

Canine brucellosis: Outbreaks and compliance

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Abstract

Canine infertility has many causes that must be considered during evaluation of abnormal reproductive function. An important infectious agent is *Brucella canis*. Classically deemed a major reason of abortion, this organism also produces infertility in stud dogs and poses a potential health hazard to dogs and humans. The State of Georgia has, out of necessity, instigated regulations to manage outbreaks and seek compliance by educating the pet owner population about this disease. A review of its etiology, methods of transmission, pathophysiology, clinical signs, diagnosis, serology and culture, pathology, treatment options, and regulated prevention featured by Georgia, are presented.

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1. Introduction

Dogs can be infected by four of the six species of *Brucella* (*Brucella canis*, *Brucella abortus*, *Brucella melitensis* and *Brucella suis*, excluding *Brucella ovis* and *Brucella neotomae*). The first case of *B. suis* infection in a dog was published in 1931 [1]. Outbreaks of canine abortions had been reported in 1963, but it was not until 1966–1967 that *B. canis* was isolated from canine tissue and vaginal discharges [2–5].

B. canis is a small, rough or mucoid, Gram-negative intracellular bacterium with the dog as its reservoir host. It has similar antigenic properties to *B. ovis*, which has been used in the rapid slide agglutination test (RSAT) described later. *B. abortus* can infect dogs that ingest aborted or fetal tissue from infected livestock. Intact dogs have the potential to

harbor and transmit this organism through natural mating, oronasal contact and ingestion of contaminated tissue or fluid. Clinical signs range from asymptomatic, lymphadenopathy, orchitis and epididymitis, embryonic loss, to abortion and testicular atrophy. No single antibiotic should be used, but combination therapy has been helpful. However, with the possibility for relapse (even if neutered or spayed), the best treatment is removal from the facility or euthanasia. Better management skills are imperative to avoid financial indebtedness from months of quarantine and lost income. Serological testing before entry and breeding will lower the anxiety among owners and also reduce the incidence in many breeds. For >2 years, the State of Georgia implemented requirements for licensed kennels that enforced depopulation in the face of outbreaks and testing to remove suspected dogs until cleared. With the cooperation and education of animal owners and practitioners, this silent bacterium can be monitored and prevented from creating spontaneous abortion and sterile males.

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2. Epidemiology

Members of the canidae family are the reservoir hosts of *B. canis*. Historically, infection was originally associated in the mid 1970s with the beagle breed [6–9]. This may have been accentuated because of this breed's popularity as research animals and in field trials. The list of breeds now includes Labrador Retrievers, Cocker Spaniels, German Shepherds, Boston Terriers, Poodles and more [Appendix A]. Despite a higher prevalence in purebred dogs, the mixed breed mongrel or any sexually mature, reproductively active dog is susceptible [10,11]. In the environment, stray and feral dogs remain predominant reservoirs [12–16].

A predominant route is venereal transmission where the likelihood for spread remains high due to large numbers of organisms shed in reproductive secretions. One study suggested that dogs do not seem to infect the same gender when housed in close contact [17]. However, other reports had male kennelmates becoming infected by being housed for an extended time in close quarters with a shedding male [18,19]. Urine may be a less important route in its natural spread [18,20,21] but does not contain a low amount of bacteria during the first weeks to 3 months following infection. The urine becomes the contaminated vehicle by the close anatomical connection of the bladder to the secretory prostate and epididymis.

B. canis infection has been diagnosed in many geographical areas, with a particular prevalence for rural southeastern United States. It occurs in wild dog packs, new untested animals, kennels, 'puppy mills' and even backyard mistakes. A study of stray dogs in Tennessee demonstrated a greater than three-fold rate of infection versus non-stray dogs [22,23]. Reports document worldwide outbreaks from Alabama [24], Mexico [25], Britain [26], Europe [27], Brazil [28], Texas [29], Colorado [30], Illinois and Wisconsin [31,32], Michigan [33], Ontario [34] and Quebec Canada [35], Japan [8], China [36], and Georgia [37–39].

Asymptomatic dogs harbor *B. canis* organisms for prolonged intervals. The time from initial exposure to a bacteremia is approximately 3 weeks and then the organisms localize in targeted genital tissues to seed a continuous or recurrent release that can last from months to years. The male prostate and epididymides serve as effective sites for bacterial emissions. These two tissues are focal sites for widespread dissemination if the male remains actively breeding. Initial semen sampling has a higher concentration of bacteria for the

first 2 months post infection, followed by a sporadic output of lower numbers for years, and the host displaying no apparent illness.

In a kennel environment, the aborting bitch is at high risk for the spread of infection. A characteristic, lengthy vaginal discharge of infective uterine secretions persists for 4–6 weeks following a single abortion. Large numbers of organisms are present in the aborted placental tissues and fluids; two million colony forming units in an infective dose or "10¹⁰ organisms/mL in discharge which constitutes 500 oral infective doses/mL" [20].

B. canis is also found in the milk of infected lactating bitches [6,40] that might lead to the potential infection of nursing pups. Artificial sources for transmission are blood transfusions, vaginoscopy, AI, and contaminated syringes [6].

