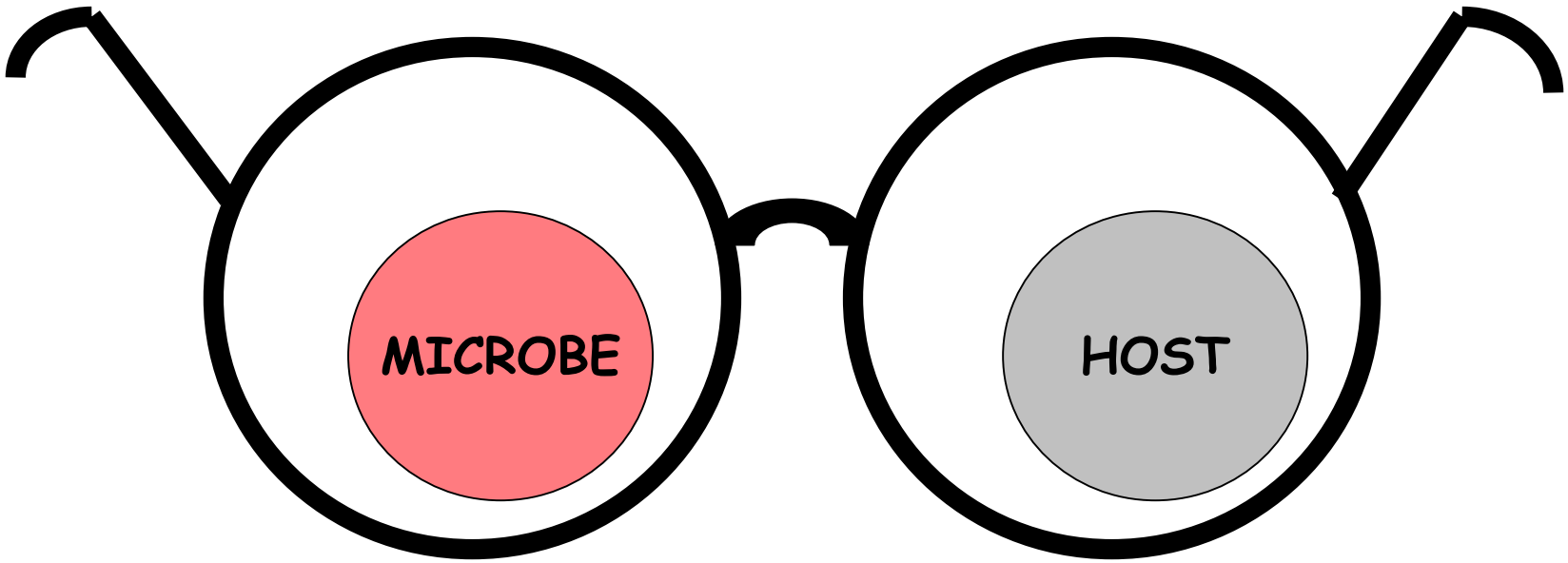
1. EACH MICROBES HAS SOME WEAPON POTENTIAL

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.

