Imported Animals and Unexpected Diseases

Brianna Skinner, DVM, DACLAM
Senior Clinical Veterinarian

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National Center for Zoonotic and Infectious Diseases
Division of Scientific Resources, Animal Resources Branch
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Examples of Outbreaks at CDC

• *Giardia* species – Prairie dogs, 2011
• *Burkholderia pseudomallei* – Pigtail macaques, 2012 and 2013
What is an Outbreak?

- Cases of illness in a place and time above what is normally expected
- Outbreaks are more localized
- Epidemic is more widespread; possibly longer duration
The Disease Triad

Agent Factors

Husbandry and Management

Host Factors

Environmental Factors

Vector Factors
Public Health Triad in Outbreak

Animals

Environment

Humans

Loss of Biodiversity

Habitat Modification

Close Contact

Zoonosis

Habitat Modification, Pollution of Air, Land, Water

Loss of Resources
Management Essentials for a Vivarium to Reduce/Contain an Outbreak

- Risk Analysis
- Standard Operating Procedures
- Personnel Training
- Vendor Importation Requirements
- Health Monitoring
- Husbandry practices

- Quarantine and Validation
- Biosecurity/Biocontainment
- Equipment Processing and Sanitation
- Engineering Controls
Additional Measures to Contain an Outbreak

- Reporting to appropriate authorities
- Occupational health screenings for staff
- Diagnostic screening for animals and staff
- Personal Protective Equipment
- Limited access in animal rooms/facility
- Disinfection/Decontamination
- Single housing /Separate housing
- Housing in barrier facilities
- Use of ventilated cages or isolators
Why are these Management Essentials Needed?

- Detects the presence of a pathogenic organisms within an animal colony.
- Countermeasures can be put into effect before or after a pathogen is detected in your facility.
- To obtain and maintain healthy animals in a research facility.
- Allows a periodic check of the health status of the animals.
Possible Risk Factors

- Animals
- Environment
- Biological Materials
- Vectors
- Equipment
- Staff
Impact of Risks

- Time
- Institutional Mission
- Scientific Research
- Cost
Components of Import Risk Analysis

- Hazard Identification
- Risk Assessment
- Risk Management

Risk Communication
Controlling Risk Factors with Husbandry and Management

- Educate and Train staff
- Screening incoming animals and biological materials
- Quarantine and Isolation
- Establish a foot pattern
- Pest control program
- Disinfecting and sanitizing supplies
Vendors and Importation

- Obtain animals from approved sources
- Obtain colony health report
- Site visitation
- Ensure receipt authorization
- Husbandry requirements
- Shipment and delivery date
- Transportation and Environmental criteria
- Notification of changes in health status
What is Biosecurity?

• “…Security of microbiological agents and toxins and the threats posed to human and animal health, the environment, and the economy by deliberate misuse or release.” – *BMBL*

• “…Consists of all measures taken to prevent, contain and eradicate adventitious infections.” – *Laboratory Animal Medicine.*
Quarantine

- Period which animals are held in isolation and health status is confirmed
- Measures should be species specific
- Restrict contact and access
- Time period will vary based on risk
- May incorporate testing and treatment
Options for Animals

• Quarantine and Test
• Quarantine, Test, and Treat
• Quarantine, Test, and Cull
• Rederivation
• Cull
Other Methods

- Depopulation and disinfection
- Rederivation (Fostering, Caesarian, Embryo Transfer)
Testing

• Determine which adventitious agents to screen for each animal species
• Determine the appropriate number of animals to test.
• Determine a frequency schedule for testing.
Testing Options

• Perform Diagnostic testing
  – Serology (IFA, ELISA, HAI)
  – PCR
  – Parasitology (external and internal)
  – Microbiology
  – Pathology (gross and histo)
Burkholderia Pseudomallei
Burkholderia pseudomallei

- Causes melioidosis
- Tier-one select agent
- Endemic in Southeast Asia, Northern Australia, Central & South America.
- Typically found in soil and standing water
- Requires CDC and/or USDA registration for possession, use, storage and/or transfer
Burkholderia Pseudomallei (con’t)

- Infects humans and animals (e.g. sheep, goats, horses, swine, monkeys, rodents)
- Can be acute to chronic
- Clinical signs vary with species and site of infection
- Organs commonly affected: lungs, spleen, liver, lymph nodes
Clinical Signs

• Three major exposure routes:
  – inhalation, cutaneous inoculation, and ingestion

• The acute form has a rapid onset (days to weeks)
  – fever, pneumonia, dyspnea, and sepsis.

• Chronic infections (months to years)
  – chronic pneumonia; suppurative infections of skin, liver, kidney, or spleen
Transmission

• Infection usually is opportunistic from the environment.
• Prolonged contact with contaminated water and soil
• Outbreaks are subsequent to seasonal weather conditions in endemic areas
• Common routes of infection:
  – percutaneous inoculation, contamination of wounds, ingestion of soil or contaminated carcasses, or inhalation.
Laboratory Hazards

- Contaminated antiseptics, injections, or equipment.
- Infected tissues, purulent drainage, blood, and sputum
- Infectious aerosols or droplets
  - Confine to BSL-3
- Respiratory protection
  - if manipulated outside of a BSC (e.g. centrifugation or handling infected animals).
Burkholderia Pseudomallei in Imported Pigtail Macaques
Case Report#1
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
  – Diagnostics performed
    • Culture and Sensitivity
    • CBC/Chemistry
  – Treatment
    • Flushed wound with betadine sol’n
    • Treatment (Clavamox, Metacam)
# Culture and Sensitivity

