Human *Brucella canis* Infection Associated with a Puppy, New York City, 2012

CM Dentinger, K Jacob, L Lee, HA Mendez, K Chotikanatis, PL McDonough, D Chico, BK De, R Traxler, R Tiller, ER Campagnolo, D Schmitt, M Guerra, S Slavinski
Brucellosis

- Zoonotic bacterial disease
  - Worldwide
  - Gram negative, aerobic, intracellular coccobacilli
- Several species
  - Abortus, meletensis, suis, ovis, canis
- Animal infection
  - Abortions, placentitis, epididymitis, orchitis
- Human Infection
  - Acute - febrile illness with non-specific symptoms
  - Chronic - arthritis, endocarditis
- Shed in animal excretions
  - Birthing fluids and tissues, animal products
**Brucella canis**

- First described in 1966
  - Reproductive failure in beagles
- Dogs are main reservoir
- Shed via oral or venereal secretions
- Surviving puppies born from infected females often have asymptomatic infections
- Prevalence
  - 1.5-19% of tested dogs sero-positive
  - May be increasing
Source of Human Infections

• Occupational (kennel workers, breeders)
• Laboratory acquired
• Naturally acquired
  – Dogs in the home
  – Stray dogs
  – Exposure to birthing products
**B. canis** in Humans

- **Transmission**
  - Contact with conjunctiva or broken skin
  - Inhalation of infectious aerosols
- **Incubation varies**
  - 7 days to several months
- **Illness**
  - Acute, nonspecific febrile illness
  - Recurrent fever, arthritis, endocarditis
- **Diagnosis**
  - Culture
Review of Reported Human Cases

• 52 human cases reported from 1967-present
  – Laboratory identification of B. canis, CDC
    • 1973-2000: 18 human isolates
    • 2002-present: 34 human isolates

• Seroprevalence
  – 3 studies done in the 1970s
  – 0.2-2% of serologic specimens tested positive
Investigation

• Human, Puppy
  – Clinical
  – Epidemiologic
• Laboratory
• Breeder
Clinical Illness in Child

- Child to ER on April 26, 2012
- History of illness
  - 2 days of dry cough, nasal congestion & clear discharge
  - Fever 38.3° C
  - 1 day of dyspnea
- Physical exam
  - Respiratory distress, no wheezing or rhonchi
  - Heart rate ~160
  - O2 saturation 93% on room air
- Laboratory exam
  - Hgb 11.7, WBC 9,200 cells/mm³ (62% neutrophils, 24% lymphocytes)
  - RSV and Influenza rapid test negative
  - Blood and respiratory specimens cultured
Clinical Illness in Child (cont)

• CXR
  – Peribronchial thickening (focal atelectasis vs consolidation)

• Treatment
  – Nebulized albuterol x 2
  – Parenteral ceftriaxone x 1
  – Admitted
  – Nebulized saline q 4

• Discharged home after 48 hours
  – No medications
Investigation Time Line
April – October, 2012

Child to ER
4/26-28

5/7

5/2
G- rods growing, 2nd culture collected

5/11
B. Canis identified; Child Treated Epi & Lab Investigation
Epidemiologic Investigation

• Family’s home
  – Two Dogs
    • 8 week-old male Yorkshire Terrier purchased from NYC pet store
      – Purchased ~ 1 month prior to child’s onset
    • 7 year old spayed Lhasa Apso
  – Two parents
  – Two visitors - grandparent, aunt

• Child had no exposure to other dogs
• No family members or visitors symptomatic
Laboratory Investigation

- NYC Public Health Laboratory
  - Brucella suspected, PCR confirmed
  - 14 exposed workers evaluated
    - 3 high-risk
- Hospital Laboratory A
  - 17 exposed workers, all high risk
- Post Exposure Prophylaxis
  - Antibiotics
  - Fever watch

Laboratory-acquired brucellosis--Indiana and Minnesota, 2006.
Puppy Investigation

