Effect of Passive Immunoglobulin Transfer on Results of Diagnostic Tests for Antibodies against *Borrelia burgdorferi* in Pups Born to a Seropositive Dam*

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**CLINICAL RELEVANCE**

The event that 8 of 12 pups born to a Lyme borreliosis–positive dam tested positive on a commonly used in-hospital Lyme borreliosis test kit at 1 week of age prompted breeder concern about the possibility and implications of transplacental Lyme borreliosis infection. Almost 2 weeks after the initial serologic test results were obtained, blood was collected from the puppies for comprehensive testing. Assessment of the findings indicate the possibility that passive transfer of maternally derived antibody to the in vivo expressed C₆ peptide of *Borrelia burgdorferi* can temporarily render pups serologically positive for antibodies on the in-hospital C₆ Lyme borreliosis antibody test kit when the test is run on very young animals.

**INTRODUCTION**

In the United States, canine Lyme borreliosis is caused by infection with the spirochete *Borrelia burgdorferi* sensu stricto. The arthropod vector, pathogenesis, and pathophysiology of canine Lyme borreliosis are described elsewhere.¹⁻³ One area of canine Lyme borreliosis seldom reviewed in the veterinary literature is transplacental transmission of *B. burgdorferi* in canids. In coyotes and dogs, conclusive evidence that gestational Lyme borreliosis leads to in utero infection and pathology is lacking. Early research on the subject published in 1989 noted that *B. burgdorferi* was isolated from one of five fetuses in a coyote trapped as part of a coyote ecology research project in southern Texas. Those authors, however, used the indirect immunofluorescent antibody test and noted that there was some serum cross-reactivity with *Borrelia hermsii*, an organism not known to cause clinical disease in canids to date. Additionally, it was noted that spirochetes did not grow to sufficient numbers to allow strain identification.⁴

In 1992, Gustafson and colleagues⁵ manually injected 1,000 *B. burgdorferi* spirochetes intradermally into shaven skin on the dorsum of the neck of each of 10 female beagles every 2 weeks from the time of breeding to parturition. Eight beagles delivered litters of three to seven pups; each litter had at least one pup with *B. burgdorferi* DNA–positive tissue on polymerase chain reaction analysis, and spirochetes were cultured from pups in two litters. Notable

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differences were seen between infected and control beagles in duration of gestation, number of fetal resorptions, and number of dystocias. No clinical signs, physical examination abnormalities, or hematologic abnormalities were detected in infected pups.5

In a study using natural tick transmission,6 Appel and associates infected two pregnant dams with 15 adult female and 7 adult male B. burgdorferi–infected ticks either 3 days or 2 weeks after breeding. Both dams seroconverted but did not develop lameness or fever. One dog was euthanized when due to whelp, and the other was allowed to whelp. Six normal fetuses were removed from the euthanized dam and were negative for B. burgdorferi on culture and polymerase chain reaction analysis. No antibody specific to B. burgdorferi was found in heart blood from any pup. The second dam whelped five normal pups that did not develop signs of Lyme disease during a 5-month observation period, and maternal antibodies declined to negative by 4 weeks of age.6

In summary, evidence of transplacental transmission of B. burgdorferi in the canine is inconclusive in early field and artificial inoculation studies and completely lacking in pregnant laboratory dogs naturally challenged with infected ticks. However, the topic of fetal transmission of B. burgdorferi in the well-cared-for companion dog population has not been addressed. This article highlights questions that arise when trying to interpret serologic test results on pups born to a Lyme-positive dam.

**CASE PRESENTATION**

A clinically healthy, 39-month-old intact chocolate Labrador retriever dam was examined by a veterinarian in Wiscasset, Maine, on April 27, 2004. On physical examination, the dam was clinically healthy and pregnant. Routine diagnostic screening using whole blood with the in-hospital C6 Lyme antibody test kit (SNAP 3Dx Test, Idexx Laboratories) according to label directions revealed the dog to be negative for heartworm antigen, negative for Ehrlichia canis antibody, and positive for B. burgdorferi C6 antibody. The dog had not been vaccinated previously with any B. burgdorferi vaccines. The veterinarian treated her with 750 mg of amoxicillin twice daily for 21 days. The dog was clinically asymptomatic for manifestations of Lyme borreliosis before, during, and after gestation.

