California Association for Medical Laboratory Technology

Distance Learning Program

THE GREAT IMPOSTER
Lyme Disease

by

Martha Kunkel, BS, CLS Retired UCD Medical Center Sacramento, CA	Judy Davis, MA, CLS Retired UCD Medical Center Sacramento, CA

Course DL-988
2.0 CE/Contact Hours
Level: Intermediate

CAMLT is approved by the California Department of Health Services as a CA CLS Accrediting Agency (#0021) and this course is approved by ASCLS for the P.A.C.E.® Program (#519)

1895 Mowry Ave, Suite 112, Fremont, CA 94538-1700
Phone: 510-792-4441 FAX: 510-792-3045

Notification of Distance Learning Deadline

All continuing education units required to renew your license must be earned no later than the expiration date printed on your license. If some of your units are made up of Distance Learning courses, please allow yourself enough time to retake the test in the event you do not pass on the first attempt. CAMLT urges you to earn your CE units early!

THE GREAT IMPOSTER – Lyme Disease

OBJECTIVES

1. List the ticks, organisms, and geographical locations associated with tick-borne diseases.
2. Correlate disease symptoms, laboratory tests, and timing with Lyme disease.
3. Compare and contrast early and late treatments of Lyme disease.
4. Describe ways to prevent the initial tick contact.
5. Discuss the prevalence of tick-associated diseases and their recognition within the medical community.
6. Discuss other potential tick-borne co-infections.

ABSTRACT

Tick-borne diseases are poorly understood and often misdiagnosed. Lyme disease has been studied extensively and is the best known. However, there are a growing number of diseases that have been associated with tick bites. Although Lyme disease was originally discovered in the Northeastern part of the United States, tick-borne diseases have been identified in all 50 states with all but two counties in California being infested. Many patients have had more than one organism identified from a single tick bite. Treatment for these diseases is highly controversial. Within the infectious disease community, most physicians adhere to the very strict Centers for Disease Control (CDC) guidelines for diagnosis and treatment (1). However, there are a growing number of primary care physicians who have found a wider variety of treatment options and testing modalities to be necessary to treat their patients (2). These organisms and their resultant diseases continue to elude diagnosis with many patients going months or years without a diagnosis.

This course is a case-study approach to Lyme disease.

I. CASE STUDY

A 55-year-old Asian female was bitten by a tick on her right leg just below the knee while hiking in the Santa Cruz Mountains in early spring. Concerned, she called her primary care physician. The physician indicated that if there was no rash at the site or flu like symptoms within a week there was no need for concern. Since the patient did not have any of the indicators within the week, she did not give the encounter any further thought.

Approximately 5-8 weeks later the patient noted a severe itch on her upper chest, particularly in the evenings. The rash disappeared after 7–10 days. Sometime later the patient broke out with hives on the upper chest. These disappeared when treated with Benadryl. Approximately one month later the patient developed a severe toothache in her lower jaw. The dentist could find no reason for the tooth pain. At the same time the patient experienced the sensations of her tongue swelling and slight numbness on one side of her face. The primary care physician could find no obvious reason for the symptoms. She was referred to an ear, nose and throat (ENT) physician who ran many blood tests and an MRI on the head and neck region. Everything was benign except for a low zinc level and low white count (3,200/µl). The patient began to notice some degree of short term memory loss and using the wrong word in conversations. These symptoms subsided and approximately four months later a rash appeared on the lower left abdomen that lasted about a week. In October the patient began to experience chest pain that was diagnosed as muscular strain. In November the patient again experienced excessive chest pain and was diagnosed with possible pneumonia. The patient was treated with antibiotics for 10 days. After completion of the antibiotic treatment, the patient experienced tachycardia and irregular heart beats seen as pre-ventricular contractions (PVC). The patient was placed on a beta blocker before leaving for Maui to visit family. While in Maui she felt faint, tightness in the chest, and increased tachycardia. A visit to the emergency room (ER) revealed an elevated D-dimer. The beta blocker was discontinued and she was discharged with instructions to return if symptoms did not go away. The discharge diagnosis was extreme anxiety. Seven days later the patient returned to the ER with the same symptoms and fatigue and was admitted to the hospital for a cardiac work-up. The D-dimer was still elevated. A treadmill echo test was normal. With no obvious indicators of why she had symptoms and positive D-dimer, she was discharged to fly home.

After returning home she was again admitted to the hospital with the same symptoms along with numbness on one side of the face, extreme exhaustion, and low body temperature (93ºF). The diagnosis was now listed as cardiac arrhythmia and cardiac neurosis (panic disorder). She was discharged and cardiac catheterization was scheduled and a Lyme test was ordered. The cardiac catheterization was normal and the patient was given a low dose of beta blocker by the cardiologist. The ELISA Lyme test came back negative.

A fortuitous visit with a dental hygienist friend, who had just attended a continuing education seminar on Lyme infections and dental patients, encouraged the patient to seek further information on Lyme infections. The patient accessed the internet and found "Doctor" Google. After much research the patient requested more extensive testing by a reference laboratory specializing in tick-borne diseases.

Laboratory Tests performed one year after exposure:

Test	Organism/test	Result	Reference Range
IFA	*B. burgdorferi*	1:40	<1:40 Negative
			1:40 Indeterminate
			= or > 1:80 Positive
Antibody Panel	*B. duncani* IgG	<1:40	<1:40 IgG Negative
	B. duncani IgM	1:20	1:20 IgM Suggestive
Western Blot	Lyme IgG	31,39,41	Positive Bands
	Lyme IgM	28,30,39,41,58	Positive Bands
IFA-HGE panel	Ehrlichiosis –IgM	1:20	<1:20 Negative IgM
	IgG	<1:40	<1:40 Negative IgG 1:20 (IgM) or 1:40 (IgG) to 1:160 suggestive of disease and/or treatment >1:160 suggestive disease state
ALT		85	3-6 U/L
AST		49	8-42 U/L
CBC	WBC	2.9	3.8 - 10.8 K/uLSC
	Hemoglobin	12.2	12.4 -15.8 g/dL
	Hematocrit	38.5	36 – 46 %

Questions:

1. Does the laboratory data support tick-borne infection and if so what organism(s)?
2. What do the elevated liver enzymes suggest?
3. Is the decreased WBC of significance?
4. What effect does the delay in performing testing have on the final diagnosis?