3. Pathophysiology

The bacteria attach to an exposed mucous membrane, penetrate the tissue; thereafter, more bacteria attach, phagocytosis continues, and virulence increases. These bacterial inclusions travel to the lymph nodes, replicate and start a bacteremia within 7–30 days. These intracellular *B. canis* bacteria target reproductive (steroid-dependent) tissue. In the male, these are the prostate, testicle, and epididymides. The female has the fetus, gravid uterus, and placenta. Bacteria are found in fetal stomach contents, suggesting that pups swallow the amniotic fluid and bacteria in utero [20]. The aborted placenta has focal coagulative necrosis of the chorionic villi, necrotizing arteritis, and numerous bacteria in trophoblastic epithelial cells.

Other body systems are seeded by the blood-borne bacteremia such as the intervertebral disks, and kidney or form antigen–antibody complexes in the anterior uvea of the eye [41,42]. Histopathologic findings are reticular cell hyperplasia in lymph nodes and a granulomatous response in skin, testis and organs [20].

Bacteremic episodes can last for years. Experimentally infected dogs remained positive in blood cultures for 5.5 years [43]. In the first 3–4 months of infection, bacteremia declines, and titers [3] reflect persistent bacteremia and/or organisms in sequestered organs or targeted gonads [12]. The cellular damage from inflamed epididymides induces a sperm granuloma from leakage of antigenic material into the surrounding tunic that stimulates antisperm antibodies, humoral and cellular immune responses. Consequently, the semen consists of sperm with abnormal morphology, agglu-

mination or absence of sperm [44,45]. The antibody is directed against sperm and not *B. canis*.

Spontaneous recovery can occur naturally 1–5 years after the initial infection [46]. The dog becomes abacteremic with low agglutination titers of 1:25 or 1:50 [20,47] that suggest clearance of the bacteria. The titer does not rise again if challenged, and no reinfection occurs because of a developed cellular immunity from this natural recovery. Negative blood culture correlate with decreased serum agglutination titer, even in some cases where *B. canis* persists in body tissue.

4. Clinical signs

The range of signs varies from asymptomatic to mild despite an ongoing systemic infection. Morbidity is high but mortality is low. Owners notice subtle or vague signs, such as a poor hair coat for a show animal, listlessness, fatigue, lethargy, exercise intolerance during a field trial, weight loss, lameness, back pain, lymphadenopathy, and behavioral changes (i.e., not alert, poor performance of trained tasks).

Symptoms not just associated with canine brucellosis for the bitch include infertility, apparent failure to conceive, early embryonic death (EED), fetal resorption, failure to whelp or worse, late-term abortion without any indication or not detected by the owner. The intact stud dog will have a painful scrotal enlargement or testicular atrophy, moist scrotal dermatitis, a decreased volume of ejaculate, loss of libido, reluctance to breed, or poor semen quality with WBCs and a high percentage of morphologically abnormal sperm, especially if examined within the first 3 months of an infection [48]. Either gender can develop signs of diskospondylitis, meningoencephalitis, or uveitis. These signs may be noticed during routine survey films or on a physical examination for a scheduled certification.

No clinical sign(s) is/are pathognomonic for canine brucellosis, but it should always be a primary consideration in dogs examined for reproductive failure or infertility. Infected dogs are not seriously ill; no deaths are directly caused by its inflammatory cellular response. Fever is uncommon because this bacterium lacks the lipopolysaccharides present in the other strains of *Brucella* to produce endotoxins [20,49]. The bacteria have little somatic antigen and eludes the immune system.

The organisms are imbedded in macrophages (intracellular) which cause generalized lymph node enlargement from diffuse lymphoid and reticuloendothelial cell hyperplasia. Besides lymph nodes, the

spleen and liver may become enlarged. The granulomatous reaction also makes the spleen firm and nodular.

4.1. Specific signs

Reproductive failure means the certainty of abortion, epididymitis, orchitis, and testicular atrophy. The most common, visible clinical sign witnessed by the owner is the spontaneous abortion of a supposedly healthy pregnant bitch. The loss occurs between 45 (mid) and 59 days (late) gestation. Brucellosis can also result in resorption or early embryonic death within the early weeks after breeding. The female is then deemed infertile, since no outward signs of fetal death were seen and since every bitch is outwardly diestrus whether internally pregnant, pseudopregnant or not. Pups are lost as early as 20 days or are carried nearly to term. She may deliver a normal litter the next pregnancy [20] or give birth to living, partly autolyzed, stillborn and normal pups that die within hours. Any surviving pups are bacteremic for a minimum of several months.

Following abortion, a prolonged, viscous, serosanguinous vaginal discharge can last for 1–6 weeks. The large number of bacterial colonies contained within this lochia is of great concern for possible contact with owners and other dogs. Extreme caution should be taken to prevent humans or dogs from ingestion, inhalation and contact with aborted tissue or fluid.

Brucellosis does not change the exhibition of estrus [50] and breeding. The bitch can abort two to three litters in succession, continue to be bred and have a normal litter later.

