


Review of April 2009 Educational Bioterrorism Proficiency Exercise Agent

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WSLH WISCONSIN STATE LABORATORY OF HYGIENE

Review of April 2009 Educational Bioterrorism Proficiency Exercise Agent

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Objectives

- Describe the epidemiology, clinical manifestations, and treatment of *Brucella spp.* infections.
- Discuss the public health and potential biothreat aspects of *Brucella spp.* infections.
- Describe the laboratory detection and identification methods for *Brucella spp.*

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Overview of WSLH September 2008 Educational Bioterrorism Proficiency Exercise Results

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Overview of WSLH Bioterrorism Proficiency Exercise

- Two samples sent to 115 Wisconsin laboratories
 - 104 reported test results (90%)
 - 11 did not report results
- Sample BPE 08-2-1:
Bordetella bronchiseptica
- Sample BPE 08-2-2:
Aggregatibacter aphrophilus
- Samples intended to simulate *Brucella spp.* in “rule out” testing

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Results of “Rule Out” Tests

Test:	Sample 1: Expected Result	% of 104 Reporting Labs	Sample 2: Expected Result	% of 104 Reporting Labs
Gram Stain	GNR	93.3%	GNR	64.4%
Other Responses:				
Gram Stain	GNCB	2.9%	GNCB	28.8%
Gram Stain	GVR	2.9%	GPR	2.9%
Gram Stain	NOS	1.0%	GVR	1.9%
Gram Stain			NOS	1.9%

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Results of "Rule Out" Tests (continued)

Test	Sample 1: Expected Result	% Reporting Labs	Sample 2: Expected Result	% Reporting Labs
α-hemolysis	Negative	97.5% (of 81)	Negative	60.2% (of 83)
β-hemolysis	Negative	97.6% (of 82)	Negative	100% (of 85)
Catalase	Positive	98.8% (of 84)	Negative	94.3% (of 88)
Indole	Negative	100% (of 75)	Negative	100% (of 74)
Oxidase	Positive	92.2% (of 88)	Negative	96.6% (of 88)
Urease	Positive	96.6% (of 58)	Negative	98.2% (of 56)
Motility	Positive	37.0% (of 27)	Negative	96.3% (of 27)
Growth on Mac/EMB	Positive	96.5% (of 86)	Negative	98.9% (of 88)

Results of "Rule Out" Tests (continued)

Sample 1	Response* %(total #) Reporting Labs
Laboratory Responses: Were you able to "Rule Out"?	
Bacillus anthracis	YES* / 94.9% (of 99)
Brucella abortus	YES* / 64.0% (of 100)
Brucella melitensis	YES* / 65.0% (of 100)
Brucella suis	YES* / 65.0% (of 100)
Burkholderia mallei	YES* / 57.6% (of 99)
Burkholderia pseudomallei	YES* / 61.6% (of 99)
Francisella tularensis	YES* / 90.0% (of 100)
Yersinia pestis	YES* / 85.0% (of 100)

* Dependent of laboratory testing capability

Results of "Rule Out" Tests (continued)

Sample 2	Response* %(total #) Reporting Labs
Laboratory Responses: Were you able to "Rule Out"?	
Bacillus anthracis	YES* / 95.0% (of 101)
Brucella abortus	YES* / 87.0% (of 100)
Brucella melitensis	YES* / 87.0% (of 100)
Brucella suis	YES* / 87.0% (of 100)
Burkholderia mallei	YES* / 81.0% (of 100)
Burkholderia pseudomallei	YES* / 83.0% (of 100)
Francisella tularensis	YES* / 66.7% (of 99)
Yersinia pestis	YES* / 84.8% (of 99)

* Dependent of laboratory testing capability

Results of Organism ID

Sample 1 – Organism ID:	% of Reporting Labs (n=75)
<i>Bordetella bronchiseptica</i>	
Acceptable Laboratory Responses:	90.7%
Bordetella bronchiseptica	22.7%
Unable to identify, referred	40.0%
Gram negative rods	24.0%
Gram negative coccobacilli	4.0%
Other Responses for Organism ID:	
Oligella urealytica	2.7%
Oligella urethralis	1.3%
Pseudomonas species	1.3%
Burkholderia mallei	1.3%
Bacillus anthracis ruled out	1.3%
Growth	1.3%