II. LYME DISEASE

Lyme disease is caused by *Borrelia burgdorferi*, a spirochete transmitted by a tick bite. The disease has three categories, acute, early disseminated, and chronic.

The acute stage is characterized by a circular rash around the tick bite that appears within 1-2 weeks. Not everyone develops the rash. The person may also have flu-like symptoms such as swollen lymph nodes, fatigue, headache, and muscle aches. In some untreated persons the initial illness may resolve, but in others the infection can spread.

In the disseminated stage the organism spreads through the blood to other parts of the body. Symptoms of this stage appear several weeks after the tick bite. The organism may affect the heart, musculoskeletal system, nervous system and other areas.

Chronic disease: In this stage there is breakdown of the immune system, allowing the disease to progress. According to Burrascano (2) chronic disease has the following criteria:

1. Illness present for at least one year (approximately when immune breakdown attains clinically significant levels.).
2. Persistent major neurologic involvement (i.e., encephalitis, encephalopathy, meningitis) or active arthritic manifestations.
3. Still have active infection with *B. burgdorferi*, regardless of prior antibiotic therapy.

The severity of Lyme disease is proportional to the spirochete load, the presence of co-infections, and the duration of the infection.

III. SYMPTOMS

The classic sign of acute Lyme disease is a circular, outwardly expanding rash called erythema chronicum migrans (also known as EM or just erythema migrans) that occurs at the site of the tick bite 1 to 32 days after being bitten. This rash is not an allergic reaction, but rather an actual skin infection caused by the Lyme bacterium, *Borrelia burgdorferi*. The rash is generally 5 to 7 cm in diameter with the innermost portion remaining dark red and the outer edge red with the portion in between clearing, giving the appearance of a “bull’s-eye.” The appearance of this rash is considered a pathognomonic sign: a physician-identified rash warrants an instant diagnosis of Lyme disease and immediate treatment. These rashes are characteristic of *Borrelia* infections and no other pathogens are known to cause this form of rash; however, care must be taken to distinguish this rash from ring-worm infection, which has a similar appearing rash.

The rash is estimated to develop in 50% to 80% of cases with some literature suggesting the percentage might be even lower. In addition, the true bull’s-eye appearance occurs in as few as 9% of the cases. The development of the EM rash at other sites or multiple sites indicates disseminated infection.

Without the classical “bull’s-eye” rash, the symptoms of tick-borne diseases mimic many conditions and diagnosis must include a search for alternative and concurrent conditions. As most of the cases that do not present in the classical format evolve into the next stage, early disseminated infection, the presenting symptoms are wide-ranging.

Many physicians who treat Lyme disease use the following checklist of possible symptoms.

Primary signs:

Have you had any of the following in relation to this illness?	Yes	No
Tick bite		
Spotted rash over large area		
"EM" rash (discrete circle)		
Linear, red streaks		

Secondary signs/symptoms:

Chills, fever, night or day sweats, flushing	Yes	No
Unexplained weight change (loss or gain)		
Poor stamina, fatigue, tiredness		
Unexplained hair loss		
Swollen glands		
Sore throat		
Unexplained menstrual irregularities Unexplained breast pain/milk production		
Upset stomach		
Change in bowel function (constipation, diarrhea)		
Chest pain		
Shortness of breath, coughing		
Heart palpitations, skipping pulse, heart block		
Joint pain or swelling		
Stiffness of the joints, neck, or back		
Twitching of the face or other muscles		
Headache		
Tingling, numbness, burning or stabbing sensations, shooting pains		
Facial paralysis (Bell's palsy)		
Eyes: blurry vision, double vision, sensitivity to light, increased floaters		
Ears: ear pain, buzzing, ringing, sensitivity to sound		
Increased motion sickness, vertigo, poor balance		
Difficulty in thinking		
Lightheadedness, wooziness		
Forgetfulness, poor short-term memory		
Speech difficulties, vocalization, word block		
Disorientation, getting lost, going to wrong places		
Mood swings, irritability, depression		
Disturbed sleep: too much/ too little		

Although symptoms alone cannot make a diagnosis of Lyme disease, the greater the number of "yes" answers, the greater the possibility that the patient has Lyme disease.
It has been postulated that the number of symptoms and their severity is directly related to the presence of co-infections. As ticks are known to carry more than one infectious

organism, the search for more than one infection is necessary. Tick-borne diseases are often called the great **“Imposter**” due to the variety of symptoms that mimic other disease conditions.

IV. IMMUNE RESPONSE

Initially PMN neutrophils can phagocytose the spirochetes. However, the saliva of the tick contains elements that decrease the effectiveness of the host immune defenses. The binding ability of PMNs is reduced, which reduces spirochete killing.

Antibodies are formed against *B. burgdorferi* by about 4 weeks after infection, though the range can be 2-6 weeks or longer. Early antibiotic treatment decreases antibody formation. This early antibiotic therapy may limit the exposure time of the host’s immune system to *B. burgdorferi* antigens. In other patients the antibody response may be delayed for as yet unknown reasons. It is postulated that the antibodies may have been isolated in immune complexes.

The first antibodies produced are IgM followed by IgG. The serum titer of IgM peaks between 20-40 days after infection. With involvement of T-cells and their response to Borrelia antigens, the class switch to IgG occurs more slowly. IgM titers may decrease or remain persistently elevated in patients with Lyme disease (3). In chronic disease IgM can last for years.

In addition to the humoral immune response, T-cells acquire cytotoxic activity after exposure to *B. burgdorferi* and have the ability to produce cytokines. These cytokines are involved in regulation of the immune system as well as being pro-inflammatory. Tick saliva also inhibits T-cells by binding interleukin 2 (IL-2), effectively reducing the T-cell response.