On June 1, the dog delivered 13 pups over 13 hours. One pup died within hours after birth; the remaining 12 were vigorous and healthy and nursed well. No malformations were seen in the pup that died. Neither necropsy nor diagnostic tests were performed on the dead pup. The mother and the surviving pups were examined 7 days after whelping. The dam was vaccinated with a conventional combination vaccine against canine distemper, adenovirus type 2, parainfluenza, parvovirus, and leptospira (Recombitek C6, Merial) and a recombinant OspA Lyme vaccine (Recombitek Lyme, Merial). She was retested using the same test kit as before and was again found to be serologically positive for B. burgdorferi antibodies, although she remained asymptomatic. (Note: Lyme borreliosis quantitative C6 antibody testing was not conducted as the assay was not commercially available until late 2004.) Out of concern and curiosity on the part of the breeder and veterinarian, the twelve 7-day old pups were individually screened for B. burgdorferi antibody status using whole blood with the in-hospital C6 Lyme antibody test kit according to label directions. The mother and 8 of 12 (67%) clinically normal pups were identified as seropositive for B. burgdorferi C6 antibodies (Table 1). The dam and all 12 pups were negative for Ehrlichia canis antibody and Dirofilaria immitis antigen. The C6 Lyme-positive pups were not treated with antibiotics in the postpartum period at any time.
Follow-Up Diagnostic Testing

Upon hearing of the in-hospital Lyme borreliosis test results, which implied that these pups might have been or perhaps were still being exposed to *B. burgdorferi*, I asked the veterinarian to collect additional blood samples for further serologic testing. On June 18 (18 days after whelping and 11 days after the initial testing in all pups), blood was collected from the dam and all 12 pups. Serum from each dog was submitted to the New York State Diagnostic Laboratory (NYSDL) on the campus of the New York State College of Veterinary Medicine at Cornell University. *B. burgdorferi*–specific quantitative antibody testing was initiated using a computerized kinetic ELISA (KELA) as described elsewhere. In addition, a qualitative assessment of *B. burgdorferi*–specific antibodies within each serum sample was performed using the Western blot. The Western blot is typically used when KELA values exceed 100, indicating a preliminary possibility of natural exposure. For the purpose of this inquiry, all serum samples were assayed using the Western blot even though most were KELA negative. Western blots were

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**TABLE 1. Test Results for Serologic Evidence of *B. burgdorferi* Natural Exposure in a Seropositive Adult Labrador Retriever Dam and Her 12 Pups at 7 and 18 Days after Whelping**

<table>
<thead>
<tr>
<th>Dog</th>
<th>In-Hospital Lyme C₆ Antibody Test Kit* (day 7)</th>
<th>Quantitative C₆ ELISA† (day 18; U/ml)</th>
<th>KELA‡ (day 18; KELA units)</th>
<th>Western Blot (day 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dam</td>
<td>+</td>
<td>112 (+)</td>
<td>355 (strong +)</td>
<td>+ Low</td>
</tr>
<tr>
<td>Pup 1</td>
<td>+</td>
<td>&lt;10 (–)</td>
<td>60 (–)</td>
<td>– –</td>
</tr>
<tr>
<td>Pup 2</td>
<td>+</td>
<td>&lt;10 (–)</td>
<td>88 (–)</td>
<td>– –</td>
</tr>
<tr>
<td>Pup 3</td>
<td>+</td>
<td>&lt;10 (–)</td>
<td>55 (–)</td>
<td>– –</td>
</tr>
<tr>
<td>Pup 4</td>
<td>+</td>
<td>&lt;10 (–)</td>
<td>63 (–)</td>
<td>– –</td>
</tr>
<tr>
<td>Pup 5</td>
<td>+</td>
<td>10 (–)</td>
<td>100 (equivocal)</td>
<td>– –</td>
</tr>
<tr>
<td>Pup 6</td>
<td>+</td>
<td>&lt;10 (–)</td>
<td>43 (–)</td>
<td>– –</td>
</tr>
<tr>
<td>Pup 7</td>
<td>–</td>
<td>&lt;10 (–)</td>
<td>28 (–)</td>
<td>– –</td>
</tr>
<tr>
<td>Pup 8</td>
<td>–</td>
<td>&lt;10 (–)</td>
<td>0 (–)</td>
<td>– –</td>
</tr>
<tr>
<td>Pup 9</td>
<td>+</td>
<td>&lt;10 (–)</td>
<td>71 (–)</td>
<td>– –</td>
</tr>
<tr>
<td>Pup 10</td>
<td>+</td>
<td>&lt;10 (–)</td>
<td>54 (–)</td>
<td>– –</td>
</tr>
<tr>
<td>Pup 11</td>
<td>–</td>
<td>&lt;10 (–)</td>
<td>95 (–)</td>
<td>– –</td>
</tr>
<tr>
<td>Pup 12</td>
<td>–</td>
<td>&lt;10 (–)</td>
<td>52 (–)</td>
<td>– –</td>
</tr>
<tr>
<td>Control (+)</td>
<td>NA</td>
<td>NA</td>
<td>400 (strong +)</td>
<td>+ –</td>
</tr>
</tbody>
</table>

