



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

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Brucellosis

- Zoonotic bacterial disease
 - Worldwide
 - Gram negative, aerobic, intracellular coccobacilli
- Several species
 - Abortus, melitensis, 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
 - 7days 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

Canis
identified;
child Treat
& Lab
investigati



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



[MMWR Morb Mortal Wkly Rep.](#) 2008 Jan 18;57(2):39-42.

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

isolate to
NYC PHL

4/26

5/7

5/25

6/5

5/2

G- rods
growing;
2nd
culture
collected

5/11

B. Canis
identified
Child Treated
Epi & Lab
investigation

5/31



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



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

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Child to ER
4/26

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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;
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5/11

B. Canis identified

Child Treated
Epi & Lab investigation

5/31

puppy from Iowa breeder ;
littermate in PA

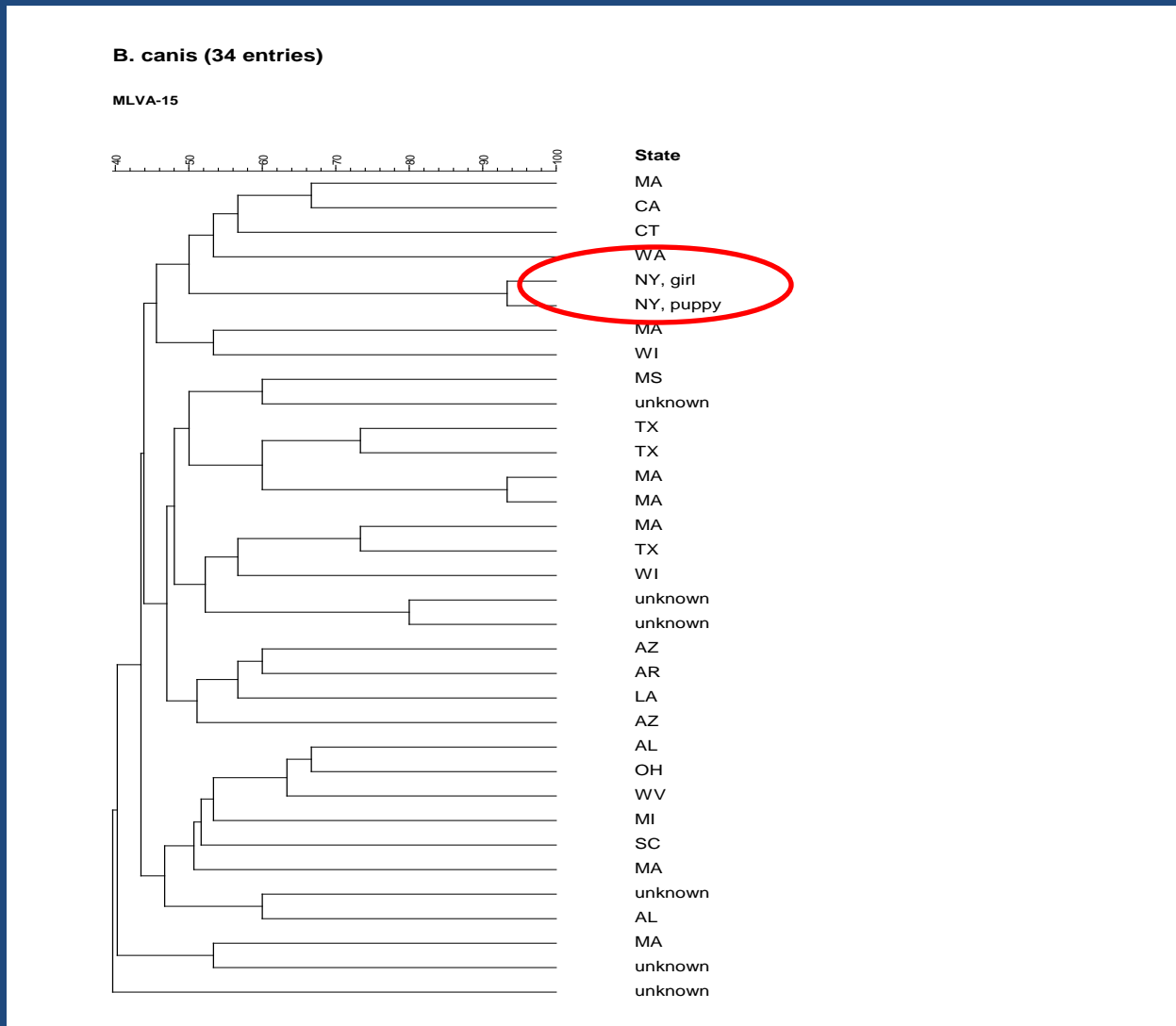
6/9

Breeder Quarantined

10/29



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



*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

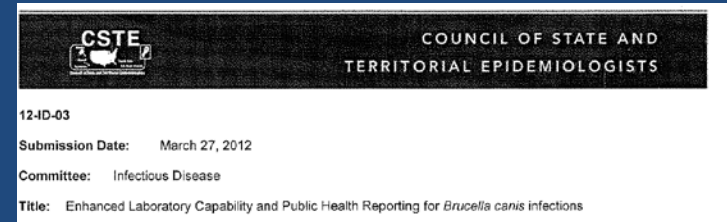
B. canis Surveillance, U.S.

- 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



Extra Slides

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.

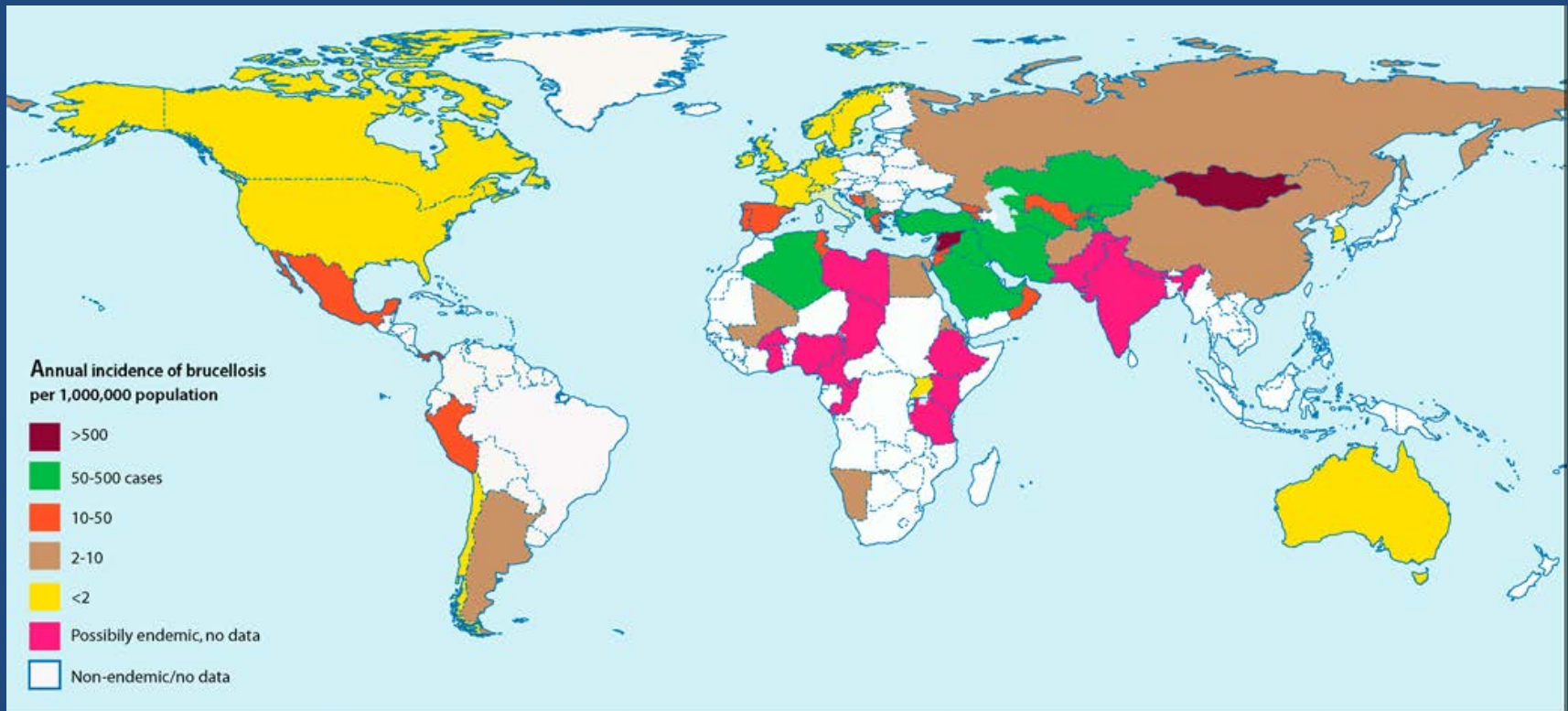
BOX 2. Recommendations for surveillance and postexposure prophylaxis (PEP) after laboratory exposure to *Brucella* isolates