<table>
<thead>
<tr>
<th>Lab Animal Aerobic Culture</th>
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</thead>
<tbody>
<tr>
<td><strong>AEROBIC CULTURE</strong></td>
</tr>
<tr>
<td><strong>COMMENTS</strong></td>
</tr>
<tr>
<td></td>
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<tr>
<td><strong>REPORTED BY</strong></td>
</tr>
<tr>
<td><strong>REPORT DATE</strong></td>
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<table>
<thead>
<tr>
<th>Antibiotic Susceptibility Pattern</th>
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<tbody>
<tr>
<td><strong>AMIKACIN</strong></td>
</tr>
<tr>
<td><strong>AMOX/ CLAV</strong></td>
</tr>
<tr>
<td><strong>AMPICILLIN</strong></td>
</tr>
<tr>
<td><strong>CEFOTAXIME (3RD GEN)</strong></td>
</tr>
<tr>
<td><strong>CEFOTETAN (2ND GEN)</strong></td>
</tr>
<tr>
<td><strong>CEFFODOXIME (3RD GEN)</strong></td>
</tr>
<tr>
<td><strong>CEPHALOTHIN (1ST GEN)</strong></td>
</tr>
<tr>
<td><strong>CHLORAMPHENICOL</strong></td>
</tr>
<tr>
<td><strong>DOXYCYCLINE</strong></td>
</tr>
<tr>
<td><strong>ENROFLOXACIN</strong></td>
</tr>
<tr>
<td><strong>GENTAMICIN</strong></td>
</tr>
<tr>
<td><strong>TETRACYCLINE</strong></td>
</tr>
<tr>
<td><strong>TICARCILLIN</strong></td>
</tr>
<tr>
<td><strong>TOBRAMYCIN</strong></td>
</tr>
<tr>
<td><strong>TRIMETHOPRIM/SULFA</strong></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
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

<table>
<thead>
<tr>
<th>Diagnostics</th>
<th>Treatments</th>
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<tbody>
<tr>
<td>• CBC/Chemistry</td>
<td>• NACL Fluids</td>
</tr>
<tr>
<td>– Anemia,</td>
<td>• Dexamethasone</td>
</tr>
<tr>
<td>– Neutrophilia</td>
<td>• Penicillin</td>
</tr>
<tr>
<td>– Lymphopenia</td>
<td>• Metronidazole</td>
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<tr>
<td>– Hyperglycemia</td>
<td>• Ensure via OG tube</td>
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<tr>
<td>• Urinalysis</td>
<td>• Regular Insulin</td>
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<tr>
<td>– Glucosuria</td>
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<tr>
<td>– Ketonuria</td>
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<tr>
<td>Parameter</td>
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<td>Volume, Urine</td>
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<tr>
<td>Color, Urine</td>
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<tr>
<td>Clarity, Urine</td>
<td>Clear (12)</td>
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<tr>
<td>SG, Urine</td>
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<tr>
<td>Sed, Urine</td>
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<tr>
<td>pH, Urine Dipstick</td>
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<tr>
<td>LEU, Urine Dipstick</td>
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<tr>
<td>PRO, Urine Dipstick</td>
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<tr>
<td>GLU, Urine Dipstick</td>
<td>50 mg/ dL (14)</td>
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<tr>
<td>KET, Urine Dipstick</td>
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<tr>
<td>UBG, Urine Dipstick</td>
<td>Normal</td>
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<tr>
<td>BIL, Urine Dipstick</td>
<td>Negative</td>
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<tr>
<td>BLD, Urine Dipstick</td>
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## Diagnostic - CBC/Chemistry

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<tr>
<th></th>
<th>Value</th>
<th>Lower Limit</th>
<th>Upper Limit</th>
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<tr>
<td>WBC</td>
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<tr>
<td>RBC</td>
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<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>
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<td>MCHC</td>
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<td>24.8-27.8</td>
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<tr>
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<td>RDW</td>
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<td>MPV</td>
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<td>PDW</td>
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<tr>
<td>%NEUT</td>
<td>88.4 (1)</td>
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<td>%LYMPH</td>
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<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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<tr>
<td>%BASO</td>
<td>1.1</td>
<td>0.0-0.3</td>
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<tr>
<td>%LUC</td>
<td>2.0 (5)</td>
<td>1.0-3.9</td>
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<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
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.
Burkholderia Pseudomallei in Imported Pigtail Macaques
Case Report #2

National Center for Emerging and Zoonotic Infectious Disease
Division of Scientific Resources, Animal Resources Branch
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 Responsible Official
• Submission of CDC/APHIS forms 4 and 3 to Division of Select Agents and Toxins (DSAT)
• Reported incident to Division of Global Migration and Quarantine (DGMQ)
• Report incident to OSHE
• Informed vendor
• Consult with Bacterial Special Pathogens Branch (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 research
- Acquire animals from vendors within the US
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• Division of HIV/AIDS Prevention-Surveillance & Epidemiology – Dr. David Garber.
The End
Questions?

For more information please contact Centers for Disease Control and Prevention

1600 Clifton Road NE, Atlanta, GA 30333
Telephone: 1-800-CDC-INFO (232-4636)/TTY: 1-888-232-6348
E-mail: cdcinfo@cdc.gov Web: http://www.cdc.gov

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