*SNAP 3Dx Test, Idexx Laboratories.
†Quantitative C₆ ELISA (Lyme Quant C₆ Test; conducted at a later date using banked frozen serum); cutoff point at IDEXX Laboratories is <30 U/ml. Antibody levels below the cutoff are considered insignificant.
‡Kinetic (whole cell lysate) ELISA antibody (KELA); cutoff point at New York State Diagnostic Laboratory, Cornell University, is <100. Antibody levels below the cutoff are considered insignificant.
– = negative; + = positive; NA = not available.
considered positive if bound antibody was detected on at least three bands from among p39, p29-30, p28, p25-26, p22, and p19. Unfortunately, the in-hospital C₆ Lyme antibody test was not conducted on these day 18 samples; however, the day 18 sera from the dam and all 12 pups were submitted to IDEXX Laboratories for Lyme C₆ quantitative antibody ELISA testing at a later date. Quantitative C₆ antibody analysis can be used as the second of two tiers of diagnostic C₆ tools used to define infection with or natural exposure to B. burgdorferi. It is often used to define the total level of C₆ antibodies in a given patient. Serial monitoring of C₆ antibody levels before and after treatment may give some indication of treatment success.⁹⁻¹¹

Results and Interpretation of Follow-Up Testing

Kinetic ELISA

Of the eight pups that had tested positive for antibodies specific to B. burgdorferi natural exposure using the in-hospital C₆ Lyme antibody test kit at 7 days of age, seven were negative and one was designated as “equivocal” when serum collected 11 days later was subjected to KELA at the NYSDL. Antibody levels in the “equivocal” range need to be interpreted with caution because they may not be specific for the Lyme agent. Antibodies to vaccination or infection with Leptospira spp may yield a false-positive result for Lyme antibodies on KELA. Presumably, if levels are high enough, passively acquired maternal antibodies to B. burgdorferi from dam to pup may also contribute to a false-positive result when using an ELISA. All three C₆ Lyme antibody-negative pups were also negative by KELA.

Western Blot

The results of Western blot testing confirmed that the dam had developed antibodies to B. burgdorferi via natural exposure to live
ticks as well as antibody to vaccine antigen (OspA). Western blot testing on all eight C₆ Lyme antibody–positive pups (from previous testing), including the one that was defined as “equivocal” on KELA, were negative for a specific antibody banding pattern normally indicative of natural exposure in a mature animal. In this case, if bands had been observed in these young pups, they would in all likelihood be characteristic of the dam’s natural exposure to B. burgdorferi that were passed to the pups via maternal antibody transfer (Figure 1). All C₆ Lyme antibody–negative pups were also negative on Western blot testing.

**Lyme C₆ Quantitative Antibody ELISA**

Studies have shown excellent correlation between the ability of C₆ antibody testing and immunoblotting to detect Lyme borreliosis–infected dogs. The results of quantitative C₆ antibody testing in this case study correlated precisely with Western blot testing and confirmed that the dam had developed antibodies specific to B. burgdorferi natural exposure. Her quantitative C₆ value was 112 U/ml. All 12 puppies—including the eight that were C₆ Lyme positive on the in-clinic test kit—had quantitative C₆ antibody levels of 10 U/ml or lower, which is defined as insignificant. Because Lyme C₆ quantitative antibody testing was conducted after KELA and Western blotting had been performed, the volume of serum available to repeat the in-hospital Lyme C₆ was insufficient. See Table 1 for a complete review of all day 18 postweaning values.

