Xenodiagnosis

The search for reliable diagnostics may return to the tick

By Betty Maloney, MD

Evidence for persistent *B. burgdorferi* infection continues to mount, and the human xenodiagnostic trial[1] provides the latest addition. This article highlights important aspects of the trial and, this being Lyme disease, some curiosities about how investigators presented their findings.

It's important to understand the connection between xenodiagnosis and why it may be useful in Lyme disease as well as how investigators presented their findings.

Premise

Xenodiagnosis is based on the relationship between vectors (the carriers) and the pathogens (disease agents) they transmit. Pathogens depend on vectors to move between hosts, and vector-pathogen pairs evolve together. This allows signaling mechanisms to develop such that pathogens in one host move towards their vector's "call," become ingested and, ultimately, are transmitted to a new host when the vector next feeds. As depicted in the diagram, xenodiagnosis uses a vector (a tick) to "probe" a host (a mouse) suspected of being infected and, once feeding is completed, the vector is examined by PCR for evidence of the pathogen.

In humans, post-treatment serologic tests for Lyme disease are diagnostically worthless because neither positive nor negative results can be taken at face value. Results may be negative because an ineffective course of antibiotics failed to clear the infection but kept the immune system from producing a full antibody response to *Bb*. In patients who have been treated and feel well, positive results may simply reflect the old infection and not an ongoing one. This explains the interest in xenodiagnosis. The saliva of black-legged ticks contains chemicals that attract *Bb* to a bite site. Knowing that xenodiagnosis successfully demonstrated persistent infection in animal models, it was logical to investigate whether it could identify persistent Lyme infection in humans.

1 Marques A, Telford SR 3rd, Turk SP, *et al*. "Xenodiagnosis to detect *Borrelia burgdorferi* infection: a first-in-human study." *Clin Infect Dis*. 2014 Apr;58(7):937-45

Study design

Officially titled "Searching for Persistence of Infection in Lyme Disease," the study sought to determine whether xenodiagnosis can be used to successfully investigate the presence of Lyme bacteria. The primary outcome measure specifically focused on determining whether xenodiagnosis could detect the continued presence of *Bb* in patients with persistent, post-treatment manifestations of Lyme. Assessing human xenodiagnosis safety was the secondary outcome measure.

Findings

The study included 36 patients separated into five categories; outcomes for all groups are listed in the table. Each had 25-30 *Bb*-free ticks placed on their skin that fed until they naturally detached. If xenodiagnosis works, the patient with a current EM should be positive (he was) and the healthy controls should be negative (they were). Researchers found two other subjects PCR+ but thought the results represented contamination and were therefore labeled indeterminate.

Subject type	Subjects	Untestable ticks	Outcome
Current EM, on treatment	1	0	+ PCR
Post-treatment EM	5	1	No + PCR
Post-treatment, well, high C6 index	10	3	No + PCR
Post-treatment, ill	10	2	+ PCR in 1 of 8
Healthy controls	10	5	No + PCR

Given that the xenodiagnostic test was appropriately positive and negative in the subjects and the healthy controls respectively, the positive result in a persistently ill post-treatment subject is highly significant evidence of persistent infection. In fact, the study's record had declared prior to the trial's start that: "evidence that *Bb* can be recovered by xenodiagnosis after antibiotic therapy in subjects with continued symptoms would change the current paradigm for potential mechanisms of disease and provide researchers and clinicians a tool for identifying patients with persistent infection." (Emphasis added)

Results

Although one might think this resolves the question of persistent infection in humans, when it comes to Lyme disease it appears that the ground rules are subject to change. The study details (*clinicaltrials.gov*) state that the primary endpoint was to "determine whether xenodiagnosis can detect the continued presence of *Bb* in patients with Lyme disease after antibiotic therapy" and list safety as the secondary outcome measure. Instead, the authors claimed, "The primary goals of this study were to develop procedures for xenodiagnostic testing of patients with Lyme disease and to determine the safety of tick xenodiagnosis in humans." This allowed them to shift their discussion away from their most important finding — a persistent *Bb* infection in a post-treatment patient with ongoing manifestations of Lyme disease — and towards the more mundane safety finding.

The authors went to great lengths to discount the significance of their own findings. Although neither of the positive xenodiagnostic specimens produced *Bb*-positive cultures or allowed for the transmission of *Bb* to immunodeficient mice, the authors only mentioned those findings in relationship to the post-treatment ill patient. They rightfully questioned whether the recovered DNA was actual evidence of *Bb* viability but the discussion on this point seemed unbalanced. The authors hypothesized that the ticks simply acquired DNA remnants from dead *Bb* (which just happened to be in the vicinity of the bite site), and offered results from a Lyme arthritis study as scientific support. However, there are significant differences between the current and previous studies. Although the arthritis study found joint fluid specimens remained PCR+ for up to 11 months positive post-treatment, the post-treatment patient in the xenodi-

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