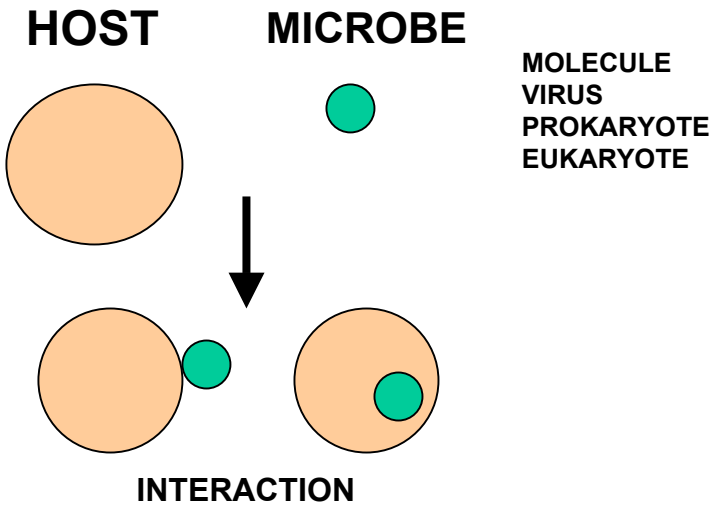
FOR TUNNEL AND TUNNEL-MYOPIA VISUAL DISTURBANCES…



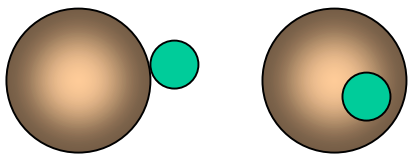
PRESCRIPTION: DAMAGE-RESPONSE FRAMEWORK (AND ITS IMPLICATIONS)

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

Casadevall & Pirofski, Nature Micro Rev. 2003

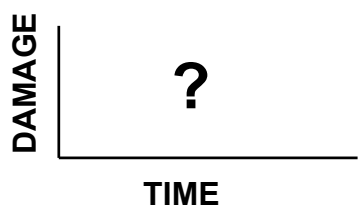
DAMAGE-RESPONSE FRAMEWORK

TYPE OF HOST-MICROBE INTERACTION

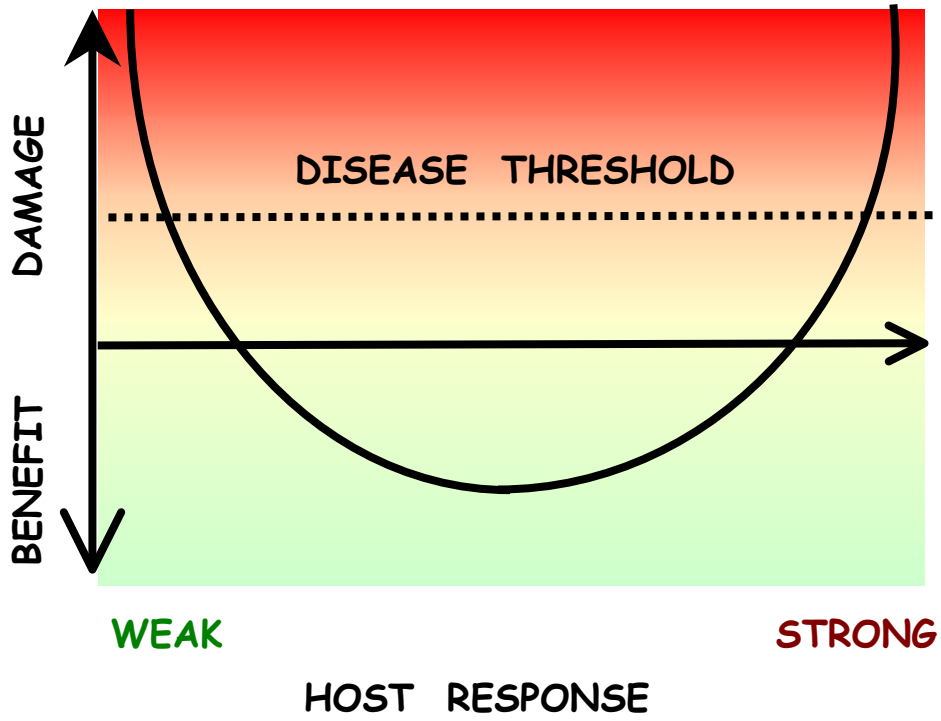
DAMAGE = f(HOST RESPONSE)

STATE OF HOST-MICROBE INTERACTION

DAMAGE = f(TIME)



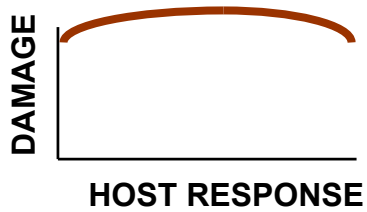
BASIC RELATIONSHIP FOR 'DAMAGE-RESPONSE FRAMEWORK'