Results of Organism ID (continued)

Sample 2 – Organism ID:	% of Reporting Labs (n=78)
<i>Aggregatibacter aphrophilus</i>	
Acceptable Laboratory Responses:	69.2%
Aggregatibacter aphrophilus	10.3%
Aggregatibacter species	2.6%
Unable to identify, referred	39.7%
Gram negative rods	11.5%
Gram negative coccobacilli	5.1%
Other Responses for Organism ID:	
Pseudomonas species	2.6%
Acinetobacter lwoffii/spp.	3.9%
Shigella species	1.3%
Haemophilus aphrophilus/paraphrophilus/spp.	5.1%
Klebsiella species	1.3%
Pasteurella species	3.8%
Sphingomonas species	9.0%
Growth/no growth	2.6%

REMIND/REFRESH/THINK

Brucella spp.

Francisella tularensis

Bacillus anthracis

Yersinia pestis

Others

RARE ISOLATES IN THE CLINICAL LABORATORY

History of *Brucella*

- 1886---Sir David Bruce isolates *Brucella*
 - *Micrococcus melitensis*
 - Malta fever
- 1895---Bernhard Bang isolates *B. abortus*
 - *Bacillus abortus*
 - Bovine abortion
- 1918---Alice Evans demonstrates close similarity of the two organisms
 - New genus proposed, *Brucella*,
- 1929---*B. suis* described by Huddleston
 - Associated with aborted swine
- 1950s---Two additional species described
 - *B. ovis* and *B. neotomae*
- 1968---*B. canis* identified as a cause of canine abortion

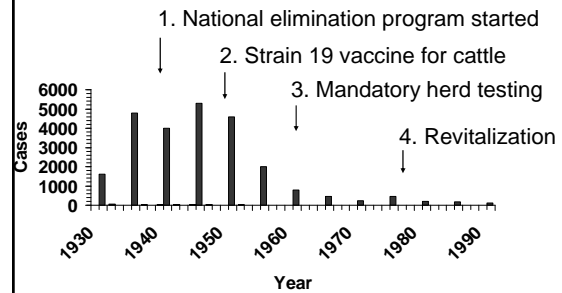
Brucella species

Organism	Animal Reservoir	Geographic Distribution
<i>B. melitensis</i>	Goats, sheep, camels	Mediterranean, Asia, Latin America, parts of Africa and some southern European countries
<i>B. abortus</i>	Cows, buffalo, camels, yaks	Worldwide
<i>B. suis</i>	Pigs (biotype 1-3)	South America, Southeast Asia, United States
<i>Brucella canis</i>	Canines	Cosmopolitan
<i>Brucella ovis</i>	Sheep	No known human cases
<i>Brucella neotomae</i>	Rodents	Not known to cause human disease
<i>Brucella pinnipediae</i> and <i>Brucella cetaceae</i>	Marine animals, minke whales, dolphins, seals	Recent case reports describing some human cases (mainly neurobrucellosis)

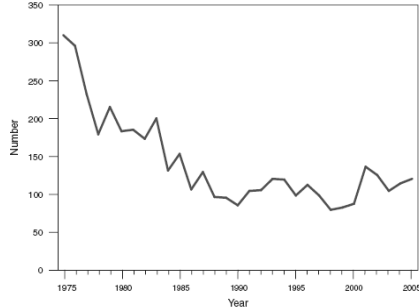
Brucellosis

- A zoonotic disease caused by any of 4 *Brucella* sp.: *abortus*, *melitensis*, *suis*, and *canis*
 - *B. melitensis* most common
- A systemic infection characterized by an undulant fever pattern
- Relatively rare in the U.S. with approximately 120 cases/yr

