Detection of Cryptic Burkholderia Pseudomallei Infections in Imported Pig-tail Macques (Macaca nemestrina): Implications for Animal Laboratory Biosafety and Security

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Centers for Disease Control and Prevention

56th Annual Biosafety Conference

October 21, 2013

Disclaimer

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Overview of Topics

- Organism
- Transmission
- Risk Factors
- Clinical Signs
- Diagnosis
- Treatment
- Prevention and Control
- Regulatory Issues
- Animal Models
- Case Reports
- Recommendations

Why is it Important?

- A Tier 1 overlap select agent
- Considered a potential biological weapon
- Several countries studied *B. pseudomallei* for use as a bioweapon.
- Lack of a vaccine
- Ability to manufacture strains resistant to multiple antibiotics
- Lack of familiarity and reporting

HHS AND USDA SELECT AGENTS AND TOXINS
7 CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73

HHS SELECT AGENTS AND TOXINS
Abrin
Botulinum neurotoxins*
Botulinum neurotoxin producing species of *Clostridium**
Conotoxins (Short, paralytic alpha conotoxins containing the following amino acid sequence $X_1CCX_2PACGX_3X_4X_5X_6CX_7$)
Coxiella burnetii
Crimean-Congo haemorrhagic fever virus
Diacetoxyscirpenol
Eastern Equine Encephalitis virus[1]
Ebola virus*
*Francisella tularensis**
Lassa fever virus
Lujo virus
Marburg virus*
Monkeypox virus[1]
Reconstructed replication competent forms of the 1918 pandemic influenza virus containing any portion of the coding regions of all eight gene segments (Reconstructed 1918 Influenza virus)
Ricin
Rickettsia prowazekii
SARS-associated coronavirus (SARS-CoV)
Saxitoxin
South American Haemorrhagic Fever viruses:
- Chapare
- Guanarito
- Junin
- Machupo
- Sabia

Staphylococcal enterotoxins A,B,C,D,E subtypes
T-2 toxin
Tetrodotoxin
Tick-borne encephalitis complex (flavi) viruses:
- Far Eastern subtype
- Siberian subtype

Kyasanur Forest disease virus
Omsk hemorrhagic fever virus
Variola major virus (Smallpox virus)*
Variola minor virus (Alastrim)*
*Yersinia pestis**

OVERLAP SELECT AGENTS AND TOXINS
Bacillus anthracis *
Bacillus anthracis Pasteur strain
Brucella abortus
Brucella melitensis
Brucella suis
*Burkholderia mallei**
*Burkholderia pseudomallei**
Hendra virus
Nipah virus
Rift Valley fever virus
Venezuelan equine encephalitis virus[1]

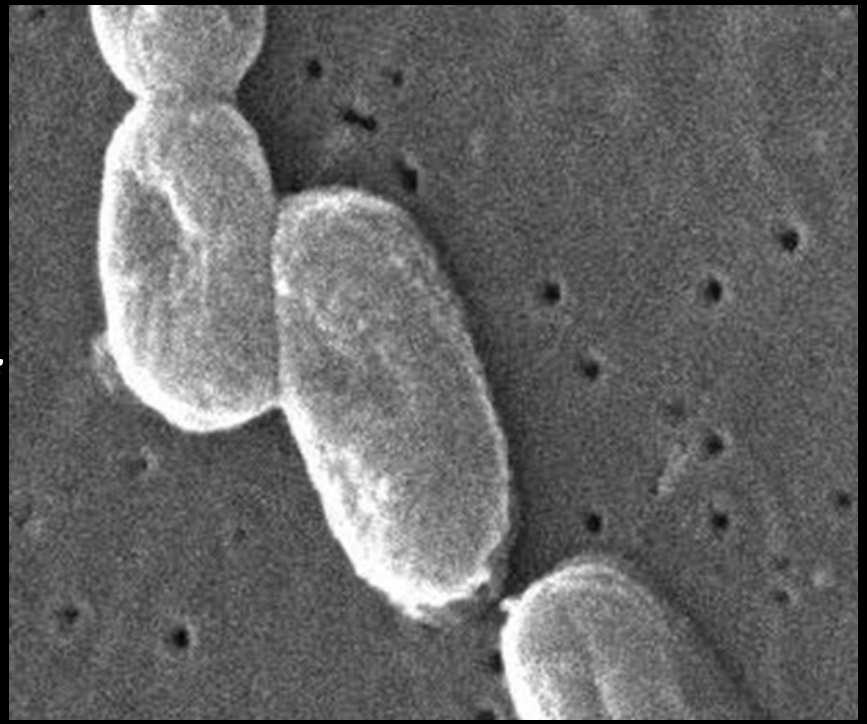
USDA SELECT AGENTS AND TOXINS
African horse sickness virus
African swine fever virus
Avian influenza virus[1]
Classical swine fever virus
Foot-and-mouth disease virus*
Goat pox virus
Lumpy skin disease virus
Mycoplasma capricolum[1]
Mycoplasma mycoides[1]
Newcastle disease virus[1,2]
Peste des petits ruminants virus
Rinderpest virus*
Sheep pox virus
Swine vesicular disease virus

USDA PLANT PROTECTION AND QUARANTINE (PPQ) SELECT AGENTS AND TOXINS
Peronosclerospora philippinensis (Peronosclerospora sacchari)
Phoma glycinicola (formerly Pyrenochaeta glycines)
Ralstonia solanacearum
Rathayibacter toxicus
Sclerophthora rayssiae
Synchytrium endobioticum
Xanthomonas oryzae

*Denotes Tier 1 Agent

What is *B. pseudomallei*?