B. canis targets androgen-dependent organs in the stud dog (i.e., epididymis, prostate). Acute onset of inflammation with associated pain and swelling produces the orchitis or epididymitis [51–54] detectable on physical exam. Scrotal dermatitis develops from the constant licking by the male seeking relief [52]. Chronic or prolonged infection in the stud dog eventually lead to uni- or bilateral testicular atrophy [46,54] along with a reluctance to breed and/or loss of libido from the painful experience. Early inflammation of bacterial infections stop spermatogenesis in the seminiferous tubules; in some dogs, fertility is restored, whereas others become sterile. The male breeding soundness evaluation records a history of failure to achieve intromission from pain, unwillingness to ejaculate, or successful internal ties without a pregnancy. Semen collection and examination determine whether the male has oligozoospermia, teratozoospermia or azoospermia in the ejaculate, and an unsatisfactory classification. After 3 months, *B. canis* can be grown from the semen of infected dogs [56].

Within 5 months of onset, a majority of abnormal sperm appear with head-to-head spermagglutination from serum antibodies in the seminal plasma [54,55].

5. Other organs

Body systems are influenced by the bacteremia. Discospondylitis of the thoracic and/or lumbar vertebrae [57–60] are visualized by radiography, sometimes an incidental finding while performing a survey film for a diagnostic workup. The dog may be presented with a history of stiffness, lameness or paraspinal pain with paresis or paralysis, which may occasionally be linked directly to a previously treated orchitis [58], or an osteomyelitis subsequent to a total hip replacement [61]. Ophthalmological exams may reveal endophthalmitis and recurrent uveitis from immune complex deposits in the eye [41,42,62], even in a spayed female [63], which are confirmed by a positive culture or serology. A low-grade, nonsuppurative meningitis has been reported [20]. A spayed female with a chronic vaginal discharge had a positive serum titer and hemoculture from an abscessed uterine stump 3 years after a hysterectomy [64].

6. Diagnosis

Canine brucellosis cases have histories of infertility, abortion, enlarged lymph nodes(s), swollen scrotum or tail of the epididymis, abnormal sperm, testicular atrophy or no apparent clinical signs [47]. A differential diagnoses for infectious infertility to rule out a viral agent (canine herpes), protozoan (*Neospora caninum*, *Toxoplasma gondii*), and bacterial etiology (*Mycoplasma*, *Ureaplasma*, *E. coli*, *Streptomyces*, *Salmonella*, *Campylobacter*, and *B. canis*) [65]. Definitive diagnosis rests with obtaining the bacterium from a tissue, discharge, blood, semen, vertebra, or eye. Supportive evidence comes from positive serologic agglutination testing, other serologic titers and hemoculture results. Difficulty lies with the fluctuant level and length of the bacteremia in the dog [66]. Just because one blood culture is negative does not eliminate *B. canis* as the etiology.

A thorough physical exam provides base information on weight, vision, locomotion, discharge and palpable swellings. Routine samples of blood for CBC and *B. canis*, chemistry profile and cystocentesis should be submitted. Hematologic findings may be within reference ranges and unremarkable. A breeding soundness examination (BSE) for the bitch includes a vaginal culture and cytology, vaginoscopy and trans-

abdominal ultrasound of the visible reproductive tract. The stud dog's BSE pursues the cause(s) for a swollen scrotum, scrotal dermatitis, firm epididymides, uni or bilateral testicular atrophy and pain by serial semen collections and testicular ultrasound. A testicular biopsy should be delayed until the initial test results are returned. It is not necessary if serologic results indicate brucellosis and instead, castration is recommended. Lower motility (asthenozoospermia) and sperm abnormalities are observed within 2 months of infection. Immature spermatozoa contain proximal and distal cytoplasmic droplets and acrosome deformities. By 4 months the severity is noticed with head-to-head agglutination and inflammatory WBCs. As time allows, the necrotizing vasculitis and inflammation to invade the testes (orchitis), a palpable enlargement of the tail of the epididymis and testicular atrophy ends with loss of seminiferous tubules and absence of sperm in the ejaculate.

6.1. Serology

B. canis has a rough and not a smooth cell wall antigen as do *B. suis*, *B. abortus* and *B. melitensis* [67]. The serology examines the agglutinating reaction to that cell wall or cytoplasmic protein antigen. Results may be negative during the first 3–4 weeks of infection, although the dog is undergoing a bacteremia by 2 weeks [46]. Possible tests are the rapid slide agglutination test (RSAT and ME-RSAT), the tube agglutination test (TAT), indirect fluorescent antibody (IFA), cell wall agar gel immunodiffusion (AGIDcwa), cytoplasmic agar gel immunodiffusion (AGIDcpa), enzyme-linked immunosorbent assay (ELISA), and polymerase chain reaction (PCR). Blood culture is definitive proof of infection, and it may yield positive results as early as 2–4 weeks post infection. The dog can remain positive for several years. A delay in serum titer is possible for 8–12 weeks after exposure to the antibody; the titer can fluctuate even with a persistent bacteremia. The magnitude of the titer does not reflect the stage of disease.