• Clinical
  – Asymptomatic

• Laboratory
  – Blood cultures at AHDC, Cornell University College of Veterinary Medicine, NY
    • Puppy’s culture grew *B. canis*
  – Isolate forwarded to CDC
Investigation Time Line
April – October, 2012

Child to ER
4/26

Isolate to NYC PHL
5/7

Puppy blood cultures grow B. canis
5/25

Child Treated Epi & Lab investigation
5/31

G- rods growing; 2nd culture collected
5/2

B. Canis identified
5/11

From Iowa breeder
6/5
Breeder Investigation

- Breeder investigated
  - Dam and sire positive for *B. canis* by serology
  - Quarantine issued
    - Test all sexually-intact dogs > 6 months
    - Sera negative x 2, 30 days apart
    - 20 positive dogs euthanized
  - No records of whereabouts of offspring of positive dogs
Investigation Time Line
April – October, 2012

Child to ER
4/26

Isolate to NYC PHL
5/7

Puppy blood cultures grow B. canis
5/25

IA investigates breeder
6/5

5/2
G- rods growing; 2nd culture collected

5/11
B. Canis identified

Child Treated Epi & Lab investigation

5/31
Puppy from Iowa breeder

6/19

[Logo: Pennsylvania Department of Health]
Pennsylvania Investigation

- **PA family**
  - 5 year old child, pregnant woman
  - No illness
- **Puppy positive by serology**
  - Family chose not to euthanize
    - Ovariohysterectomy
    - Antibiotics
    - Retest
- **Family members**
  - Avoid contact until puppy negative
  - Medical evaluation
  - Family declined testing
Investigation Time Line
April – October, 2012

Child to ER
4/26

isolate to NYC PHL
5/7

Puppy blood cultures grow B. canis
5/25

IA investigates breeder
6/5

PA DOH investigation
6/19

5/2
G- rods growing; 2nd culture collected

5/11
B. Canis identified
Child Treated Epi & Lab investigation

5/31
puppy from Iowa breeder; littermate in PA

6/9
Breeder Quarantined

10/29
Phylogenetic Tree, 34 *B. canis* isolates, US, 2002-2013

*B. canis* (34 entries)

MLVA-15

State
- MA
- CA
- CT
- WA
- NY, girl
- NY, puppy
- MA
- WI
- MS
- unknown
- TX
- MA
- MA
- TX
- WI
- unknown
- unknown
- AZ
- AR
- LA
- AZ
- AL
- OH
- WV
- MI
- SC
- MA
- unknown
- AL
- MA
- unknown
- unknown
- unknown

*unweighted pair-group method using arithmetic averages

**Multi-locus variable analysis
Follow-up, Child

- Blood cultures
  - Negative at 1 and 6 weeks post treatment
- Liver enzymes
  - Normal 1 week post treatment
- Clinical
  - Asymptomatic
Follow-up
Hospital and PHL Laboratories

• Hospital Laboratory A
  – In-service for all workers emphasis on safety
  – Revised protocol for handling and forwarding isolates for identification

• NYC PHL
  – Handled in the General Microbiology Unit
  – Dedicated a BSL2+ room in GMU
    • Preliminary testing of unknown isolates
    • Rule-out *B. anthracis*, *F. tularensis*, *Brucella* spp., *B. mallei/pseudomallei*, *Y. pestis* and *N. meningitidis*
  – Room access limited
B. canis Surveillance, U.S.