Brucellosis in the U.S., 1930-1990: Public Health Success Story

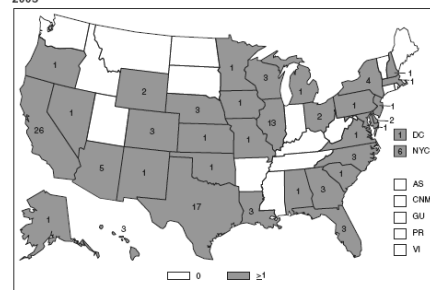


BRUCELLOSIS. Number of reported cases, by year — United States, 1975–2005

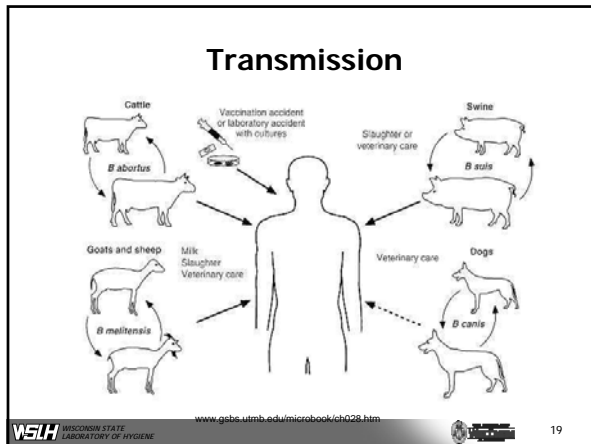


The incidence of brucellosis has remained stable in recent years, reflecting an ongoing risk for infection with *Brucella suis* acquired through contact with feral swine in the United States, and *B. melitensis* and *B. abortus* acquired through exposure to unpasteurized milk products in countries with endemic brucellosis in sheep, goats, and cattle.

BRUCELLOSIS. Number of reported cases — United States and U.S. territories, 2005



The incidence of brucellosis has remained stable in recent years, reflecting an ongoing risk from feral swine in the United States, and exposure to unpasteurized milk products from countries with endemic brucellosis.



Transmission

- **Ingestion**
 - Unpasteurized dairy products
 - The most common mode of transmission
- **Direct skin contact**
 - Occupational hazard for farmers, butchers, veterinarians, and laboratory personnel
- **Aerosols**
 - Highly infectious (Infective Dose 10-100 organisms)

Infectious Dose

Bacteria	Dose	Route of Inoculation
<i>F. tularensis</i>	10	inhalation
<i>C. burnetii</i>	10	inhalation
<i>M. tuberculosis</i>	<10	inhalation
<i>Brucella spp.</i>	10-100	inhalation
<i>S. typhi</i>	10 ⁵	ingestion
<i>F. tularensis</i>	10 ⁸	ingestion

Pathogenesis

- Patterns of illness similar in all forms of infection
 - Brucella are intracellular organisms
 - Survive and multiply within mononuclear phagocytes
 - Become localized in reticuloendothelial system e.g. lymph nodes, liver, spleen, and bone marrow
 - Illness reflects distribution of macrophages in bone, joints, brain, liver, spleen, and lung
 - Abscesses and granulomas
 - LPS is the major determinant of virulence

Clinical Manifestations

Acute onset in 50% of cases
Incubation period of 5-60 days

Symptom	% (Total No. in Study)	Sign	% (No. in Study)
Fever	98 (930)	Hepatosplenomegaly	41 (400)
Constitutional symptoms*	94 (930)	Hepatomegaly	38 (930)
Sweats	85 (930)	Splenomegaly	22 (930)
Chills	79 (930)	Osteoarticular	23 (930)
Arthralgias	53 (930)	Relative bradycardia	21 (530)
GI symptoms†	51 (400)	Adenopathy	9 (930)
Headache	42 (400)	Neuro/CNS‡	8 (930)
Lumbar pain	39 (930)	Orchitis/epididymitis	6 (400)
Myalgias	35 (930)	Cutaneous	3 (530)
Cough/dyspnea	19 (400)		
Weight loss	18 (400)		
Neurological ‡	14 (400)		
Testicular pain	5 (930)		

*Anorexia, asthenia, fatigue, weakness, malaise
†Abdominal pain, constipation, diarrhea, vomiting
‡Anxiety, confusion, depression, insomnia
§Paralysis, nuchal rigidity, papilledema

From Wafa Al-Nassir, emedicine.com/topic248.htm

Treatment

- Mortality 5% in untreated patients
 - Usually from CNS infection and endocarditis
- Treatment
 - Adults---Combination of doxycycline plus an aminoglycoside for 4 weeks; followed by doxycycline and rifampin for 6 weeks
 - Children---TMP-SMX plus rifampin 4-6 weeks