6.1.1. RSAT or card test

The RSAT or card test is a rapid screening test developed in 1974 [68] and is commercially available (D-Tec CB, Synbiotics, San Diego CA, USA) and practical with results within 2 min. Agglutination is detectable 3–4 weeks after the onset of infection. *B. ovis* is used as the antigen since its similarity to *B. canis* and ease in manufacturing [37,68]. The patient's serum is mixed with a rose-bengal stained heat-killed *B. ovis*

suspension on a card. If the precipitate on the test side is similar to the known standard, a positive interpretation is made and a more specific test ordered. RSAT is considered highly sensitive (i.e., detects truly infected) but not specific (i.e., defines difference between true positive and true negative). It is rare for false negatives [47,69], but as many as 50–60% false positives do occur [37,47]. RSAT is not definitively diagnostic since cross-reaction occurs between the *B. ovis* cell wall antigen IgG IgM and the antibody of *Bordetella*, *Pseudomonas*, *Moraxella*-type organism, and other Gram-negative bacteria [12,56]. If the test is negative, the dog does not have brucellosis. Conversely, if the test is positive, the dog should be isolated and rechecked with a more specific test.

6.1.2. ME-RSAT

The modified RSAT (ME-RSAT) adds 2-mercaptoethanol (2-ME) drops to inactivate IgM mentioned above and thereby increases the specificity of the test. ME-RSAT is another screening test and useful as a subsequent test for a positive RSAT serum [70]. *B. ovis* antigen is replaced by *B. canis* cells to reduce the number of false positives. It is semiquantitative. An animal can be positive for 30 months after becoming abacteremic, although false negatives can occur during the first 8 weeks post infection.

6.1.3. TAT

The Tube Agglutination Test (TAT) detects antibodies to *B. canis* in dogs that test positive with RSAT or ME-RSAT. A confirmatory titer from this test is positive 2–4 weeks following exposure or bacteremia. Graded amounts of test serum are added to the diluted *B. canis* antigen. The antigen solution is a suspension of heat-killed and washed *B. canis* bacteria. Results are semiquantitative with a 1:200 titer presumptive evidence of an active infection [23,32,47,57]. There is good correlation between a titer $\geq 1:200$ and the organism being recovered via blood culture. Measurable titers below 1:200 should be rechecked 2 weeks later. The test is sensitive but not specific (allows for false positive results).

Although the RSAT, ME-RSAT and TAT detect agglutinating antibodies from the dog, those antibodies do not protect the dog itself from infection [6]. In that regard, the dog remains bacteremic for years despite a positive agglutinin titer [20,71].

6.1.4. AGIDcwa and AGIDcpa

The Agar Gel Immunodiffusion test (AGID) is used to confirm suspected cases from RSAT, ME-RSAT and

TAT [47]. Seven wells, six peripheral and one central, are cut into agarose gel to which test serum, a known positive, or a negative sera are placed in peripheral wells with the *B. canis* antigen in the middle. Antibodies diffuse inward to meet the outbound antigen and form a distinct, visible, smooth precipitin arc [72]. Two antigens are used, a cell wall or a cytoplasmic protein. A lipopolysaccharide antigen from the bacterial cell wall is less specific than the internal cytoplasmic antigen. The AGIDcwa is a highly sensitive test but still allows false positives. Results are positive at 8–12 wk and negative from 3 to 4 years. The cytoplasmic antigen is comprised of soluble proteins that were extracted from *B. canis* or *B. abortus*. This most specific, least sensitive antigen reacts with antibodies against *Brucella* species (*canis*, *abortus*, *suis*). Tests are negative in early infections when other analyses are positive. Similar to the cell wall antigen, a dog has reactive antibodies 4–12 weeks after infection that persist, and the dog reacts positively 8–12 weeks later and for ≥ 5 years.

A limited number of veterinary diagnostic laboratories are capable of conducting AGID, which requires trained personnel and special media. Currently, the Tifton Veterinary Diagnostic and Investigational Laboratory in Georgia has been added to Cornell University and the University of Florida as qualified locations.

6.1.5. IFA and ELISA

The Indirect Fluorescent Antibody (IFA) test and enzyme-linked ELISA became alternatives as test materials for RSAT and TAT were unavailable. Because the sensitivity of the IFA is uncertain, some infected dogs may go undetected [46]. The ELISA has a cell wall antigen for specificity, and results are positive within 30 days of infection.

6.1.6. Diagnostic imaging

Radiographic findings can be suspected lesions of *B. canis*. Unifocal or multifocal inflammation of intervertebral disk (i.e., disk space) with unaffected vertebral architecture is visual evidence of discospondylitis. Skeletal limb abnormalities may be indicative of osteomyelitis, or a soft tissue involvement diagnostic for stump pyometra. Any nonreproductive lesion should be confirmed by antibody testing and/or culture.

Thorough ophthalmological exams done either for visual deficit or breed certification can detect a uveitis and accompanying lesions. Real-time ultrasonography during the male breeding soundness evaluation identifies changes associated with inflammation or atrophy of the epididymides and testes, respectively.