If the disease is not treated in the early stages, it may become chronic. In chronic disease there is an inhibitory effect on the immune system: There is reduction of both B- and T-cells. The T3 subset of CD-57 is notable decreased. The use of CD-57 counts has become useful in the diagnosis of chronic Lyme disease.

Consequences of the reduction of the immune response in chronic disease are

1. Activation of co-infection organisms. Ticks have been shown to carry other pathogens in addition to Borrelia. These organisms may have been contained by the immune system early on, but decrease in the immune response in chronic patients allows these organisms to become active.
2. Other latent infections, such as herpes viruses, which pre-date the tick bite may become reactive, adding to the illness.
3. Serologic tests become less sensitive because of the decreased antibody response.
4. Immune complexes form, trapping *B. burgdorferi* antibodies. These complexed antibodies are not detected by serologic testing.

V. LABORATORY TESTS FOR LYME DISEASE

Lyme disease is diagnosed on clinical findings, as no currently available test, no matter the source or type, is definitive in ruling in or out infection with this pathogen. The diagnosis is based on symptoms, physical findings, and a history of possible exposure to infected ticks. The development of the EM rash is diagnostic for Lyme disease and generally treatment at this time provides the highest rate of success. In these cases additional laboratory testing is not required unless the patient continues to present symptoms which could represent co-infections.

Two categories of laboratory tests are used for symptomatic, non-rash presenting patients: (1) *Borellia burgdorferi* (Bb) antibody detection (2) direct detection of Bb organism in the body fluids or tissues. The CDC recommends a two-tier approach, using antibody detection systems, for symptomatic patients who do not present with the typical EM rash. The initial screening test recommended is the ELISA or IFA. All positive results are to be followed up with a specific antibody Western Blot test.

ELISA

The utilization of the ELISA (enzyme-linked immunosorbent assay) or IFA (indirect fluorescent antibody) as screening tests presents several problems with tick-borne diseases.

First, timing is critical. The detection of the antibody requires sufficient time for the immune system to respond, first with Immunoglobulin M (IgM) and then Immunoglobulin G (IgG) antibodies. As mentioned above, the IgM is an immediate response to the invading organism while IgG antibodies with enhanced avidity for the organism develop later. Generally, IgM levels fall as IgG levels increase. Since the development of antibody-specific IgG requires 4-6 weeks, the timing of the screening test is critical. However, a positive serology only indicates exposure and cannot differentiate between current and previous exposure. Additionally, chronic unresolved Bb frequently has both high titers of IgM and IgG due to the continued presentation of the antigen to the lymphocytes.

Second, a screening test should have a high degree of sensitivity and accuracy (specificity). The ELISA test has a sensitivity of 65% therefore 35 out of 100 are false negatives. In comparison the HIV screening test has a false negative rate of 5 out of 100 or 95% sensitivity. The IFA for Lyme disease has about the same sensitivity or less, with the human subjectivity adding an additional element of complexity. A good screening test should have a 95% or higher degree of sensitivity.

Third, low levels of detectable antibodies. There are several causes of low antibody detection. The Bb bacteria have the ability to change their surface characteristics, thus preventing recognition with the reference strain used by the testing laboratory. Known as pleomorphism, this is a common characteristic of infectious organisms, particularly viruses. Another common problem is the early intervention treatment with antibiotics, which halts or significantly reduces the number of antibodies present.

For these reasons a negative ELISA result does not rule-out infection with Bb or other tick-borne disease. However, a positive result does not prove Bb infection either. Therefore, many Lyme disease practitioners recommend the Western Blot and CD-57 counts in later and chronic disease states.

Western Blot

The Western Blot is more specific and is recommended as the confirmatory test by the CDC. A positive ELISA and Western Blot are considered diagnostic for exposure to the Bb infective agent; however, diagnosis of active disease is still a clinical determination.

Western Blots are reported by indicating which bands are reactive. 41KDa bands appear the earliest but there can be cross-reaction with other spirochetes. The 18KDa, 23-25KDa, 31KDa, 37KDa, 39KDa, 83KDa, and 93KDa bands are species-specific, but appear later or may not appear at all. At a minimum the 41KDa and one of the specific bands should be seen. Bands 55KDa, 60KDa, 66KDa, and 73KDa are non-specific and non-diagnostic. Bands 31 and 43 are specific for Bb. Many commercial laboratories omit these bands since these bands were originally identified for use in the development of a Lyme vaccine. GlaxoSmithKline developed a recombinant vaccine using the outer surface protein A (OspA) of Bb. The vaccine was called LYMErix and was released in December 1998. It proved effective in 76% of adults and 100% of children. Unfortunately, there were reports of autoimmune disorders developing after taking the vaccine. Although the CDC found no connection, the company pulled the product from the market in 2002. Anyone receiving the vaccine could anticipate having bands 31KDa and 43KDa positive. The 31 and 43 bands should be included in the Western Blot for all other patients, as these bands are specific for *Borrelia*.

PCR

The polymerase chain reaction (PCR) is 90% specific (accurate) but less than 30% sensitive.

PCR is used to test for Bb bacteria's DNA, therefore substantially increasing specificity. Sensitivity is decreased due to Bb not being primarily a blood-borne organism but instead a deep tissue infection. Tissues containing collagen such as joints, tendons, fascia, and connective tissue are most often involved. Therefore, it is recommended that multiple samples be tested, as is done with blood cultures. Although PCR is relatively expensive, multiple sampling increases the success rate. The test can be run on whole blood, buffy coat, serum, urine, spinal and other body fluids, and tissue biopsies. Some recommend running all whole blood samples, while others will run a combination of body fluids. More important is the timing of the specimen collection. The disease tends to have a four-week cycle of waxing and waning of symptoms. Therefore, it is critical that specimens be collected when there are active symptoms and the patient is antibiotic free. Antibiotic treatment should be stopped at least six weeks prior to testing. If unexplained rashes/skin lesions are present, it is highly recommended that a biopsy be done with PCR and histology ordered. The pathologist should be notified that spirochetes are suspected. A negative result, however, does not rule out infection, but a positive one is significant.