• Underreporting suspected
  – Lack of current validated serological tests
    • Cannot use available tests for B. abortus, suis, melitensis
    • Only culture and isolation for confirmation
• Brucellosis is nationally notifiable, but species is not reported so incidence due to B. canis is unknown
• B. canis is not listed as select agent so no reporting through Laboratory Response Network (LRN) occurs
Conclusion

• First documented transmission of *B. canis* from canine to child in US
  – Frequent close contact between puppy and child
  – No additional human cases
• 31 laboratory workers evaluated after exposure
• Areas for improvement in biosafety at two laboratories identified
• Multi-state, multi agency investigation
• Variable state and interstate regulations in the commercial dog-breeding market
CSTE Position Statement on *B. Canis*

- Probable under-recognition
- Evidence of increasing seroprevalence in dogs
- Need to develop human diagnostic assay
- Variable state regulations for control
  - Improved inter-state communication
- Report species when reporting *Brucella* cases in electronic national surveillance systems
Acknowledgements
BOX 1. Recommendations for safe laboratory practices to avoid exposure to *Brucella* spp.

- When brucellosis is suspected, clinicians or forwarding laboratories should note on the laboratory submission: “Suspect or rule out brucellosis.”
- Review laboratory containment methods and microbiologic procedures to ensure compliance with recommendations in the *Biosafety in Microbiological and Biomedical Laboratories, Fifth Edition*.
- Use primary barriers (i.e., safety centrifuge cups, personal protective equipment, and Class II or higher biological safety cabinets [BSCs]) for procedures with a high likelihood of producing droplet splashes or aerosols.
- Use secondary barriers: restrict access to the laboratory when work is being performed and maintain the integrity of the laboratory air-handling system by keeping external doors and windows closed.
- Avoid causing splashes or aerosols when performing procedures on unidentified isolates.
- Prohibit sniffing of open culture plates to assist in the identification of isolates.
- Manipulate isolates of small gram-negative or gram-variable rods initially inside a BSC.
BOX 2. Recommendations for surveillance and postexposure prophylaxis (PEP) after laboratory exposure to *Brucella* isolates

- Evaluate all workers exposed to *Brucella* isolates* and classify exposures as either high risk or low risk.†
- Recommend PEP for workers with high-risk exposures to *Brucella* isolates. PEP should be offered as soon as *Brucella* exposure has been identified, up to the end of the 6-month incubation period.
  - Administer doxycycline 100 mg twice daily and rifampin 600 mg once daily for 3 weeks or doxycycline alone if exposed to *Brucella abortus* RB51 strain, which is resistant to rifampin.
  - Trimethoprim–sulfamethoxazole (160 mg/800 mg) should be considered for patients with contraindications to doxycycline.
  - Pregnant workers with high-risk exposures should be considered for PEP in consultation with their obstetricians.
- Discuss potential PEP with workers who have low-risk exposures to *Brucella* isolates.
- Obtain baseline serum samples from all workers exposed to *Brucella*, unless exposed to *B. abortus* RB51 strain, which does not elicit a measurable serologic response using available assays.
- Arrange for serologic testing on all workers exposed to *Brucella* (e.g., 2, 4, 6, and 24 weeks postexposure) using agglutination testing (e.g., tube or *Brucella* microagglutination testing) at the state public health laboratory or CDC; serologic testing is not recommended for workers exposed to *B. abortus* RB51 strain.
- Arrange for regular (e.g., weekly) active surveillance for febrile illness among all workers exposed to *Brucella* isolates for 6 months after last exposure.

* A *Brucella*-exposed worker is defined as any worker present in the microbiology laboratory during workup and identification of a *Brucella* isolate from the time the culture is first manipulated until all culture isolates are destroyed or removed from the laboratory.
† A high-risk exposure is defined as 1) having direct personal exposure to *Brucella* (e.g., skilling bacteriologic culture; direct skin contact, pipetting by mouth, instillation, or spouting into the eyes, nose, or mouth), 2) performing work on an open bench (i.e., outside of biosafety level 3 containment equipment) with an open culture plate containing a *Brucella* isolate or being in close proximity to such work (e.g., across an open bench top or within 6 feet), or 3) presence in the laboratory during any procedure conducted on a *Brucella* isolate that might result in generation of aerosolized organisms and inhalational exposure (e.g., vortexing or catalase testing). A low-risk exposure is defined as being present in the laboratory during an exposure but not meeting the definition for a high-risk exposure.
The Global Incidence of Human Brucellosis