- Gram-negative, aerobic, motile, bacillus
- Causes melioidosis
- Formerly known as *Pseudomonas pseudomallei*
- Rare in the United States
- Can remain in macrophage without phagocytosis



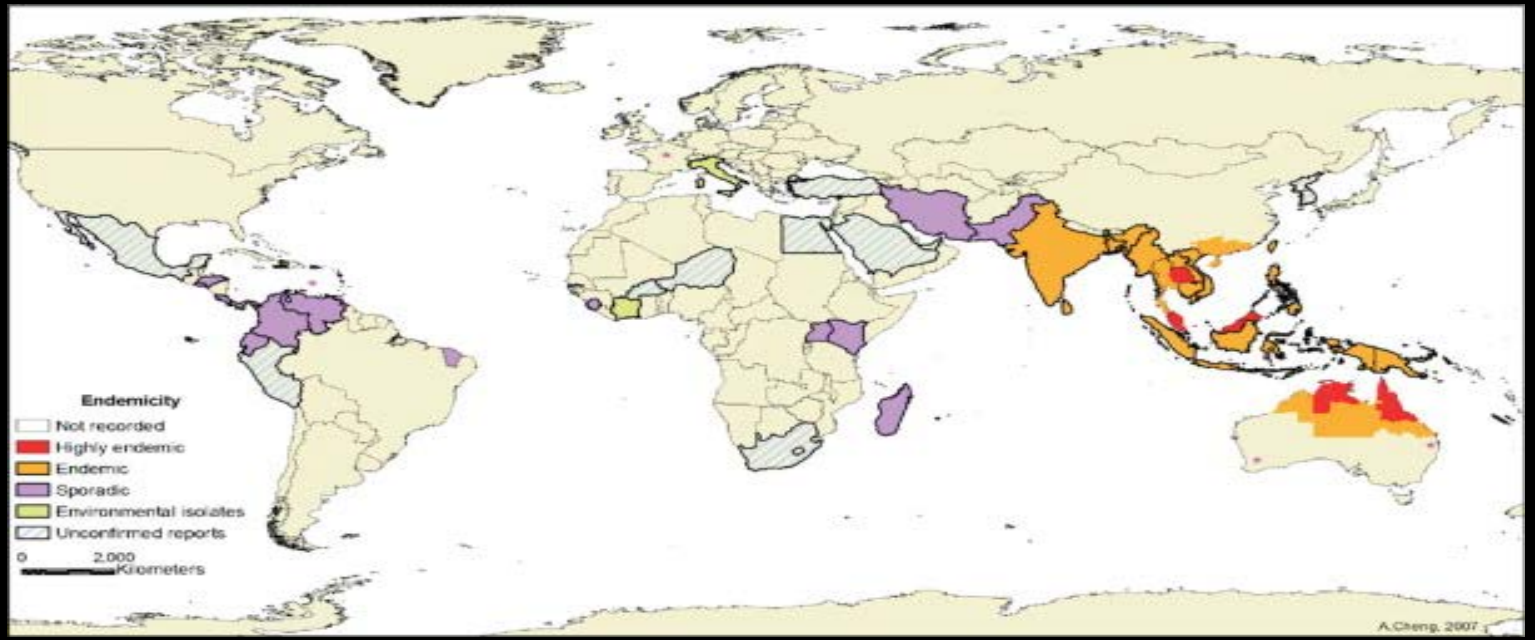
Burkholderia pseudomallei

- Emerging pathogen usually found in soil, standing water, roots of plants.
- Endemic in Southeast Asia, Northern Australia, Central & South America.
- Seasonal outbreaks usually occur in endemic areas
- Reported in indigenous groups, farmers, travelers, military personnel

Burkholderia pseudomallei(con't)

- The cause of Melioidosis (aka Whitmore's disease).
- Clinical signs varies with species and site of infection.
- Infects humans and animals
- Associated with suppurative or caseous lesions that can occupy any body organ.
- Organs most commonly affected: lungs, spleen, liver, lymph nodes.

The Global Distribution of *Burkholderia pseudomallei* and Melioidosis (2008)



Source: Transactions of the Royal Society of Tropical Medicine and Hygiene Volume 102, Supplement 1, December 2008, Pages S1–S4

Transmission

- Infection usually is opportunistic from the environment rather than from animal to animal
- Three major exposure routes (humans):
  - Cutaneous inoculation
  - Inhalation
  - Ingestion

Laboratory Transmission

- Contaminated antiseptics, injections, or other hospital or surgical equipment
- Other sources of infection include:
  - Infected tissues and purulent drainage from cutaneous or tissue abscesses
  - Blood and sputum

Risk Factors

- Prolonged contact with contaminated water and soil
- Seasonal outbreaks subsequent to typhoon season and flooding in several endemic areas