6.1.7. Blood culture

A positive blood culture is the definitive diagnosis for the suspected animal [24,30,46,73]. Dogs are bacteremic 2–4 weeks after oral nasal exposure and for the next 1–3 years. The number of organisms circulating in the leukocyte portion of the blood is often small, therefore, multiple samples of whole blood may be required. Direct culture is difficult because *B. canis* is a fastidious organism that may not be present in that drawn sample, especially if the animal has received previous antibiotic therapy.

Bacterial growth would be important proof for kennel situations. Organisms can be isolated from milk, a vaginal discharge after abortion, placental and fetal tissue, semen, prostatic fraction, lymph nodes, bone marrow, and urine. Organisms have been cultured from discospondylitis, eye lesions or a uterine stump [41,42,64]. The urine from experimental dogs was culture positive [4] with males shedding greater number of organisms than females [21], and some dogs having a negative blood culture [17]. A caution is given with the culture from voided urine samples and possible contaminant overgrowth. Negative cultures do not rule out the disease.

7. Pathology

Gross pathological findings are enlarged lymph nodes and spleen. The majority of diagnostic lesions are found by histopathologic examination. The bacteria is an intracellular organism that causes cellular inflammation which makes granulomatous lesions by the associated necrotizing vasculitis; this response produces the lymphadenopathy, splenomegaly, scrotal edema and dermatitis, epididymitis, orchitis with subsequent uni- or bilateral testicular atrophy, anterior uveitis, and discospondylitis.

8. Treatment

Treatment for *B. canis* is not encouraging. The bacteria is sequestered inside cells for extended periods, and bacteremia is episodic. The organism is sensitive to a variety of antibiotics, but drug therapies have had failures and relapses. Studies report that a single antibiotic regime is unsuccessful [46,47,74] and not recommended. Specific antibiotics are better, but antimicrobials are not a curative process. Better success has come from a combination of antibiotics of the tetracyclines (tetracycline HCl, chlortetracycline, doxycycline, minocycline) and dihydrostreptomycin. Due to the unavailability of streptomycin, gentamicin has

been substituted, but first check for renal disease. The disadvantages of antibiotics are expense, lengthy regime, declining owner compliance, and uncertain results with accessibility to an intracellular organism [75] or the prostate of the stud dog. Two or three courses of therapy separated by 1–2 months may be required. A 90 days treatment is then followed by more testing, and retreat if positive. If the AGID is negative, then stop antibiotics and retest in 3–6 months. Relapse of the disease is possible. Testing will continue indefinitely. Treatments with antibiotics lower the bacteremia and thus give a false-negative serologic result. Two weeks of tetracycline therapy will cause antibody titers to decrease; however, after the therapy is stopped, a relapse occurs, and the antibody titer rises again.

9. Prevention

Serologic testing is more accurate near or during estrus since the bacteremia is elevated under hormonal influence [46]. All positive dogs must be eliminated or removed by euthanasia. New additions to a kennel should be isolated for at least 1 month [18,24,32,73,76] and have two negative titers 1 month apart before being introduced into a kennel [24,32]. Intact positive dogs should not be bred. Exposure to an infected dog is avoided and separation by partial walls is inadequate [76]. Aborting females may deliver subsequent normal litters and transmit infection to her offspring. Infected intact animals should be spayed or neutered, however, each is likely to carry a positive titer for years.

Brucellosis does not acutely threaten a dog's life, but the results from its reproductive failure can alter an owner's stability. The financial loss from aborted progeny is compounded by depletion of breeding potential for a professional kennel. Stray and feral dogs change a family pet's longevity while maintaining a reservoir of infection through incidental matings. A canine vaccine is unavailable, unlike the recombinant strain for cows. Breeding facilities should be required to test annually. If a kennel is contaminated, all dogs are tested. Positives animals are isolated and monthly tests submitted until all positive dogs have been removed from further contact. Removal of dead or potentially infected tissue is handled with gloves and appropriate clothing. Direct contact by dogs through wire fencing is modified. Two negative tests at a monthly interval release the facility from quarantine. *B. canis* cannot survive very long outside the dog. Disinfectants will cleanse the premises (e.g., quaternary ammonium, 1% sodium hypochlorite or bleach, iodophor solutions, 70% ethanol, or formaldehyde).

Sample and testing consistency should be maintained by referring serum or cultures to a competent laboratory. Every dog that has been boarded, shown or in field trials for a lengthy time, any male with scrotal enlargement, dermatitis, pain, reluctance to breed and poor semen quality, every dog prior to breeding, and every female with a history of abortion or suspected infertility should be checked. It is safe to repopulate a kennel after two negatives tests on all dogs at the premises.

10. Public health

Transmission to humans is rare, with only 30 cases reported worldwide since the isolation of *B. canis* in the late 1960s [2–5]. *Brucella* causes undulant fever in humans [15] or non-specific signs of recurrent fever, headache and weakness [77]. A laboratory worker was exposed to a less virulent M-strain of *B. canis* as an antigen for serological testing [77]. Through close contact to the family pet, an owner became infected [[15], GDA communication]. The disease has been associated with ocular lesions [78] and endocarditis [79] in humans. Brucellosis has greater impact for the immunosuppressed individual (e.g., cancer patient, HIV or transplantation), children and pregnant women. Public health concern becomes manifested in the prevention of contact and potential transmission to other humans through the improper transfer of untested breeding stock, the naïve acquisition of a pregnant purebred, or the entrance into a closed yard by a stray female or male. Kennel personnel and dog owners place themselves at risk with unknown medical histories of their brood animals.