- 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., staffing bacteriologic cultures, direct skin contact, pipetting by mouth, inoculation, or spraying 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 5 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



Reproduced from: Gutierrez Ruiz C, Miranda JJ, Pappas G (2006) A 26-year-old man with sternoclavicular arthritis. *PLoS Med* 3(8): e293. doi:10.1371/

journal.pmed.0030293 Derived from: Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsianos EV (2006) The new global map of human brucellosis.

Lancet Infect Dis 6: 91-99.

Brucella spp

| Species | Biovar/Sero var | Natural Host | Human Pathogen |
|----------------------|------------------------|---------------------|-----------------------|
| <i>B. abortus</i> | 1-6, 9 | cattle | yes |
| <i>B. melitensis</i> | 1-3 | goats, sheep | yes |
| <i>B. suis</i> | 1, 3 | swine | yes |
| | 2 | hares | yes |
| | 4 | reindeer, caribou | yes |
| | 5 | rodents | yes |
| <i>B. canis</i> | none | dogs, other canids | yes |
| <i>B. ovis</i> | none | sheep | no |
| <i>B. neotomae</i> | none | Desert wood rat | no |
| <i>B. maris</i> | | marine mammals | ? |

Common Clinical Presentation in Humans

* Symptom % with symptom (n=32)

| | | |
|-------------|-----|------|
| Fever | 66% | (21) |
| Fatigue | 34% | (11) |
| Headache | 31% | (10) |
| Chills | 28% | (9) |
| Weight loss | 28% | (9) |
| Malaise | 22% | (7) |
| Sweats | 22% | (7) |
| Vomiting | 16% | (5) |
| Cough | 13% | (4) |
| Diarrhea | 6% | (2) |

* Sign % with sign (n=32)

| | |
|---------------------------------|---|
| Splenomegaly | 5 |
| Lymphadenopathy | 7 |
| Osteomyelitis (frontal bone) | 1 |
| Endocarditis | 2 |
| Septic arthritis | 1 |
| Mycotic aneurysms | 1 |

- 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
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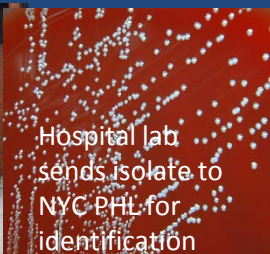
Prevention and Control

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



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



NYC Case, Time Line

April – October, 2012



| | | | | | |
|-------------|--|---|-------------------------|----------------------|----------------------------|
| Child to ER | 1 st isolate to PHL; 2 nd isolate G-rods | Puppy blood cultures grow <i>B. canis</i> | IA investigates breeder | PA DOH investigation | CDC confirms isolates same |
| 4/26 | 5/7 | 5/25 | 6/5 | 6/19 | 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.

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