CD-57

The CD-57 count measures a sub-set of the immune system's natural killer cells. Chronic Lyme infections are known to suppress the immune system, resulting in a decrease of the CD-57 cells. In fact, many believe that only *Borrelia* (all species) will significantly decrease the CD-57 count below 60. Normal value is usually considered to be above 200. Effectiveness of the treatment can be measured by the CD-57 counts. If the count does not improve to near normal values, the prognosis for recovery is poor and relapse often follows. High CD-57 counts usually indicate that a sick patient has something other than *Borrelia* infection or the patient has a co-infection that results in an elevated CD-57.

Reasons for False Negative Lyme Disease Blood Test Results (4)

Antibodies against Bb may not be present in detectable levels in a patient with Lyme disease because

1. The patient is currently on, or has recently taken, antibiotics. The antibacterial effect of antibiotics can reduce the body's production of antibodies.
2. The patient is currently on or has previously taken anti-inflammatory steroidal drugs. These can suppress the immune system, reducing or preventing an antibody response.
3. The patient's antibodies may be bound with the bacteria with not enough free antibodies available for testing.
4. The patient could be immunosuppressed for a number of other reasons, and the immune system is not reacting to the bacteria.
5. The bacterium has changed its makeup limiting recognition by the patient's immune system.
6. The patient's immune response has not been stimulated to produce antibodies-the blood test is taken too soon after the tick bite.
7. In the chronic stage *B. burgdorferi* inhibits the immune system. The organism has been demonstrated *in vitro* to inhibit and kill B- and T-cells.
8. The laboratory sets the cut-off too high or the laboratory is unable to detect the antibodies.

Other Tests

A wide variety of other tests may prove valuable to rule-out other conditions, follow side effects of treatment, and provide supporting diagnostic information.

As the number of tests that might be of value in the diagnosis and treatment of tick-borne disease could be exhaustive, the following is a list of those most closely associated with Bb and co-infections and treatment.

Test	**Results associated with Bb/co-infections**
Ionized magnesium	low levels
CBC	low white blood cell counts
Complete Metabolic Panel (CMP)	elevated liver enzymes
C-Reactive Protein (CRP)	increased
Erythrocyte Sedimentation Rate	elevated at beginning of treatment
Anti-Nuclear Antibody (ANA)	autoimmune disorders are often triggered by Bb or co-infections
Vitamin B12	decreased
Vitamin D	decreased
Vascular endothelial growth factor (VEGF)	increased in co-infection with *Bartonella*
Buffy coat	detailed examination for presence of the organisms

VI. TREATMENT

Treatment of Bb is varied depending on the stage of the disease, morphologic form, and co-infections present. After an infective tick bite, Bb is rapidly disseminated throughout the body including the central nervous system.

Early Localized Infection (Stage 1)

Symptoms occur within one month after the infective tick bite with replication of the spirochetes taking place in tissues adjacent to the bite. The earlier treatment is begun, the higher the success rate. If the classic EM rash is observed, immediate treatment with high doses of antibiotics is begun. Generally, early disease is treated with high doses of one of the tetracyclines, such as doxycycline or minocycline, for four to six weeks. It is important to achieve bacteriostatic levels. Of note is that kill kinetics of antibiotics and *B. burgdorferi* indicate that a large spike in blood and tissue levels is more effective than a sustained level, unlike most antibiotic regimes. Therefore higher doses are given twice a day to achieve the spikes.

Several days after antibiotics are begun there may be increased symptoms. The increased symptoms, which can be quite severe depending on the bacterial load, are called Jarisch-Herxheimer-like reaction. This is probably due to lysis of the spirochetes and release of antigenic material and possibly toxins. It takes approximately 48 to 72 hours for effective killing of the spirochetes to take place, thus correlating with the Jarish-Herxheimer reaction.

Early Disseminated (Stage 2)

Stage 2 usually occurs within weeks to months of the bite as the organism moves via the blood stream and invades other tissues of the body. This movement is reflected in the wide variety of conditions that patients experience. Symptoms generally associated with Stage 2 include inflammatory arthritis, cardiovascular changes, and neurological (photophobia, phonophobia, etc). Antibiotics should be continued for 4 to 8 weeks until no active disease is observed. Typically, this is 4 – 6 months. However, there is a great deal of controversy surrounding long term treatment with antibiotics.

Late or Chronic Infection (Stage 3)

Stage 3 can occur months or years later. Involvement of the central nervous system is the classical symptom for Stage 3. These symptoms can disappear for months or years and then reappear. Late or chronic infections require much longer treatment with a combination of antibiotics, not dissimilar to tuberculosis. There are a number of reasons for this:

<u>First</u>: The organism can exist in two or maybe three different morphologic forms. The one most commonly associated with the disease and seen in body fluids is the spirochete. The spirochete has a 12 to 24 hours generation time *in vitro* and maybe longer *in vivo*. The organism may go dormant with the development of the spheroplast (I-form) or, as recently postulated, even a cystic form during the course of the infection.

As the cystic forms do not contain cell walls, the treatment will need to include different classes of antibiotics.

<u>Second</u>: Bb can be found in body fluids and tissues. As no one antibiotic is effective in both compartments, a multi-dimensional approach is required.

<u>Third</u>: Bb has the ability to invade cells and evade capture. Thus both intracellular and extracellular regimes may be required.
<u>Fourth</u>: There is some evidence that when antibiotics are present, the spirochete will change into a cyst form, returning to the spirochete form when antibiotics are no longer present. Therefore, chronically ill patients who do not respond to treatment may require an antibiotic that attacks the spirochete and another drug that will disrupt the cyst.
<u>Fifth</u>: Immune suppression occurs because of the inhibitory effect of Bb on the immune system. Co-infections are no longer suppressed and become active. Co-infections result in a more severe clinical presentation, with more organ damage, and the pathogens become more difficult to eradicate.