11. Georgia

The State of Georgia acquired regulatory authority of licensed facilities in 2003 (www.agr.georgia.gov Animal Protection Act). Personal pets are now included in those regulations. Reports of positive tests for *B. canis* were being received from both veterinary diagnostic laboratories and veterinarians (Appendix B). In an effort to protect the public health and consumer, the State Veterinarian's office in the Georgia Department of Agriculture (GDA) initiated a protocol and began enforcing those guidelines through quarantine by inspectors of the Animal Health Division. Sixteen facilities and/or kennels have been placed under quarantine since 2003. The standard operating procedure became the fact sheet (Appendix C), a public document sent to veterinarians and owners. Dog owners

bought the problem into the state with the entry of new brood stock, through contact with infected dog(s) at a show or field trial, and by acquiring an animal from another owner going out of business. Data is being tabulated from the epidemiological studies of kennels that were quarantined from June 2004 to September 2005.

Positive serology has been reported from random testing for prebreeding evaluations, abortions, mismatings, suspected infertile dogs, certification examinations for eyes and hips, and new additions to a kennel. Once a positive outcome is reported to the GDA by the veterinarian or laboratory, the facility is placed under immediate quarantine. Dogs are separated into the best isolation areas possible. Serum samples from false positive dogs are submitted to the Tifton Veterinary Diagnostic and Investigational Laboratory for the AGID test or blood culture. Dogs with a positive report are euthanized. The premises are disinfected with appropriate chemicals. Another series of blood tests are sent from the remaining dogs. Dogs with a subsequent positive report are euthanized. Dogs with negative results are retested in 30 days. Two negative tests are required to remove the quarantine. Therefore, the process takes a minimum of 60 days (8 weeks).

In-house testing using the screening card test by Georgia veterinary practices has given inconsistent results. One negative card test was positive on AGID test, and positive again on retest. Therefore, the state has recommended that veterinarians not do the card test, instead that they send samples to one of the veterinary diagnostic laboratories for quality assurance.

No visitors are allowed during the quarantine; no dogs are moved on or off the premises for a show or sale. Dogs whether young or old are individually housed. Designated personnel manage the breeding of animals. Gloves are worn during and after a delivery of puppies, with an abortion or for vaginal discharges. Immunosuppressed people, children or pregnant people are prohibited from contact with the animals. Concerns have been voiced about the loss of income during the quarantine. A kennel can be isolated for more >2 months if tests continue to be positive. Economic issues arise with the delay in natural breedings, postponed arrival of new dogs, cost of monthly testing, and the necessitated physical and personnel modifications to the facilities. Some kennels will go out of business.

Appendix A

Breeds of dogs testing positive for *B. canis* (courtesy of the Georgia Department of Agriculture):

- Golden Retriever.
- Boston Terrier.
- Lhasa Apso.
- Dachshund.
- Chihuahua.
- Miniature Pinscher.
- Yorkshire Terrier.
- Poodle.
- Shih-tzu.
- Australian Shepherd.
- Pomeranian.
- Mix breeds.

Appendix B

- Georgia Department of Agriculture www.arg.georgia.gov;
- GA Reportable Animal Disease System (RADS).
- Rules of Georgia Department of Agriculture, Animal Health Division.
- Chapter 40-13-4 Infectious and Contagious Diseases.
- Chapter 40-13-4-.02 Reportable Diseases.

(4) “Clinical diagnosis or laboratory confirmation of any of the following disease in an animal residing in or recently purchased from an animal shelter, kennel, or pet dealer licensed under the Animal Protection Act or a bird dealer licensed under the Bird Dealers Act shall be reported within 24 h or by the close of the next business day to the State Veterinarian.” Brucellosis (canine).

Appendix C. Canine brucellosis (*B. canis*)

C.1. Agent

Canine brucellosis is caused by the intracellular bacterium *B. canis*—a small, Gram-negative coccobacilli or short rod. The disease is found worldwide, but is especially common in Central and South America and in the Southeastern United States. Canine brucellosis has been diagnosed in commercial and research breeding kennels in several other countries including Japan.

C.2. Brief description

Clinical signs vary from asymptomatic infertility to overt abortion. The dog may show no signs of clinical infection before having a sudden onset of infertility or abortion. Approximately 75% of infected females abort

after 45–55 days gestation. A prolonged period of vaginal discharge follows abortion. Early embryonic death and resorption or undetected abortion 10–20 days after breeding may result in observed conception failure. If undiagnosed, affected females may abort repeatedly.