**DISCUSSION**

Passive transfer of immunoglobulins (particularly IgG) via colostrum is essential for the protection of neonates against a variety of illnesses, and the importance of passively acquired antibodies, cytokines, and interleukins on the development of a young animal’s immune system is described elsewhere. Relating to Lyme borreliosis, passive transfer of antibodies from dam to offspring likely induces no immunity to B. burgdorferi infection because naturally infected dogs harboring the same antibodies often remain persistently infected and natural infection antibodies do not confer borrellicidal activity ex vivo. However, research exploring the possible effect of passive maternal transfer of Lyme borreliosis–specific antibodies on Lyme serology and diagnostic interpretation is lacking. It is known that passively acquired antibodies interfere with early immunization success in veterinary medicine. Additionally, passively acquired antibodies may interfere with commonly used commercial test kits that are geared toward detecting infection antibodies in animals past the age of weaning. Although heretofore not documented in the realm of in-hospital serologic testing for evidence of B. burgdorferi exposure, an important example of this interference occurs with FIV testing. Kittens readily absorb antibodies against FIV in colostrum of FIV-vaccinated queens. These antibodies have been shown to interfere with commercially available test kits, creating the inability to distinguish truly infected from noninfected kittens.

In the realm of canine Lyme borreliosis testing, traditional whole-cell ELISA-based diagnostic test kits are quantitative in nature and cannot differentiate vaccinal from infection or even maternally derived antibodies. In adult dogs, this fact necessitates the use of immunoblotting and/or the C₆ peptide test to discern the difference. The identification and the relatively recent advance of C₆ antibody testing in the form of an in-hospital rapid assay ELISA format and the Lyme C₆ quantitative antibody ELISA have revolutionized serologic testing for infection with Borrelia spp because C₆ antibodies are generated relatively quickly.
after infection, and results are not falsely impacted by vaccination.12

The goal of this study was to more thoroughly investigate information pertaining to the serologic status of pups born to a B. burgdorferi–seropositive dam and the temporary diagnostic conundrum that ensued. The fact that 8 of 12 pups born to a Lyme borreliosis–positive dam tested positive on a commonly used in-hospital Lyme test kit prompted owner concern and precipitated many questions about the possibility of fetal exposure. After subsequent follow-up testing over an 18-day period, it appears that passively transferred maternal antibody to the C6 peptide of B. burgdorferi may have temporarily rendered pups serologically positive for B. burgdorferi antibodies on the in-hospital C6 Lyme antibody test kit, falsely indicating the possibility of active infection. Although extra serum from the day 7 blood collection was not available for subsequent testing, serum taken 11 days later was subjected to a battery of serologic tests, including KELA, Western blot, and Lyme C6 quantitative antibody ELISA (Table 1). These tests confirmed the serologic observation of natural exposure to B. burgdorferi in the dam but showed no serologic evidence of exposure in all pups. Although the possibility exists that serum from the 7-day-old pups may have revealed the same information had the samples been subjected to these same confirmatory tests, the reality is that additional blood was not drawn on these week-old pups at the time of initial diagnosis.

Given the testing information, the possibility also exists that the initial results in the eight 7-day old pups were falsely characterized as “positive” owing to end-user or test-kit error. A thorough review of test-kit procedure, the fact that all 12 pups were tested simultaneously using the same commercial lot, that results in four pups were negative, and the long-standing hospital familiarity with using the test kit diminish the likelihood of a false-positive result due to end-user or test-kit error.

Another conceivable hypothesis for the eventual absence of serologic evidence of B. burgdorferi exposure in these pups is the possibility of bacterial clearance afforded by the dam being placed on 250 mg amoxicillin twice daily in the fifth week of pregnancy. That the timing of antibiotic administration may have coincided with the theoretic arrival of B. burgdorferi across the placenta and allowed for bacterial clearance is interesting but speculative. Results from gestational studies in dogs and the possible persistence of B. burgdorferi infection in the dam despite antibiotic therapy speak to both the lack of evidence that transplacental infections occur and the difficulty in clearing B. burgdorferi despite antibiotic treatment.6,18,19 Perhaps a more plausible explanation is that the level of transferred maternal antibodies specific for the C6 peptide was high enough to trigger a positive C6 Lyme antibody test result in the hospital setting at 7 days of age but subsequently waned. Thus the level of passively acquired C6 antibodies—in fact, the level of all Lyme-specific maternal antibodies—just 11 days later was not high enough to provide serologic evidence of exposure when subjected to KELA, Western blot, and Lyme C6 quantitative antibody ELISA. In keeping with these results, when pups were

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rechecked at the veterinary hospital between 58 and 76 days of age, four previously Lyme “positive” dogs had turned Lyme “negative” on the in-clinic C₆ Lyme test kit, furthering the notion that maternally acquired C₆ antibody levels had waned below the limit of detection on that test.