The longer that identification and treatment are delayed, the more severe the complications will be.

VII. CO-INFECTIONS

Frequently a tick will carry more than one organism. The presence of co-infections should always be considered. It is not uncommon to have more than one co-infection. Lyme disease with co-infections typically presents with a greater number of symptoms and the disease is more severe. The more common co-infections that can be transmitted along with the *Borrelia* species from a tick bite are:

Babesiosis (piroplasmosis): Piroplasms are not bacteria; they are protozoans. There are at least thirteen and possibly more than two dozen known piroplasm species. It is believed that the tick may carry most of these and potentially can transmit them to humans. However, at the present time we can test for only two of the species, *Babesia microti* and *Babesia duncani* (isolate WA-1). *Babesia* infections are becoming recognized more frequently in patients with Lyme disease. Recent studies have reported as many as 66% of Lyme patients show serologic evidence of co-infection with a *Babesia* species. *Babesia* infections, even mild ones, may recur even after treatment up to several years after the initial infection. *Babesia* infections, like Bb, are immunosuppressive. *Babesia* carriers pose a risk to the blood supply as this infection has been reported to be passed on by blood transfusions.

The organism can be seen on standard Giemsa-stained blood smears early in the infection (up to two weeks). After that an acridine orange stained buffy coat preparation or fluorescent in-situ hybridization assay (FISH) is recommended. A Maltese cross (tetrad) configuration in erythrocytes is diagnostic for *B. microti* infection. Ring forms closely resembling those seen in malaria are also present. PCR is very sensitive for *B. microti* but is not available for other species.

***Bartonella* organism**: *Bartonella henale*, associated with “cat-scratch disease,” reacts differently from the *Bartonella* found with tick bites. For this reason, *Bartonella*-like organism (BLO) is used to describe this infection. The diagnosis is made on clinical symptoms as the standard PCR or serology test does not pick up BLO. Indicators of BLO infection are primarily central nervous system symptoms and red rashes. The rash differs from that seen in Lyme disease in that it has the appearance of red streaks like stretch marks.

***Ehrlichia*:** Human Erlhichiosis is caused by rickettsial-type organisms. Antibody titers are determined by IFA and PCR specific tests and are used to identify Human Granulocytic

Anaplasmosis (formerly Ehrlichiosis), caused by *Anaplasma phagocytophila*, and Human Monocytic Ehrlichiosis, caused by *Ehrlichia chaffeensis*. More species are known to be present in ticks than can be tested for at this time. Seroconversion usually occurs from two to four weeks after infection. It is recommended that acute and convalescent specimens be collected. When present alone or without *B. burgdorferi*, persistent leucopenia is an important clue. Thrombocytopenia and elevated liver enzymes are commonly present in early disease.

Other known tick-borne infectious organisms that may be present with Lyme disease are **Colorado tick fever, Encephalitis, Q fever, Rocky Mountain spotted fever, tick paralysis, tick-borne relapsing fever, and Tularemia.**

VIII. BRIEF HISTORY OF LYME DISEASE

In the early 1970s, 50 children and 12 adults from the community of Lyme, Connecticut presented with arthritic and neurological symptoms. In conjunction with Yale University researchers and the Institute of Arthritis and Musculoskeletal and Skin Diseases, a condition called "Lyme arthritis" was described. In 1978 Dr. Allen Steere published his research linking ticks and Lyme disease. A few years later (1982) Dr. Willy Burgdorfer identified the etiological agent as a spirochete that was named *Borrelia burgdorferi*. Originally, the ticks associated with Lyme disease were thought to occur only in the Northeastern part of the United States. As a result, for many years the diagnosis of Lyme disease was only considered amongst those individuals living or traveling in the Northeast.

However, this is not the case. In fact, Lyme disease was first described in Europe by Dr. Alfred Buchwald in 1883 as a degenerative skin disease. In 1909 Dr. Arvid Afzelius of Sweden published in his research on the expanding "bull's eye" so characteristic of early Lyme disease. In 1929 Dr. Afzelius speculated that the tick *Ixodes scapularis* was responsible for the disease. Between the 1920s and 1940s, a number of publications identified and linked the EM rash with many of the problems seen in patients with Bb infections including arthritic, neurological, cardiac, and ophthalmologic conditions.

The World Health Organization (WHO) lists Lyme disease as a significant health problem in Canada, Great Britain, Australian, Japan, New Zealand, Russia, and most of the European continent. In Germany it is estimated that 15% of the population is infected.

In Europe and Asia the organisms causing the disease are *Borrelia garinii* and *Borrelia afzelli*. The organism found in the United States, and also in Europe, is *Borrelia burgdorferi sensu stricta.*

In the United States every state with the exception of Hawaii has had Lyme disease as a reportable disease since 1982. There is a concentration of cases in the Northeast and the Upper Midwest with Northern California also showing a significant numbers of cases. The following table shows the states having the highest number of cases, with California ranking number 13.

STATES SHOWING HIGHEST NUMBER OF LYME DISEASE CASES 2008*

RANK	STATE	NUMBER OF CASES
1	Pennsylvania	6958
2	New York	5714
3	New Jersey	2801
4	Maryland	2076
5	New Hampshire	1465
6	Minnesota	1183
7	Wisconsin	1146
8	Massachusetts	1039
9	Maine	868
10	Virginia	809
11	Delaware	766
12	Vermont	362
13	California	209

*MMWR Vol, 57 No. 53; Jan 9, 2009

The first recognized human case in California occurred in 1978 in a hiker in Sonoma County. Mendocino, Humboldt, and Trinity counties have the highest incidence. The reported cases in California have decreased from 139 in 1999 to 78 in 2007 (The last year that reports are available at the time of this article.)