- Clinical disease
  - Diabetes
  - Liver disease
  - Renal disease.
  - Immunosuppression (not HIV)
  - Alcoholism
  - Cancer
  - Chronic lung disease.
  - Thalassemia

Classifications of Infections

- **Localized infection**
  - Generally presents as an ulcer, nodule, or skin abscess and may result from inoculation through a break in the skin
- **Pulmonary infection**
  - Most common presentation of the disease and can produce a clinical picture of mild bronchitis to severe pneumonia. Chest pain is common, but a nonproductive or productive cough with normal sputum is the hallmark
- **Bloodstream infection**
  - This is typically an infection with rapid onset, and abscesses may be found throughout the body, most notably in the liver, spleen, or prostate
- **Disseminated infection**
  - Presents with abscess formation in various organs of the body, and may or may not be associated with sepsis. Disseminated infection may be seen in acute or chronic melioidosis

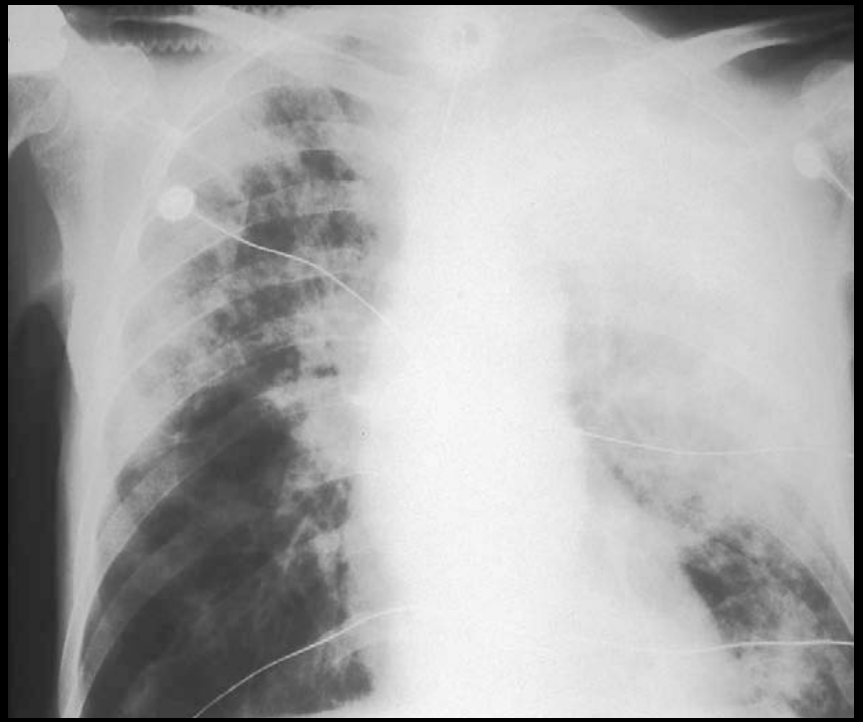
Clinical Signs in Humans and Animals

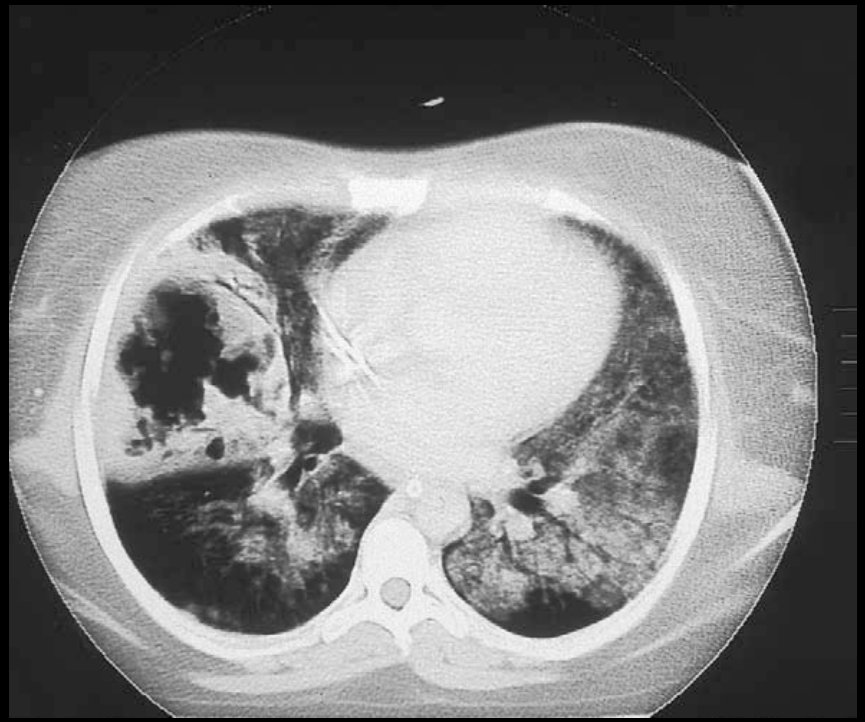
- The acute form has a rapid onset.
  - First symptoms developing within days to a few weeks after exposure.
- Acute infections:
  - Fever, pneumonia, dyspnea, and sepsis.
- Chronic infections:
  - Chronic pneumonia; suppurative infections of skin, liver, kidney, or spleen; subclinical infection.
  - May experience clinical signs months to years after the initial infection