Diagnosis

- Routine laboratory tests
 - WBC---usually normal or depressed
 - Anemia
 - Thrombocytopenia
- Serology
 - Serum agglutination test (SAT) gold standard
 - *B. abortus* 1119 antigen
 - Also reacts with antibodies to *B. melitensis* and *B. suis*
 - No single serum titer is "diagnostic"
 - Most cases $\geq 1:160$
 - Acute and convalescent serum optimal for diagnosis
 - Decrease in titer indicates good response to therapy
 - Increase titer indicates relapse

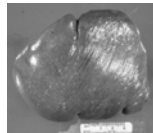
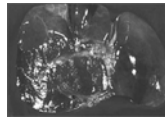
Diagnosis

- Culture
 - 15-70% isolation rate depending on methods used and specimens cultured



Brucella spp. Specimen Selection

- Blood or bone marrow
 - Sources from which Brucellae are most often isolated
- Tissue (spleen, liver)
 - Brucellae occasionally isolated



Biosafety



"I'm glad you two have finally met."



Exposure to Attenuated Vaccine Strain RB51

- Fall 2007 CAP LPS survey
- 916 laboratorians in 254 labs with potential RB51 exposure
 - 679 (74%) with high-risk exposure
- Postexposure prophylaxis recommended for high risk exposure
 - Performed a potentially high-exposure practice (e.g., sniffing plates)
 - Within 5 feet of any manipulation of RB51 on the open bench
 - Present in the laboratory during a widespread aerosol-generating event (e.g., vortexing)

Exposure to Attenuated Vaccine Strain RB51

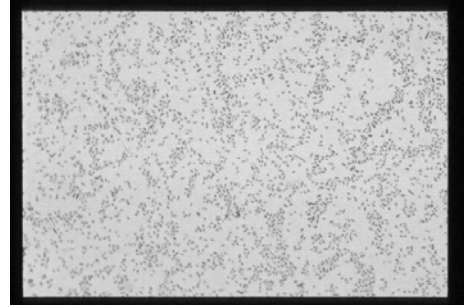
- CAP written instructions
 - Handle in Class II BSC with BSL-3 primary barriers and safety equipment
- Event emphasized importance of having written, established protocols for handling highly lethal infectious agents
 - Protocol should define laboratory findings that signal the need for increased biosafety precautions

Identification

- Gram Stain
 - Tiny gram negative coccobacillus
 - Faintly staining
 - 0.5-0.7 x 0.6-1.5 microns
- Growth on original plates
- Biochemicals
 - Automated or commercial systems
 - Standard biochemicals

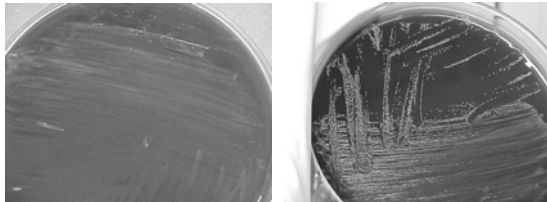


Gram Stain



Growth on Original plates

- Slow growing taking up 72 hours
- Colonies--non hemolytic & non pigmented



Possibilities Based on These Results

- Clinical lab non BT specimen
 - Haemophilus (Blood agar growth?)
 - Acinetobacter (Large but maybe treated ?)
 - Other “environmental type” organisms
 - Flavobacterium
 - Pseudomonas
 - Pasteurella
 - Rarely seen “Odd ball” BT types
 - Brucella spp.
 - Francisella tularensis
 - Yersinia



Key Rule Out Tests

- Growth on BAP looks like Staph or Diphtheroids,
 - Key test ---gram stain GNCB
- Oxidase---Positive
- Catalase---Positive
- Urea---Positive
 - Very key test
 - Rapid positive---within minutes
 - (Rare strains of melitensis show weak positive)
- Motility---Negative



Urea Reaction



Commercial Systems

- Growth too slow for rapid commercial systems (MicroScan, Vitek or Pheonix)
- API
- Fastidious ID systems
 - Non-sense or improbable identification
- Molecular---works well but not many of us have this capability
- WHEN IN DOUBT---REFER