From 1980 until December of 2008, 341,000 cases of Lyme disease have met the CDC criteria for reporting. In 2008 through December 31, 26,739 cases were reported to the CDC (MMWR Vol. 58, No.2, 1/9/09). The CDC estimates that only one in 10 cases is actually reported. Conservatively, that would translate to 267,390 cases in 2008 or 3.41 million cases since 1980 in the United States.

IX. TICKS AND PREVENTION

There are several species of deer ticks across the United States that are known to carry *Borrelia burgdorferi*. They are: *Ixodes scapularis* found in the Northeast & upper Midwest; *Amblyomma americanum* found throughout the United States; and *Ixodes pacificus* found in the West in coastal regions and along the Sierra Nevada range. *Ixodes pacificus* is commonly known as the western black-legged tick.

Ticks have three life stages: larva, nymph, and adult. During each stage the tick must attach to an animal for a blood meal. Typically, the tick will attach for several days to take one blood meal then drop off and mature into the next stage. Ticks do not pass the Lyme bacteria to their offspring. Instead, the larvae become infected when they feed on an infected animal. They then pass the bacteria on to the next animal that provides a blood meal. Larvae generally feed on small rodents, lizards, or birds. The nymph stage feeds on small animals and humans. Adults tend to feed on larger animals such as deer, hence the name “deer ticks.” Only nymphs and adult females transmit Lyme disease to humans. Many individuals do not know they have been exposed to or bitten by a tick, as ticks, particularly the nymphs, are very small and difficult to see. All three stages of the western black-legged tick can fit on the tip of your index finger. Once they have taken a blood meal and are engorged, they are easier to see, but many individuals are not aware that they have been bitten due to the location and size of the tick. Nymphs are <1/20th of an inch in size or about the size of a sesame seed. These dots represent the approximate size of the ticks at each stage.

•	•	●
Nymph	Adult Male	Adult Female

Ticks have a two year life cycle. Eggs are laid in the spring and larvae hatch out in late spring and summer. They take their first blood meal and may acquire the spirochetes at this time. They remain dormant for the fall and winter and molt into the nymph stage the following spring. The nymphs molt into adults over the fall and winter, ready to mate and lay eggs in the spring. The highest number of new cases is reported in the spring of each year as nymphs are the most active from March to July. At that time they are looking for a blood meal and are more likely to attach to humans than adult ticks are. Approximately 5-15% of nymphs are infected with *Borrelia burgdorferi* in California. Nymphs are found in cool, moist areas—in leaf litter, on logs, tree trunks, fallen branches, and under trees.

Adult ticks are most active in the fall and winter, from October to February. Adult female ticks are more likely to spread disease since they need a larger blood meal. Adult ticks position themselves on low vegetation and grasses, approximately 24 inches tall, waiting for a host meal to pass by. They tend to be located on the uphill, shady side of trails.

For Lyme disease to exist in an area the following need to be present:

1. The Lyme spirochete
2. Ticks that can transmit the disease
3. Mammals, such as mice and deer to provide food for the tick.

Migrating birds spread the disease. When the birds feed on the ground, ticks can attach to them and infect them. Then the bird carries the organism to a new area where local ticks can pick up the spirochete.

Typically ticks need to attach for two to three days to complete a blood meal. The longer they are attached, the greater the risk of acquiring an infection. Some literature suggests that ticks attached less than 24 hours are not likely to cause Lyme disease. This may be due to the fact that the spirochetes must move from the midgut to the saliva glands of the tick. In order for this to occur *B. burgdorferi* requires a protein, OSP-C (outer surface protein C) to be present. The host's response with polymorphonuclear leukocytes (PMNs) provides initial protection. However, the saliva of the tick contains elements that decrease the effectiveness of the host immune defenses. The binding ability of PMNs is reduced, which reduces spirochete killing. Also, tick saliva inhibits T-cells by binding interleukin 2 (IL-2), effectively reducing the T-cell response.

Prevention is the best way to avoid Lyme disease. If possible stay out of areas known to have ticks, particularly in the spring. The county vector control agency or department of health can provide information on the locations to avoid. If you cannot avoid these areas, protect yourself by:

1. wearing protective clothing – hat, long sleeved shirts, and long pants.
2. tucking pants into socks or boots and shirts into pants.
3. wearing light colored clothing – makes the tick more visible.
4. staying on trails and avoiding bushes and grasses.

5. using tick repellent.
6. checking pets for ticks.
7. putting clothes in dryer for 30 minutes to kill ticks.
8. examining self (especially behind earlobes, knees, in the hairline, groin, axilla, under the breast, etc.) and removing ticks promptly.
9. checking for several days since ticks are small; as they engorge they are more easily seen.

If you are bitten by a tick, remove the tick as soon as possible. The tick should be removed with a pair of tweezers. The tweezers should be placed flat on the skin and moved around the mouth parts of the tick. Pull straight out, using a firm steady motion: do not jerk or twist Do not smother, squish, or burn an attached tick: these procedures are ineffective. Wash your hands and the bite site with soap and water and apply an antiseptic. Ticks can be tested for Lyme, *Babesia*, *Bartonella*, and *Ehrlichia*, so save the tick in an airtight container or zip lock bag with a moist cotton ball.

X. CONCLUSION

Our patient presented with many of the late and chronic complications seen with Lyme disease. The severity of her disease suggested multiple infections. An examination of her laboratory work supported a diagnosis of multiple infections. The Western Blot showed positive bands at the KDa 31 and KDa 41 positions, which makes the diagnosis of *Borrelia burgdorferi* or Lyme disease. The *Babesia duncani* IgM titer of 1:20 and the IFA-HGE Ehrlichiosis-IgM titer of 1:20 add the co-infections of Babesiosis and Ehrlichiosis. The low WBC and elevated liver enzymes lend additional support to the diagnosis of multiple tick borne infections. Since ticks often carry more than one infectious organism, many patients will develop co-infections from a single encounter. Others, who are frequently outside where their exposure is greatly increased, may acquire multiple infections from more than one tick bite.