<table>
<thead>
<tr>
<th>Species</th>
<th>Biovar/Sero var</th>
<th>Natural Host</th>
<th>Human Pathogen</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. abortus</em></td>
<td>1-6, 9</td>
<td>cattle</td>
<td>yes</td>
</tr>
<tr>
<td><em>B. melitensis</em></td>
<td>1-3</td>
<td>goats, sheep</td>
<td>yes</td>
</tr>
<tr>
<td><em>B. suis</em></td>
<td>1, 3</td>
<td>swine</td>
<td>yes</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>hares</td>
<td>yes</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>reindeer, caribou</td>
<td>yes</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>rodents</td>
<td>yes</td>
</tr>
<tr>
<td><em>B. canis</em></td>
<td>none</td>
<td>dogs, other canids</td>
<td>yes</td>
</tr>
<tr>
<td><em>B. ovis</em></td>
<td>none</td>
<td>sheep</td>
<td>no</td>
</tr>
<tr>
<td><em>B. neotomae</em></td>
<td>none</td>
<td>Desert wood rat</td>
<td>no</td>
</tr>
<tr>
<td><em>B. maris</em></td>
<td></td>
<td>marine mammals</td>
<td>?</td>
</tr>
</tbody>
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## Common Clinical Presentation in Humans

<table>
<thead>
<tr>
<th>Symptom</th>
<th>% with symptom (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>66% (21)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>34% (11)</td>
</tr>
<tr>
<td>Headache</td>
<td>31% (10)</td>
</tr>
<tr>
<td>Chills</td>
<td>28% (9)</td>
</tr>
<tr>
<td>Weight loss</td>
<td>28% (9)</td>
</tr>
<tr>
<td>Malaise</td>
<td>22% (7)</td>
</tr>
<tr>
<td>Sweats</td>
<td>22% (7)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>16% (5)</td>
</tr>
<tr>
<td>Cough</td>
<td>13% (4)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>6% (2)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sign</th>
<th>% with sign (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Splenomegaly</td>
<td>5</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>7</td>
</tr>
<tr>
<td>Osteomyelitis (frontal bone)</td>
<td>1</td>
</tr>
<tr>
<td>Endocarditis</td>
<td>2</td>
</tr>
<tr>
<td>Septic arthritis</td>
<td>1</td>
</tr>
<tr>
<td>Mycotic aneurysms</td>
<td>1</td>
</tr>
</tbody>
</table>

*Compiled data of symptoms and signs from 32 human cases reported in the literature*

*Presentation similar to brucellosis caused by other species- *B. abortus, melitensis, suis*
Prevention and Control
Mitigating Public Health Risks to Staff Working in Kennels and Pet Owners

• Compilation of published recommendations*
  – Prevention of *B. canis* in kennels
  – Controlling outbreaks of *B. canis* in kennels
  – Management of pets that test positive for *B. canis*

*Hollett, 2006; Marley & Rynders, 2007; Shin & Carmichael, 1999;
Prevention and Control
Prevention Strategies in Kennels

- Quarantine new dogs entering kennel until they test negative
- Test twice at least 4-6 weeks apart
  - May also perform blood cultures
- All breeding dogs should be tested once a year
  - Optimum time for testing females- 3 wks before estrus
    - Most likely time to be shedding if infected
    - Allows for second test before breeding if first test is positive
- Dogs testing positive
  - Retest with different test
  - Euthanasia
Prevention and Control
Outbreak Management in Kennels

- Quarantine dogs by housing individually
- Serial testing of dogs
  - Dogs greater than 6 wks of age
    - Serology-RSAT or AGID
    - Culture and isolation for confirmation
  - Puppies less than 6 wks of age
    - 3 cultures taken at least 24 hrs apart
- Test adults (> 6 wks old) monthly for minimum of 3 months
- Test until all dogs are negative for 2 consecutive tests
- Euthanize dogs testing positive by serology or culture
- Disinfect premises with commonly available detergents wearing appropriate personal protective equipment (PPE)
Prevention and Control
Management of Pets