It is important to point out that the KELA and Western blot assays in laboratories like the NYSDL use *B. burgdorferi* antigens from organisms grown on culture. Cell culture–derived *B. burgdorferi* contain the appropriate genes but do not express the VlsE protein and its component C₆ peptide. Subsequently, standard whole-cell–based assays do not contain the VlsE and C₆ antigens in their test wells and are therefore unable to detect antibodies to C₆. Thus, the addition of the Lyme C₆ quantitative antibody ELISA allowed for precise measurement of C₆ antibody levels. Regardless, the purpose of using the Western blot was to discern more clearly the presence (or absence) of a distinct pattern of antibodies that would normally be specific for evidence of natural exposure to *B. burgdorferi*. However, in this case, had these young pups shown distinctive bands for infection on Western blot, it could convincingly be argued that these bands would have represented only maternal antibody transfer rather than evidence of in utero infection. Instead, the measurements revealed very low levels of total antibody (KELA), faint evidence of maternally acquired infection antibody bands (Western blot), and levels of C₆ antibody almost below the limit of quantification. In essence, the test results of the pups were merely a weak and passing serologic reflection of the serologic status of their mother.

Potential limitations of any conclusions that might be drawn include the fact that the pup that died within hours after birth was not necropsied and that no samples were collected for direct evidence of *B. burgdorferi*. Although subsequent serologic evidence of infection in the 12 surviving pups was absent, one could question whether fetal infection may be variable within a litter and had perhaps contributed in some way to early morbidity. Had the Lyme C₆ quantitative test been commercially available when the pups were 7 days of age, the level of antibody (in units/milliliter) necessary to trigger a “positive” blue dot on the in-hospital Lyme C₆ test kit could have been discerned. Likewise, had enough serum been available from the day 18 blood collection to re-run the in-hospital Lyme C₆ test kit, this information could have been matched with the resultant quantitative C₆ data.

The decay kinetics of maternal antibody in pups likely varies with, but is not limited to, maternal immune status, immunoglobulin class, and efficiency of early suckling. Accordingly, passively acquired antibodies can linger for a few weeks or many months.

This study was initiated because of results from unusually early testing. Although the majority of pups were serologically positive for *B. burgdorferi* antibodies at 1 week of age, none of the retested pups were positive at 8 to 11 weeks of age. Therefore, if presented with pups born to and suckled from a dam serologically positive for *B. burgdorferi*, I would refrain from conducting serologic tests specific for Lyme disease antibodies until the pups are older than 8 weeks. If a pup is determined to be serologically positive for *B. burgdorferi*, I would refrain from conducting serologic tests specific for Lyme disease antibodies until the pups are older than 8 weeks. If a pup is determined to be serologically positive for *B. burgdorferi* antibodies using the in-hospital C₆ Lyme borreliosis antibody test kit, the Lyme C₆ quantitative antibody ELISA can be used to follow the course of C₆ titers over time, with waning titers indicative of maternal antibody interference.

**CONCLUSION**

This report demonstrates the possibility that maternally derived antibodies to the C₆ peptide in a seropositive dam can be transferred to puppies. It appears that this level of C₆ antibody transfer can trigger a blue dot positive result on
the in-hospital C₆ Lyme antibody test kit in the absence of true B. burgdorferi infection when pups are tested at a very young age. This report also documents the observation that 12 Labrador retriever pups born to a Lyme borreliosis–positive dam ultimately tested serologically negative using a wide spectrum of available diagnostic tests conducted at approximately 3 weeks of age. All available puppies examined between 8 and 11 weeks of age remained clinically healthy and Lyme negative on the in-clinic C₆ Lyme test kit, adding confidence that transplacental infection did not take place in this instance. Information gathered from this case example may be useful for concerned breeders, veterinarians, or new puppy owners when a breeding bitch or pregnant dam is determined to be serologically positive for B. burgdorferi exposure on a commonly used in-hospital C₆ Lyme borreliosis antibody test kit.

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REFERENCES


