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

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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;CCX;PACGX;X;X;X;CX;)
Coxiella burnetii
Crimean-Congo haemorrhagic fever virus
Diacetoxyscirpenol
Eastern Equine Encephalitis virus
Ebola virus
Francisella tularensis
Lassa fever virus
Lujo virus
Marburg virus
Monkeypox virus
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)
Saxtoxin
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

*Denotes Tier 1 Agent

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

USDA SELECT AGENTS AND TOXINS

African horse sickness virus
African swine fever virus
Avian influenza virus
Classical swine fever virus
Foot-and-mouth disease virus
Goat pox virus
Lumpy skin disease virus
Mycoplasma capricolum
Mycoplasma mycoides
Newcastle disease virus
Peste des petits ruminants virus
Rinderpest virus
Sheep pox virus
Swine vesicular disease virus

USDA PLANT PROTECTION AND QUARANTINE (PPQ) SELECT AGENTS AND TOXINS

Peroonosclerospora philippinensis (Peroonosclerospora sacchari)
Phoma glycicinola (formerly Pyronochaeta glycines)
Ralstonia solanacearum
Rathayibacter toxicus
Sclerotinia rystiae
Synchytrium endobioticum
Xanthomonas oryzae
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
## SECTION 1 — TO BE COMPLETED BY ALL ENTITIES

<table>
<thead>
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<th>Details</th>
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### SUBMISSION REQUIREMENTS

- This form must be filled out in full and submitted electronically to the appropriate authorities.
- Failure to comply may result in legal action.

## SECTION 2 — TO BE COMPLETED BY ALL ENTITIES

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<td>Category (A, B, C, D)</td>
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**Instructions:**

1. **Date of Incident:**
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2. **Date of Incident:**
   - [Date]
3. **Type of Incident:**
   - [Type]
4. **Name of Laboratory:**
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5. **Address:**
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7. **State:**
   - [State]
8. **Zip Code:**
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9. **Telephone:**
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10. **Fax:**
    - [Fax]
11. **E-mail:**
    - [E-mail]
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    - [Date]
14. **Name of Laboratory:**
    - [Name]
15. **Address:**
    - [Address]
16. **City:**
    - [City]
17. **State:**
    - [State]
18. **Zip Code:**
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    - [Zip Code]
29. **Telephone:**
    - [Telephone]
30. **Fax:**
    - [Fax]
31. **E-mail:**
    - [E-mail]
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

<table>
<thead>
<tr>
<th></th>
<th>AEROBIC CULTURE</th>
<th>COMMENTS</th>
<th>REPORTED BY</th>
<th>REPORT DATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gram negative bacilli</td>
<td>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.</td>
<td>Ashley Phillips; Lab Tech III</td>
<td>03/22/2012</td>
</tr>
<tr>
<td>2</td>
<td>Staphylococcus spp.- Coagulase negative</td>
<td>Very light growth. This organism is not usually associated with disease.</td>
<td>Ashley Phillips; Lab Tech III</td>
<td>03/22/2012</td>
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## Antibiotic Susceptibility Pattern

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<tr>
<th>Antibiotic</th>
<th>Susceptibility</th>
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<tr>
<td>AMIKACIN</td>
<td>R</td>
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<tr>
<td>AMOX/ CLAV</td>
<td>S</td>
</tr>
<tr>
<td>AMPICILLIN</td>
<td>R</td>
</tr>
<tr>
<td>CEFOTAXIME (3RD GEN)</td>
<td>R</td>
</tr>
<tr>
<td>CEFOTETAN (2ND GEN)</td>
<td>I</td>
</tr>
<tr>
<td>CEPFODOXIME (3RD GEN)</td>
<td>R</td>
</tr>
<tr>
<td>CEPHALOTHIN (1ST GEN)</td>
<td>R</td>
</tr>
<tr>
<td>CHLORAMPHENICOL</td>
<td>S</td>
</tr>
<tr>
<td>DOXYCYCLINE</td>
<td>S</td>
</tr>
<tr>
<td>ENROFLOXACIN</td>
<td>I</td>
</tr>
<tr>
<td>GENTAMICIN</td>
<td>R</td>
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<tr>
<td>TETRACYCLINE</td>
<td>S</td>
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<tr>
<td>TICARCILLIN</td>
<td>R</td>
</tr>
<tr>
<td>TOBRAMYCIN</td>
<td>R</td>
</tr>
<tr>
<td>TRIMETHOPRIM/SULFA</td>
<td>S</td>
</tr>
</tbody>
</table>
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

Right Knee

Right Knee DV
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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Reference Range</th>
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<tr>
<td>WBC</td>
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<td>4.09-14.91</td>
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<tr>
<td>RBC</td>
<td>5.15</td>
<td>5.19-6.39</td>
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<tr>
<td>HGB</td>
<td>9.9</td>
<td>10.3-12.8</td>
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<tr>
<td>HCT</td>
<td>38.2</td>
<td>38.9-48.8</td>
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<tr>
<td>MCV</td>
<td>74.2</td>
<td>69.4-82.1</td>
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<tr>
<td>MCH</td>
<td>19.1</td>
<td>18.5-21.3</td>
</tr>
<tr>
<td>MCHC</td>
<td>25.8</td>
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<tr>
<td>CHCM</td>
<td>24.2</td>
<td>23.7-26.9</td>
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<tr>
<td>RDW</td>
<td>13.0</td>
<td>10.4-13.4</td>
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<td>CH</td>
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<td>HDW</td>
<td>1.61</td>
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<tr>
<td>PLT</td>
<td>376</td>
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<tr>
<td>MPV</td>
<td>10.2</td>
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<tr>
<td>PDW</td>
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<tr>
<td>%NEUT</td>
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<td>41.5-75.5</td>
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<tr>
<td>%LYMPH</td>
<td>4.9 (2)</td>
<td>19.8-51.5</td>
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<tr>
<td>%MONO</td>
<td>3.2 (3)</td>
<td>0.8-4.1</td>
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<td>%EOS</td>
<td>0.3 (4)</td>
<td>0.0-2.6</td>
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<td>%BASO</td>
<td>1.1</td>
<td>0.0-0.3</td>
</tr>
<tr>
<td>%LUC</td>
<td>2.0 (5)</td>
<td>1.0-3.9</td>
</tr>
<tr>
<td>#NEUT</td>
<td>12.41 (6)</td>
<td>1.31-10.00</td>
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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 questionnaire
• 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 integration
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?