Clinical Signs Humans

- Anorexia
- Fever
- Headache
- Weight loss
- Cough/Respir. Distress
- Disorientation
- Myalgia
- Seizures
- Skin lesions
- Subcutaneous and tissue abscesses
- Pneumonia
- Neurological infections
- Osteomyelitis
- Pericarditis
- Septic arthritis
- Genitourinary infections

Diagnostic Images of Pathology in Humans





Pathology in Humans

Liver Abscess

Subcutaneous Abscess

Clinical Signs in Animals

- Asymptomatic
- Anorexia
- Wasting
- Purulent drainage
- Subcutaneous abscesses and other soft tissue lesions in various organs
- Lymphadenitis
- Lameness
- Osteomyelitis
- Paralysis
- Neurological signs

Diagnosis

- Detection by antibody response (e.g. IHA, ELISA)
- PCR
- Isolation by culture
  - Blood
  - Urine
  - Sputum
  - Throat swabs
  - Abscesses
  - Skin and tissue lesions

Treatment

- Intravenous antibiotics (10 – 14 days)
  - Ceftaxidime
  - Meropenem
- Oral Antibiotics (3 – 6 months)
  - Trimethoprim Sulfa
  - Doxycycline (?)

Laboratory Safety

- Perform work with BSL-2 practices, containment, equipment, and facilities.
- Work should be done in a BSC and gloves worn when manipulating the microorganism.
- Respiratory protection microorganism is manipulated outside of a BSC
  - Centrifugation
  - Handling infected animals
- Confined to BSL-3 facilities In cases where infectious aerosols or droplets could be produced, or where production quantities of the organism are generated

Regulatory Issues

- Discovery of must be reported immediately to the Federal Select Agent Program
- Report identification to other appropriate authorities required by Federal, State, or local law.
- Submit APHIS/CDC Form 4 for:
  - Identification of a select agent
- Submit APHIS/CDC Form 3:
  - Transfer or release of a select agent

USDA

REPORT OF THEFT, LOSS, OR RELEASE OF SELECT AGENTS AND TOXINS (APHIS/CDC FORM 3)

FORM APPROVED
OMB NO. 0579-0213
OMB NO. 0920-0576
EXP DATE 11/30/2015

INSTRUCTIONS

Detailed instructions are available at http://www.selectagents.gov/TLRForm.html. Answer all items completely and type or print in ink. This report must be signed and submitted to either APHIS or CDC:

Animal and Plant Health Inspection Service
Agricultural Select Agent Program
4700 River Road Unit 2, Mailstop 22, Cubicle 1A07
Riverdale, MD 20737
FAX: (301) 734-3652
Email: ASAP@aphis.usda.gov

Centers for Disease Control and Prevention
Division of Select Agents and Toxins
1600 Clifton Road NE, Mailstop A-46
Atlanta, GA 30333
FAX: (404) 718-2096
Email: lrsat3@cdc.gov

Accession Number:

(For Program Use ONLY)

Submit completed form only once by either email, fax, or mail

SECTION 1 – TO BE COMPLETED BY ALL ENTITIES

1. Date of Incident:
2. Date of Immediate Notification:
3. Type of Immediate Notification: ☐ Email ☐ Fax ☐ Telephone
4. Name of Entity (entities registered with CDC or APHIS) or Name of Hospital or Laboratory (non-registered entities):
5. Entity registration number (For select agent registered entities only):
6. Physical Address:
7. City:
8. State:
9. Zip Code:
10. Responsible Official (registered) or Name of Laboratory Supervisor (non-registered):
11. Telephone #:
12. Fax #:
13. Email address:
14a. Type of Incident (Human Health): ☐ Theft ☐ Loss ☐ Release ☐ Lab Acquired Infection
14b. Type of Incident (Animal and Plant Health): ☐ Unintended Animal Infection ☐ Unintended Plant Agent Release
14c. Transfer: ☐ Transfer incident (complete Sections 1 and 2 and Appendix B)
15. Did the release result in a potential exposure? ☐ No ☐ Yes ☐ N/A (If Yes, explain in Blocks 28 or 30)
If yes, has medical surveillance been initiated? ☐ No ☐ Yes ☐ N/A (If Yes, explain in Blocks 28 or 30)
16. Time incident occurred:
17. Location of incident (building and room #):
18. Location of incident within room (e.g., freezer, incubator, centrifuge):
19. Biosafety level: ☐ BSL2 ☐ BSL3 ☐ BSL4 ☐ ABSL2 ☐ ABSL3 ☐ ABSL4 ☐ PPQ Agent ☐ BSL3 Ag
20. Date of last inventory (for reporting loss only):
21. Name of Principal Investigator:

SECTION 2 – TO BE COMPLETED BY ALL ENTITIES

	22. Name of Select Agent or Toxin	23. Characterization of Agent (e.g., strain, ATCC #)	24. Quantity / Amount
A			
B			
C			

25. Provide a detailed summary of events including a timeline of what occured. Whenever possible, conduct a risk assessment of the event and determine if the root cause can be identified. State specifically what personal protective equipment was worn and what, if any, medical surveillance was provided or planned. If incident involves a non-human primate, please state species.