Although Lyme disease was first recognized in the 1800s and has been identified in many parts of the world, many health care practioners have never seen a case or did not recognize the disease without the characteristic EM rash being present. The recognition of late and chronic forms is just beginning to be accepted and treatment modalities developed. Controversy continues to swirl around criteria to diagnose and treat the tick-borne diseases. The Connecticut Attorney General’s office antitrust investigation of the Lyme disease guidelines, published by the Infectious Diseases Society of America’s (IDSA), uncovered a number of serious flaws in the 2006 Lyme disease guidelines. The IDSA has agreed to convene another panel representing an expanded community to review the large body of knowledge on tick-borne infections. Whatever the outcome of the different opinions, it remains that these infections pose a serious threat to individuals who live and work in endemic areas--which covers most of the United States, Canada, and Europe, with South America and Africa beginning to report cases. Continued research is essential to identify the most effect diagnostic and treatment modalities.

XI. REFERENCES

1. CDC. Recommendations for test performance and interpretation from the Second National Conference on Serologic Diagnosis of Lyme Disease. *MMWR*. 1995:44:590-591.
2. Burrascano JJ. Advanced Topics in Lyme Disease: Diagnostic Hints and Treatment Guidelines for Lyme and Other Tick Borne Illnesses, *Managing Lyme Disease*. 15th ed. September, 2005.
3. Vojdani A, Raxlen B, Scott, S. The use of lymphocyte proliferation assay and cytokine production in seronegative patients with Lyme arthritis or neuroborreliosis. *Townsend Letter for Doctors and Patients*. May, 2007.
4. Kaplan M. Reasons for false negative results in Lyme disease. www.anapsid.org/lyme/lymeseroneg.html
5. Hodzic E, Sunlian F, Holden K, Freet K, Barthold SW. Persistence of *Borrelia burgdorferi* following Antibiotic Treatment in Mice. *Antimicrobial Agents and Chemotherapy*. May 2008:1728-1736.
6. Singleton KB. *The Lyme Disease Solution.* Dallas, Texas: Brown Books, 2008.
7. Stricker R. Lyme Disease: The Hidden Epidemic. California Committee on Health & Human Services. February 25, 2004.

REVIEW QUESTIONS
#DL-988
Choose the **one** best answer.

1. Lyme disease, one of the most studied and best known of tick-borne diseases, is caused by which of these organisms:
   a. *Babesia duncani*
   b. *Borrelia burgdorferi*
   c. *Rickettsia rickettsii*
   d. *Anaplasma phagocytophilum*

2. The classic sign of Lyme disease is:
   a. an oozing sore at the site of a tick bite.
   b. a red streak gradually elongating from the site of a tick bite.
   c. high fever occurring within several hours of a tick bite.
   d. the appearance of a "bull's-eye" rash at the site of a tick bite.

3. Erythema migrans:
   a. appears only at the site of the tick bite.
   b. may occur in only 50% of Lyme disease infections.
   c. is often mistaken for allergy to poison oak.
   d. is representative of infection by three separate microorganisms.

4. Which of these is not a possible symptom of Lyme disease?
   a. Sudden rise in blood pressure
   b. Unexplained hair loss
   c. Bell's palsy
   d. Vertigo

5. The number of symptoms of Lyme disease and their severity may be directly related to the presence of co-infections that were also transmitted through a tick bite. Which of the following is another tick-borne infection?
   a. *Babesia duncan*i
   b. West Nile virus
   c. *Borrelia burgdorferi*
   d. Ringworm

6. Even though Lyme disease is diagnosed on clinical findings, due to no available laboratory test being definitive, two categories of laboratory tests are used to aid in the diagnosis. These two categories are:
   a. complete chemistry panel and CBC.
   b. ESR and D-Dimer.
   c. coagulation studies and platelet count.
   d. Bb antibody detection and direct detection of Bb in the body fluids and tissue.

7. The ELISA and the IFA antibody detection tests are both recommended by the CDC as screening tests for Lyme disease. However, both are problematic because?
    a. Even with sufficient time the body may never develop any antibodies to the bacterial infection.
    b. The tests have high sensitivity but accuracy is low.
    c. Late intervention treatment with antibiotics has reduced the number of antibodies present.
    d. Low antibody detection due to Bb bacteria changing their surface characteristics.

8. Western Blots are reported by showing which bands are reactive. Which statement is <u>not</u> true?
    a. Some bands can cross-react with other spirochetes.
    b. Band 60KDa is non-specific and non-diagnostic.
    c. Band 37KDa is species specific and always present.
    d. The LYMErix vaccine could produce a positive reaction in bands 31KDa and 43KDa which are specific for Bb.

9. Which of the following is true of the polymerase chain reaction test?
    a. PCR is not used to test for Lyme disease infection.
    b. The test can be run on both urine and whole blood.
    c. The specimens can be collected at any time during infection with high sensitivity.
    d. Although PCR is expensive, a negative result will rule out infection.

10. The CD-57 count measures a sub-set of the immune system's natural killer cells. Which of these statements is <u>not</u> true?
    a. Effectiveness of treatment can be measured by CD-57 counts.
    b. Co-infections can affect the CD-57 count.
    c. Chronic infections of Bb can suppress the immune system and the CD-57 count.
    d. The CD-57 count is not used as a prognostic tool.

11. Treatment of Bb varies due to several factors. Which of these does <u>not</u> affect treatment?
    a. Presence of EM rash
    b. Co-infections present
    c. Site of tick bite on patient
    d. Whether infection is early stage or late stage

12. Early stage infection is generally treated first with:
    a. high doses of one of the tetracyclines.
    b. any antibiotic which will achieve bacteriostatic levels.
    c. a tetracycline given once a day.
    d. a tetracycline given for three weeks.

13. The Jarish-Herxheimer-like reaction is:
    a. increased symptoms due to allergic reaction to antibiotics.
    b. probably due to lysis of the spirochetes and release of antigenic material and toxins.
    c. increased symptoms after antibiotics are begun, however symptoms are almost always mild.
    d. severe symptoms due to the disease but unrelated to treatment.