In male dogs, *Brucella* organisms infect the prostate, testicles, and epididymis for several months. Epididymitis of one or both testes, testicular atrophy or orchitis, scrotal dermatitis, prostatitis, and infertility are observed. Bacteria are disseminated in the seminal fluids and occasionally urine. Abnormal sperm morphology and inflammatory cells are seen in semen samples, especially in the first 3 months following infection. Semen evaluation of chronically infected males may show aspermia (no sperm), head to head agglutination of sperm, or reduced numbers of immature sperm.

Nonspecific signs in both sexes include lethargy, loss of libido, unwillingness to breed, premature aging, and generalized lymphadenitis. *B. canis* has been associated with diskospondylitis and recurrent uveitis. Prevalence estimates of 7–8% among stray dogs have been reported in the southern United States and Japan. In affected kennels, a 75% reduction in numbers of weaned puppies may be seen. True prevalence rates among breeding dogs and facilities are unknown, and much of canine brucellosis epidemiology remains unclear.

C.3. Mode of transmission

Transmission occurs after ingestion or contact with the organism through mucous membranes or broken skin following exposure to contaminated placenta, aborted fetuses or fetal fluids, or vaginal discharges from infected bitches during heat, breeding, abortion, or full term parturition. Infected males may shed low numbers of *B. canis* in the urine unless urine is contaminated with seminal or prostatic fluids, in which case bacterial numbers may be higher. *Brucella* can be aerosolized in animal pens or microbiology laboratories or spread by fomites under conditions of high humidity, low temperatures, and no sunlight. Organisms are shed for several weeks or intermittently for months after an abortion.

C.4. Incubation period

Variable, from 2 weeks to several months. Most dogs can be positively detected by serologic testing methods within 8–12 weeks after infection.

C.5. Diagnosis

Serology, blood culture, tissue culture, and histopathology are valid modalities for detecting *B. canis* infection. Appropriate protective gear should be used when extracting specimens for laboratory submission to reduce exposure to potentially infectious material. In Georgia, the Tifton Veterinary Diagnostic and Investigational Laboratory is the reference laboratory for diagnostic testing of canine brucellosis. The laboratory must be notified prior to sample submission at <http://hospital.vet.uga.edu/dlab/tifton/index.php> or 229-386-3340.

C.5.1. Serology

Serum samples should be submitted on ice packs and via courier (ex. FedEx, UPS) within 24 h of collection. Samples should be kept cold, but not frozen. Screening tests include the tube agglutination test (TAT), indirect fluorescent antibody (IFA), rapid slide agglutination test (RAST) and agar gel immunodiffusion using cell wall antigens (AGIDcwa). Serologic screening tests are sensitive but are associated with high rates of false positive results. Consequently, a positive screening test is followed by confirmation using a more specific test. More specific tests used for confirmation include the AGID using cytoplasmic protein antigens (AGIDcpa) and serial blood cultures.

C.5.2. Blood culture

Over 50% of dogs are bacteremic for at least a year following infection; however, a single negative blood culture should not be used to rule out disease. A series of three blood cultures collected consecutively, at least 24 h apart, should be submitted for diagnosis. Blood cultures are submitted in heparinized tubes (green-topped blood collection tubes) or aerobically prepared special blood culture media. All blood specimens should be shipped on ice packs within 24 h of collection.

C.5.3. Pathology and culture of tissues

Stomach contents of spontaneously aborted fetuses, fetal tissues, and placentas are the best samples for isolating *B. canis*. Other samples include lymph nodes, spleen, liver, reproductive tissues and semen. Both chemically fixed samples for histopathology and fresh tissues for culture should be submitted.

C.6. Prevention measures/control

Breeding dogs should be purchased from brucellosis-free kennels. All newly acquired dogs should be

isolated and tested twice at least 4–6 weeks apart before they are incorporated into the breeding group. All breeding dogs in a facility should be tested yearly. Dogs bred intensively outside the facility should be tested 2–4 times per year. Tests should be conducted at least 3 weeks prior to the onset of estrus, so a confirmation test can be conducted if the screening test is positive. Testing is more accurate near or during estrus since bacteremia is heightened under hormonal influence.

C.6.1. Disinfection of contaminated premises

Brucella is susceptible to 1% sodium hypochlorite, 70% ethanol, iodine/alcohol solutions, glutaraldehyde and formaldehyde.

C.6.2. Eradication from licensed facilities

Quarantine, testing, and euthanasia of infected dogs are the primary methods necessary to eliminate and prevent the spread of disease in a commercial breeding facility. Reporting of a confirmed laboratory diagnosis in a licensed facility will result in a Georgia Department of Agriculture (GDA) quarantine of the facility. During quarantine, each breeding animal must be maintained in separate housing to avoid further transmission. Movement of dogs shall be in accordance with the requirements of GDA. Report of a “suspicious” result (positive result on screening, negative result on AGIDcpa) will require submission of three serial blood cultures collected at least 24 h apart to determine the true status of the animal. Euthanasia of suspicious dogs is a second option to minimize the time in which the facility is quarantined. The following diagnostic protocols must be followed by licensed facilities in order for the GDA to consider releasing the quarantine:

- (1) Identification of infected adult dogs in facility: serum samples must be submitted for screening (RSAT, TAT, IFA, or AGIDcwa) and AGIDcpa confirmation from all dogs over 6 weeks of age in the facility. All tested dogs must not have received antimicrobials within 3 months of sample collection. The reference laboratory in Georgia for submission of these tests is the Tifton Veterinary Diagnostic and Investigational Laboratory. The laboratory must be notified prior to sample submission:
 - a. Dogs that have positive test results on both screening and confirmation should be euthanized.
 - b. Dogs that are positive on screening and negative on AGIDcpa confirmation (“suspicious result”) should be (1) retested every 4 weeks until definitive results are obtained, or (2) have three

negative blood cultures at least 24 h apart with the first blood culture being collected no later than 7 days following the original sample date, or (3) be euthanized.