Block 25. Continued: (Use Appendix A for continuation, if necessary)

SECTION 3 – TO BE COMPLETED BY ALL ENTITIES ONLY FOR RELEASE OF SELECT AGENTS AND TOXINS OR OCCUPATIONAL EXPOSURE

26. An internal review of laboratory procedures and policies has been initiated to lessen the likelihood of recurrences of theft, loss or release of select agents and toxins at this entity.
☐ No ☐ Yes If yes, please provide additional details:

27. What were the hazards posed to humans by the extent of the release or occupational exposure?

28. What is the estimated extent of the release or exposure in relation to the proximity of susceptible humans, animals and plants?

29. Provide a brief summary of how the laboratory and work surfaces were decontaminated after the release.

30. In select agents and toxins posing a risk to humans, please state how many laboratorians were potentially exposed and provide a brief summary of the medical surveillance provided (do not provide names or confidential information).

Certification: I hereby certify that the information contained on this form is true and correct to the best of my knowledge. I understand that if I knowingly provide a false statement on any part of this form, or its attachments, I may be subject to criminal fines and/or imprisonment. I further understand that violations of the select agent regulations may result in civil or criminal penalties, including imprisonment. 7 CFR 331, 9 CFR 121, 42 CFR 73.

Signature of Respondent: _____ Title: _____

Typed or printed name of Respondent: _____ Date Signed: _____

Case Reports

Case #1

Neurological Melioidosis

Patient History

- Five-year old female *Macaca nemestrina*
- Imported into the USA in January 2012 from Indonesia
- Quarantine at a CDC-registered commercial vendor until release to the CDC vivarium in March of 2012.
- Completed quarantine at CDC(Atlanta) facility and was released into the general colony.

Clinical History

- March 2012
  - Abscess surrounding right stifle joint
    - Purulent discharge
    - Joint space not involved
    - Sample collected for culture
    - Blood collected for CBC/Chemistry
    - Flushed with dilute betadine solution
    - Treatment(Clavamox, Metacam)

Culture and Sensitivity

Lab Animal Aerobic Culture

1	**AEROBIC CULTURE**	Gram negative bacilli
	COMMENTS	Light to moderate growth. Non-reactive organism; unable to i.d. Susceptibility result presumptive: No guidelines have been approved by CLSI (NCCLS) for susceptibility breakpoints using the disk diffusion method of susceptibility testing for this organism.
2	**AEROBIC CULTURE**	Staphylococcus spp.- Coagulase negative
	COMMENTS	Very light growth. This organism is not usually associated with disease.
	REPORTED BY	Ashley Phillips; Lab Tech III
	REPORT DATE	03/22/2012

Antibiotic Susceptibility Pattern

	1
AMIKACIN	R
AMOX/ CLAV	S
AMPICILLIN	R
CEFOTAXIME (3RD GEN)	R
CEFOTETAN (2ND GEN)	I
CEFPODOXIME (3RD GEN)	R
CEPHALOTHIN (1ST GEN)	R
CHLORAMPHENICOL	S
DOXYCYCLINE	S
ENROFLOXACIN	I
GENTAMICIN	R
TETRACYCLINE	S
TICARCILLIN	R
TOBRAMYCIN	R
TRIMETHOPRIM/SULFA	S

Clinical History

- **One Week Follow-Up**
  - Bleeding present
  - Decreased range of motion
  - Minimal purulent discharge
  - Decreased swelling
- **Treatment plan**
  - Wound flushed
  - Radiographs NSF
  - Baytril added to treatment plan

Radiographs

Clinical History

- April 2012 – June 2012
  - Reoccurring clinical signs
    - Inflammation
    - Purulent discharge
    - Bleeding
- Impression smear – Hematoma
- New wound present on left knee
- Radiographs repeated on both knees
- June 2012 –resolution of wounds

Clinical History

August 2012 – Day 1

- Left head tilt
- Full Body tremors
- Muscle rigidity/Stiffness
- No pupil reflex
- Vertical nystagmus
- Teeth grinding with vocalizations

August 2012 – Day 2

- Lying down in cage
- Hypothermic
- Anisocoria
- Minimal rigidity/stiffness present

Differential Diagnosis

- Tetanus
- Streptococcus pneumonia
- Otitis interna
- Guillain–Barré syndrome

Diagnostics - Day 1

- CBC and Chemistry
- Urinalysis
- CSF Tap
- Radiographs
- Fecal culture

Diagnostics/Treatments – Day 2

Diagnostics

- CBC/Chemistry
  - Anemia,
  - Neutrophilia
  - Lymphopenia
  - Hyperglycemia
- Urinalysis
  - Glucosuria
  - Ketonuria

Treatments

- NACL Fluids
- Dexamethasone
- Penicillin
- Metronidazole
- Ensure via OG tube
- Regular Insulin