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

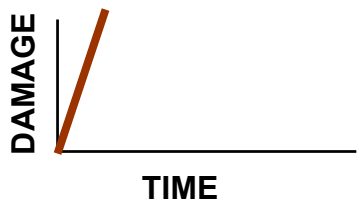
TYPE OF HOST-MICROBE INTERACTION

DAMAGE = f(HOST RESPONSE)



STATE OF HOST-MICROBE INTERACTION

DAMAGE = f(TIME)



BIOLOGICAL WEAPON = ↑ DAMAGE ↓ TIME'

A WEAPON POTENTIAL RELATIONSHIP

WEAPON POTENTIAL = [BASIC MICROBIAL PATHOGENESIS PARAMETER] [TECHNOLOGICAL CAPACITY OF AGGRESSOR] [HUMAN NATURE (PANIC…)]

f(VIRULENCE) AMPLIFICATION FACTORS

WEAPON POTENTIAL = [BASIC MICROBIAL PATHOGENESIS PARAMETER] [DELIVERABILITY 'D'] [TERROR 'X']

WEAPON POTENTIAL = [↑ DAMAGE ↓ TIME] [D = 1.0] [X = 1.0]

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

Casadevall & Pirofski, Trends in Microbiology 2004 (June)

MAXIMUN 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				
B.anthracis	A	0.008	1	1.0	1.0	14.2	5.6 x 10-4
VARIOLA	A	0.76	100	90	0.25	10	1.7 x 10-2
HIV	NOT IN LIST	0.99	1000	5	0.25	2920	4.2 x 10-7
HIV	NOT IN LIST	0.99	1000	5	0.25	1	1.2 x 10-3
C. ALBICANS	NOT IN LIST	0.29	7.9×10^8	5	0.75	5	2.7 x 10-10
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

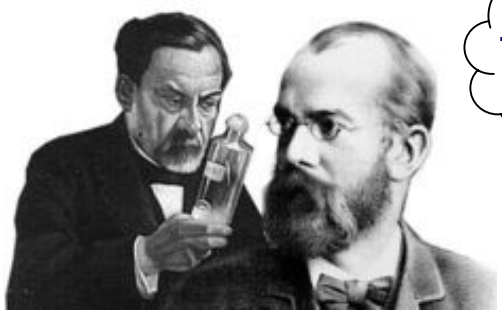
VARIOLA > HIV > B. *anthracis* >> *C. albicans*

APPLICATIONS

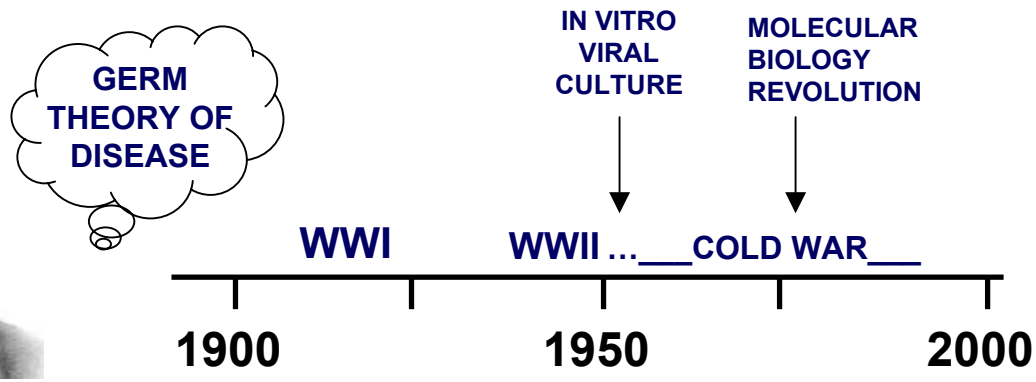
ESTIMATE WP OF NEW MICROBES…CONSIDER SARS

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

DELIVERABILITY AND IMMUNITY CHANGE WEAPON POTENTIAL OF MICROBE OVER TIME



PASTEUR & KOCH c1890



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

*ASSUMING GLOBAL ERADICATION AND DISCONTINULATION 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?

<u>THOUGHT EXPERIMENT</u>: 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