- (2) Identification of infected puppies: all puppies born to infected dams or puppies less than 6 weeks of age at the time of initial screening must have three negative blood cultures at least 24 h apart or be euthanized. Puppies with a positive blood culture should be euthanized. Puppies over 6 weeks of age at the time of initial screening should be treated as adults.
- (3) Identification of acutely infected adult dogs: 4 weeks following the identification and euthanasia of infected dogs in a facility, all remaining adult dogs must be tested again by serology. Test results will be interpreted as in (1) above.
- (4) Confirmation of brucella-free facility: all adult dogs (both sexually intact and altered) on the premises should be tested using serology or blood culture at 4 weeks intervals until all dogs on the premises have tested negative for brucellosis (i.e., negative screening and AGIDcpa or negative serial blood cultures) on two consecutive tests. The minimum period of quarantine therefore should be 8 weeks. The risks of exposure, disease transmission, and epidemiology of the particular circumstances will be taken into consideration when determining the quarantine period.

C.7. Zoonotic risk

Humans can be infected with *B. canis*, although cases are rarely diagnosed or reported even in areas where canine prevalence is relatively high. Individuals who handle breeding dogs in kennels and are exposed to reproductive tissues and fluids are at higher risk of exposure. *B. canis* causes a mild, nonspecific disease in humans. Clinical signs are vague and include prolonged fever and lymphadenopathy. Infections among laboratory workers have been documented.

C.8. Reporting requirements

- Any person who makes a clinical diagnosis or laboratory confirmation of canine brucellosis in animals residing in or recently purchased from a Georgia Department of Agriculture licensed facility such as an animal shelter, kennel or pet dealer shall report it by the close of the next business day to the State Veterinarian's office at 404 656-3667 in Atlanta or 1-800-282-5852 outside of Atlanta.

- Laboratory confirmation of brucellosis in humans is immediately reportable to the Georgia Division of Public Health, Notifiable Disease Section. For more information, or to contact the Georgia Division of Public Health, call (404)-657-2588 or go to <http://health.state.ga.us/epi/disease/index.asp>.

C.9. Georgia Department of Agriculture Program

Animal Protection Division 40-13-13-.05 Control of Disease for Licensed Facilities: http://www.agr.state.ga.us/assets/applets/PR_AP_Anim_Prot_Rules_Amended_091301.pdf.

1. In the control, suppression, prevention, and eradication of animal disease, the Commissioner or any duly authorized representative acting under his authority is authorized and may quarantine any animal or animals, premises, or any area when he/she shall determine:
 - a. that the animal or animals in such place or places are infected with a contagious or infectious disease;
 - b. that the animal (s) has been exposed to any contagious or infectious disease;
 - c. that the unsanitary condition of such place or places might cause the spread of such disease;
 - d. or that the owner or occupant of such place is not observing sanitary practices prescribed under the authority of this chapter or any other law of this state.
2. The Commissioner or his duly authorized representative is authorized to issue and enforce written or printed stop sale, stop use, or stop movement orders to the owners or custodians of any animals, ordering them to hold such animals at a designated place, when the Commissioner or his duly authorized representative finds such animals:
 - a. to be infected with or to have been exposed to any contagious or infectious disease; or
 - b. to have been held by persons in violation of this chapter, until such time as the violation has been corrected, and the Commissioner, in writing, has released such animals.

Authority Ga. L. Sec. 4-11-1 et seq.; 4-11-9.1

C.10. Disease consultants

University of Georgia College of Veterinary Medicine: Dr. Richard Fayrer-Hosken, rfh@vet.uga.edu, and Dr. Bruce Hollett, bhollett@vet.uga.edu. Both Drs.

Fayrer-Hosken and Hollett can be reached at the University of Georgia Large Animal Veterinary Teaching Hospital, 706-542-3223.

Tifton Veterinary Diagnostic and Investigational Laboratory is the reference laboratory for diagnostic testing of canine brucellosis. <http://hospital.vet.uga.edu/dlab/tifton/index.php> or 229-386-3340.

Cornell College of Veterinary Medicine: the Animal Health Diagnostic Laboratory at the Cornell College of Veterinary Medicine is recognized as the principal diagnostic laboratory in the US Dr. L. Carmichael at the Baker Institute for Animal Health at the College of Veterinary Medicine at Cornell University (e-mail: lec2@cornell.edu) is one of the leading experts in canine brucellosis.

C.11. Electronic references

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