Diagnostic- CBC/Chemistry

WBC	14.04	4.09-14.91
RBC	5.15	5.19-6.39
HGB	9.9	10.3-12.8
HCT	38.2	38.9-48.8
MCV	74.2	69.4-82.1
MCH	19.1	18.5-21.3
MCHC	25.8	24.8-27.8
CHCM	24.2	23.7-26.9
RDW	13.0	10.4-13.4
CH	17.9	17.9-20.3
HDW	1.61	1.63-2.19
PLT	376	327-739
MPV	10.2	8.4-12.3
PDW	61.7	
%NEUT	88.4 (1)	41.5-75.5
%LYMPH	4.9 (2)	19.8-51.5
%MONO	3.2 (3)	0.8-4.1
%EOS	0.3 (4)	0.0-2.6
%BASO	1.1	0.0-0.3
%LUC	2.0 (5)	1.0-3.9
#NEUT	12.41 (6)	1.31-10.00

Radiographs

Treatments

- Tetanus treatment
  - Penicillin IM (29,000u/kg)
  - Diazepam IM (0.5ml/kg)
  - Diphenhydramine IV (5mg/kg)
  - Tetanus antitoxin IV
  - LRS fluids(SQ and IV)
  - Buprenex IM(0.02ml/kg)
- Immediate improvement in range of motion of neck

Clinical History

- August 2012 - Day 3
  - Lateral recumbency
  - Unable to ambulate
  - Vomiting

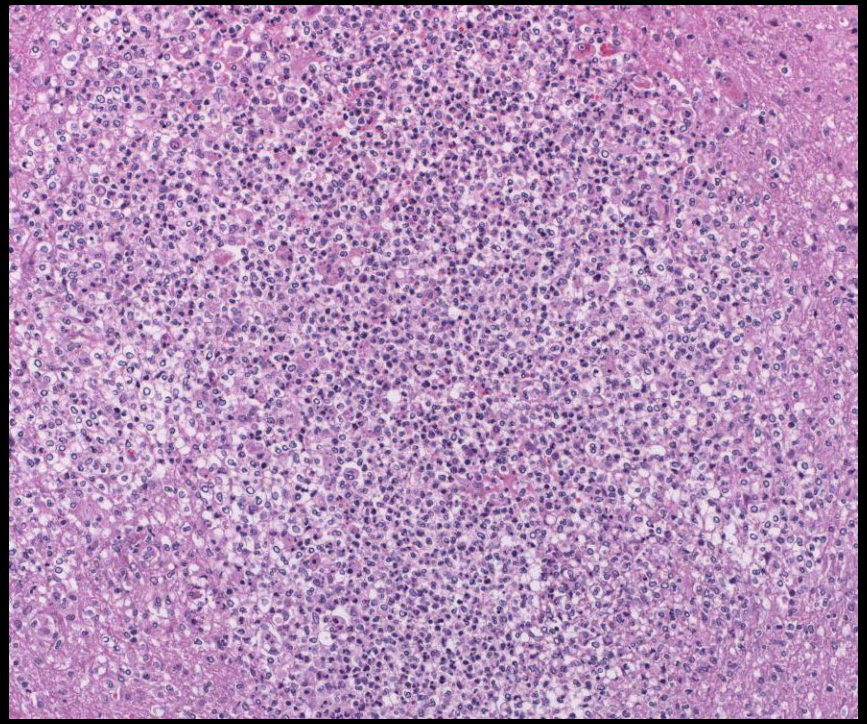
- Treatment
  - Ranitidine
  - Metoclopramide
  - Regular insulin
  - Penicillin

Post-Mortem Evaluation

- Gross and histopathological examination
- Special stains (gram-stain, IHC, and Warthin-Starry)
- Culture of isolate
- Indirect Hemagglutination Assay (IHA)
- Genotyping with Multi-Locus Sequence Typing
  - Measures DNA sequence variations by PCR amplification (data collection)
  - Characterize strains by unique allelic profiles (data analysis)
  - Nucleotide differences between strains checked with variable number of genes (multilocus sequence analysis)

Gross Necropsy

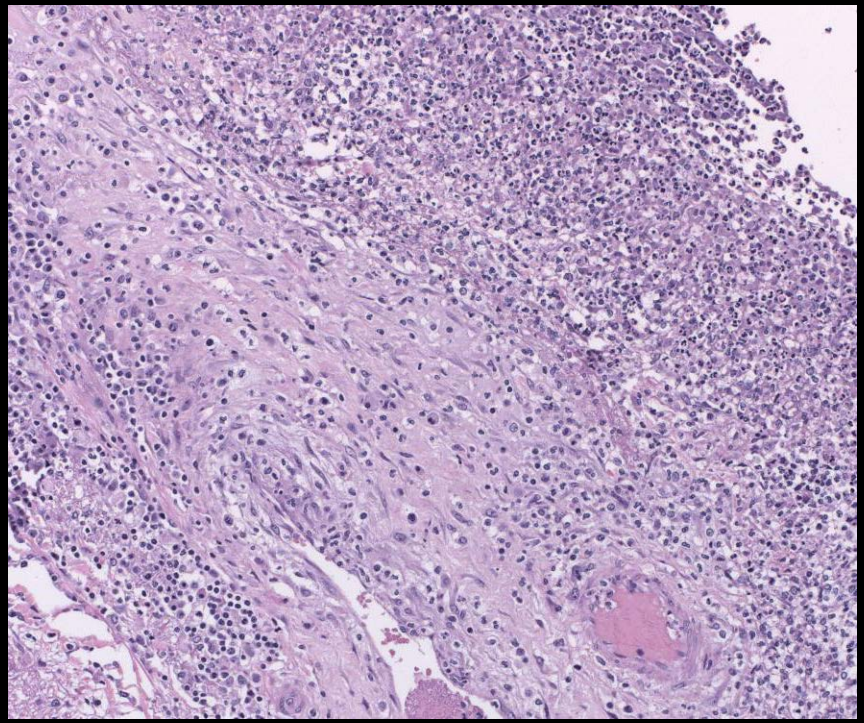
Focal Encephalitis



- Focal necrotizing pyogranulomatous encephalitis.
- Note the focus of macrophages and neutrophils destroying and replacing the brain parenchyma.