• Pets that test positive for *B. canis*
  – Euthanasia- best option for eliminating public health risk
  – If owner refuses euthanasia
    • Isolate infected dog from other dogs
    • Spay or neuter to remove organs with affinity for *B. canis* and decrease risk of transmission
    • Treatment – outcome uncertain
  – Testing- culture or AGID
    • End of treatment and 1, 3, and 6 mos. post-treatment
    • If positive, repeat treatment or consider euthanasia
Prevention and Control
Outbreak Management in Kennels

– Quarantine dogs by housing individually
– Serial testing of dogs
  • Dogs greater than 6 wks of age
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Prevention and Control
Public Health Risks- Summary

• Even with repeated testing- may be difficult to conclude that dog testing negative for *B. canis* is not infected

• In kennel situation- widely accepted recommendation is for euthanasia of dogs that test positive for *B. canis*

• For privately owned dog- owner must be informed of potential risk of transmission in spite of treatment

• Groups at higher risk of infection – children, pregnant women, immunocompromised persons
NYC Case, Time Line
April – October, 2012

Child with respiratory illness seen in NYC ER, admitted

NYC PHL notifies BCD of B. canis

CDC confirms that puppy’s and child isolate nearly identical

IA investigates breeder

PA DOH investigates PA family who purchased the puppy’s littermate

NYS DAM determined that puppy’s littermate purchased by PA family; puppy originated from Iowa breeder

Puppy blood cultures grow B. canis at NY State Veterinary Diagnostic Center

4/26/12
5/7/12
5/11/12
5/31/12
6/19/12
6/19/12
5/2/12
5/11/12
5/25/12
6/5/12
10/29/12

Hospital lab sends isolate to NYC PHL for identification
NYC PHL notifies CDC of B. canis
Puppy blood cultures grow B. canis at NY State Veterinary Diagnostic Center
CDC confirms that puppy and child isolate nearly identical
NYC Case, Time Line
April – October, 2012

Child to ER
4/26

1st isolate to PHL; 2nd isolate G-rods
5/7

Puppy blood cultures grow B. canis
5/25

IA investigates breeder
6/5

PA DOH investigation
6/19

CDC confirms isolates same
10/29

5/2
G- rods growing; 2nd culture collected

5/11
B. Canis identified; Child Treated Epi & Lab investigation

5/31
puppy from Iowa breeder; littermate in PA

6/9
Breeder Quarantined
Serologic Studies, Puppy
Animal Health Diagnostic Center, Cornell
College of Veterinary Medicine

- Microscopic slide = 4+ positive
- AGID2 = negative

- Interpretations:
  1. The dog is not infected with Brucella canis (the slide is a screening test and there are false positives)
  2. The dog is acutely infected and the AGID2 requires from 8 to 12 weeks post exposure to become evident on this test
Additional notes – environmental persistence

- Environmental persistence
  - Fomites- *B. canis* can survive well in high humidity, low temperatures, lack of sunlight

- Female dogs may shed *B. canis* for weeks to months after abortion through vaginal discharge

- Male dogs may shed *B. canis* in urine, organism present in seminal and prostatic fluid
Additional notes, serologic assays

B. canis requires a specific test as it does not have a smooth lipopolysaccharide cell wall.
Common serological tests for Brucella do not identify antibodies to B. canis because it does not have a smooth LPS.
The tests were developed in house, probably quite accurate, but definitely not licensed as diagnostic tests for human use at the time.
Investigation Time Line
April – October, 2012

- Child to ER: 4/26
- Isolate to NYC PHL: 5/7
- B. Canis identified: 5/11
- G-rods growing; 2nd culture collected: 5/2
- 2nd culture collected: 5/25
- Child Treated: 5/31
- Epi & Lab investigation: 6/5
- Puppy blood cultures grow B. canis: 6/5