14. Late or chronic infection requires longer treatment. Which of the following is <u>not</u> true of chronic infection?
    a. Requires combination of antibiotics.
    b. The spirochete may go dormant with the development of spheroplast or even a cystic form.
    c. Bb can be found in body fluids but not in tissues.
    d. The longer identification and treatment is delayed the more severe the complications.

15. *Babesia duncani* is one of the organisms transmitted to humans by ticks. This organism is a:
    a. bacterium
    b. virus
    c. mold
    d. piroplasm

16. Which of the following is true of the *Bartonella* associated with tick bites?
    a. Diagnosed with a standard serology test
    b. Diagnosed with a polymerase chain reaction test
    c. The same disease as “cat scratch disease”
    d. Often has a rash with the appearance of red streaks

17. Human Ehrlichiosis is caused by rickettsial-type organisms. Which of these statements is <u>not</u> true?
    a. Antibody titers are a tool used for diagnosis.
    b. All species of *Ehrlichia* known to be present in ticks can be tested for by using PCR specific tests.
    c. Persistent leucopenia is an important clue in diagnosing Ehrlichiosis.
    d. Seroconversion usually occurs from two to four weeks after infection.

18. Which of these statements is true of Lyme disease?
    a. The disease is found only in the Northeastern part of the United States.
    b. The World Health Organization does not consider Lyme disease to be a significant health problem.
    c. The spirochete *Borrelia burgdorferi* was identified by Dr. Willy Burgdorfer.
    d. The CDC estimates that one in five cases of Lyme disease is reported.

19. There are several species of deer ticks across the United States that are known to carry *Borrelia burgdorferi*. *Ixodes pacificus* is commonly known as the western black-legged tick. It is found:
    a. mainly in desert areas.
    b. in the coastal regions of the West and along the Sierra Nevada range.
    c. only along the Canadian border.
    d. easily due to its large size.

20. Prevention is the best way to prevent Lyme disease and other tick-borne infections. Which of these statements is <u>not</u> true?
    a. Wear protective clothing - hat, long sleeves, and long pants.
    b. Use tick repellant when outdoors in grasses and bushes.
    c. Know how to remove a tick and how to save it for testing.
    d. Put clothes in dryer for three minutes on low heat to kill ticks.

Course #DL-988 – THE GREAT IMPOSTER– Lyme Disease
Registration/Answersheet - 2.0 CE Credit

Name ___________________________________ CLS Lic. # _____________ Date ___________

Signature (Required) ____

Address ____
Street City State/Zip
Payment Method ___ Check or ___Credit Card # _________________________ Type -Visa / MC

2.0 CE Fee = $30 (members $24) Exp. Date _________ Signature _________________________

Please circle the one best answer for each question.

1.	a	b	c	d	11	a	b	c	d
2.	a	b	c	d	12	a	b	c	d
3.	a	b	c	d	13	a	b	c	d
4	a	b	c	d	14	a	b	c	d
5	a	b	c	d	15	a	b	c	d
6.	a	b	c	d	16	a	b	c	d
7.	a	b	c	d	17	a	b	c	d
8.	a	b	c	d	18	a	b	c	d
9	a	b	c	d	19	a	b	c	d
10	a	b	c	d	20	a	b	c	d

Distance Learning Evaluation Form

According to state regulations, this evaluation must be completed and returned in order to receive CE hours. Your comments help us to provide you with better continuing education materials in the distance learning format. Please circle the number that agrees with your assessment.

1. Overall, I was satisfied with the quality of this Distance Learning course.

   (strongly agree) 5 4 3 2 1 (strongly disagree)

2. The objectives of this Distance Learning course were met.

   (strongly agree) 5 4 3 2 1 (strongly disagree)

3. The difficulty of this Distance Learning course was consistent with the number of CE hours.

   (strongly agree) 5 4 3 2 1 (strongly disagree)

4. I will use what I learned from this Distance Learning course.

   (strongly agree) 5 4 3 2 1 (strongly disagree)

5. The time to complete this Distance Learning course was: __________ hours

6. What did you like or dislike about this Distance Learning course ?

Common comprehensive Medical laboratory scientist degree programs are set up in a few different ways. In 3+1 programs, the student attends classroom courses for three years and complete a clinical rotation their final year of study. In 2+2 programs, students have already completed their lower division coursework and return to complete their last two years of study in a CLS program. In 4+1 program, students who have already completed an undergraduate program return to complete a year of medical laboratory training.Â California has similar restrictions on MLTs. To accommodate California's restrictions, the American Association of Bioanalysts (AAB) developed a separate certification examination for California licensure. 1 California Association for Medical Laboratory Technology Distance Learning Program NEUTROPHILIA by Helen M. Sowers, MA, CLS Dept. of Biological Science (retired) California State University, East Bay Hayward, CA Dora W. Goto, MS, CLS, MT(ASCP) Laboratory Manager Bay Valley Medical Group Hayward, CA Course DL CE/Contact Hour Level: Basic.Â ACKNOWLEDGMENTS: Major funding for photographs used in this presentation was provided by: California Health Foundation and Trust (CHFT) Healthcare Laboratory Workforce Initiative (HLWI) of the Healthcare Foundation of Northern and Central California California Association for Medical Laboratory Technology (CAMLT) All 1. California Association for Medical Laboratory Technology Distance Learning Program Hematology Case Studies: Platelets by Dora W. Goto, MS, CLS, Helen M. Sowers, MA, CLS MT(ASCP) Dept of Biological Science (ret.) Laboratory Manager California State University, East Bay Bay Valley Medical Group Hayward, CA Hayward, CA Course Number: DL-985 1 .0 CE/Contact Hour Level of Difficulty: Intermediate Â© California Association for Medical Laboratory Technology. Permission to reprint any part of these materials, other than for credit from CAMLT, must be obtained in writing from the CAMLT Executive Offi