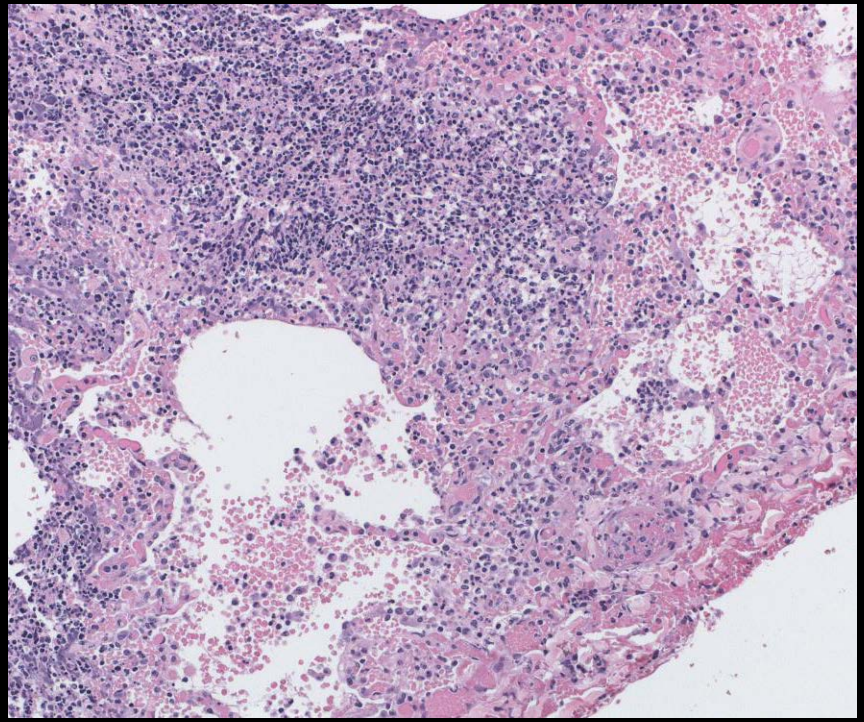
Meningitis

- Diffuse necrosuppurative myelitis with thrombosing vasculitis.
- Accumulation of neutrophils extending from what is left of dura mater on the surface of spinal cord.
- Walls of the large and small arteries in the image are infiltrated by mixed inflammatory cells and occluded by thrombi.
- The loss of normal architecture is the result of necrosis



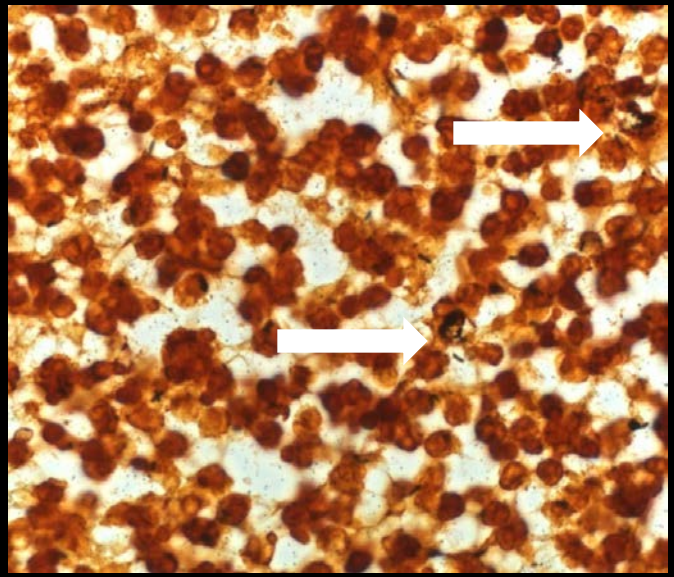
Pneumonia

- Diffuse necrohemorrhagic pyogranulomatous pneumonia with thrombosing vasculitis.
- Loss of normal alveolar architecture, replaced by inflammation and necrotic debris.
- Remaining alveoli filled with erythrocytes, adjacent to vessel walls destroyed with inflammation and thrombosis.



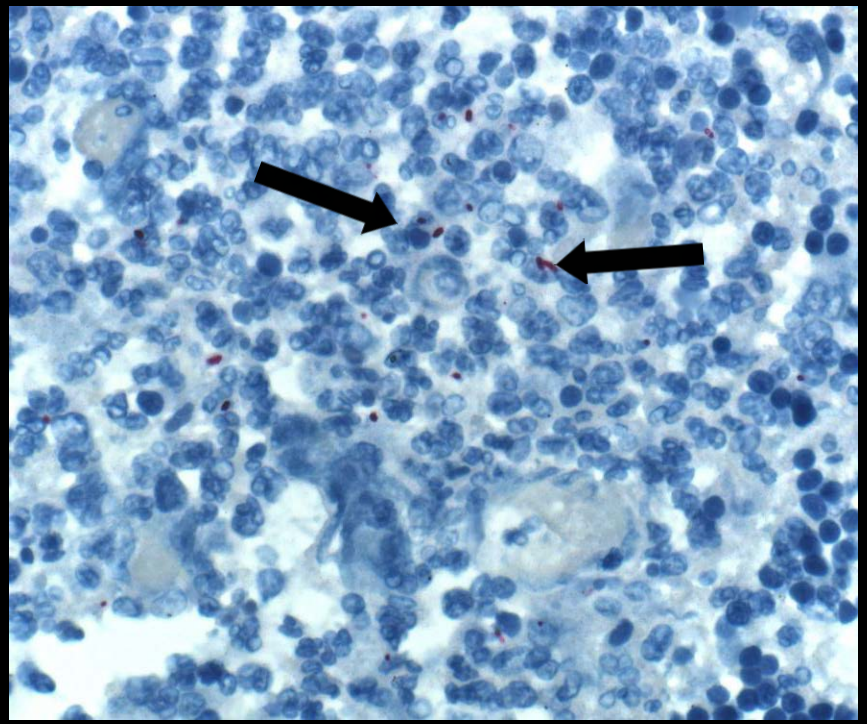
Warthin-Starry

- Rare to occasional bacterium observed within inflammatory cells.



IHC stain for *B. pseudomallei*

- Immunolocalization of *Burkholderia pseudomallei* bacterium with characteristic shape between a rod and a coccus engulfed within macrophage cytoplasm.
- Numerous neutrophils also present.



Case Reports

Case #2

Cutaneous Melioidosis

Patient History

- 5 year old female *Macaca nemestrina*
- Imported into the USA in November 2011 from Indonesia
- Approximately 5.0kg with no history of illness
- Quarantined at a CDC-registered facility until released to the CDC vivarium in January of 2012
- Completed quarantine at research facility and was released into the general colony

Clinical History

- March 2013
  - Abscess surrounding right carpal joint
    - Serosanguinous discharge
    - Joint space not involved
    - Samples collected:
      - Bacterial swab
      - Aspirate
      - Tissue
      - Blood
      - CSF tap
    - Flushed with dilute betadine solution

Abscess

Challenges

- To Treat or Not to Treat
  - Case #2 – Euthanized (sentinel case)
    - Culture confirmed with PCR
    - Immunohistochemistry staining
    - Blood and CSF culture
  - Select agent registered facility
    - Animal tissue and waste classified as select agent
    - Biosecurity of the animal and waste

Reporting/Notification Procedures

- Report incident to Responsible Official (RO)
- Submission of CDC/APHIS forms 4 and 3 to DSAT
- Report incident to DGMQ
- Report incident to OSHE
- Inform vendor
- Consult with Subject Matter Experts(SME)

Management Protocol for Animal Care Staff

- Compiled list of potentially exposed personnel
  - Techs, vets, lab, research, husbandry and pathology staff
- Informational meeting
- Subject Matter Experts
  - BSPB, OSHE, veterinary staff, senior management
- Risk Assessment
- Training
- Contact Former employees

Reporting/Notification Procedures

- Reported incident to RO
- Submission of CDC/APHIS forms 4 and 3 to DSAT
- Reported incident to DGMQ
- Report incident to OSHE
- Informed vendor
- Consult with BSPB

Management Protocol for Animal Care Staff

- Informational meeting
- Compiled list of potentially exposed personnel
  - (techs, vets, lab and husbandry staff, pathology staff)
- BSPB, OSHE (physician and microbiologist), veterinary staff, senior management.
- Contact Former employees
- Formulated a Decision tree and questionaire
- Risk Assessment
- Training

Colony Management

- Defined Potential Exposure for colony
- Complied list of exposed animals
- Restricted handling and access
- Established a foot pattern
- Established work practices for husbandry and handling of animals
- Decision for disposition of colony
  - Cull vs. not cull
  - Separation vs intergration

Colony Management (con't)

- Post-exposure prophylaxis
  - Doxycycline (50 mg/ml SID)
  - Trimethoprim Sulfa (250mg BID)
  - Florastor (250mg SID
- Serological diagnostics IHA
  - 4 week and 6 week testing
  - Annual testing
  - 1:40 vs. 1:160 titers vs. 1:320 titers

Recommendations

- Knowledge of clinical signs and diagnostics
- Include melioidosis in differentials
- Occupational health and safety procedures
- Colony management procedures
- Knowledge of regulatory and reporting procedures
- Consideration for scientific integrity of researchs

Acknowledgements

- Dr. Crystal Johnson
- Dr. George Lathrop
- Dr. Nathanial Powell Jr.
- Dr. Sharon Dietz
- Dr. Robyn Engel
- Pathologists in the BSPB
- Zoonoses and Select agent Laboratory(ZSAL)
- ARB Animal Care Staff


The End
Questions?