Managing Lyme Disease and Ehrlichiosis

A roundtable discussion

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Roundtable Speaker Biographies

Managing Lyme Disease and Ehrlichiosis

A. Rick Alleman, DVM, PhD, DABVP, DACVP, is an associate professor of clinical pathology at the University of Florida College of Veterinary Medicine in Gainesville, Fla. He received his DVM in 1980 from the Louisiana State University School of Veterinary Medicine, Baton Rouge, La. He practiced for nine years in New Orleans, La., at a small-animal practice. Dr. Alleman received his PhD in the area of molecular biology of tick-transmitted disease at the University of Florida College of Veterinary Medicine in 1995. Dr. Alleman's research interests are the molecular diagnosis of animal diseases and identification of immunogenic surface antigens for the serologic diagnosis of *Haemobartonella felis.* Past and present work involves the use of molecular tools such as cloning, PCR, and hybridization studies in the diagnosis of rickettsial hemoparasites. Dr. Alleman is the recipient of numerous awards, including the Florida Veterinary Medical Association's Clinical Investigator Award.

Edward B. Breitschwerdt, DVM, DACVIM, graduated from the University of Georgia's School of Veterinary Medicine in 1974. He completed an internship and residency in internal medicine at the University of Missouri in 1977.Currently, Dr. Breitschwerdt is a professor of medicine and infectious diseases at the North Carolina State University College of Veterinary Medicine, Raleigh, N.C. He is also an adjunct associate professor of medicine at Duke University Medical Center. Dr. Breitschwerdt's clinical interests include infectious diseases, immunology, and nephrology. His research emphasis has been on vector-transmitted, intracellular pathogens. Since 1984, he has supervised a biosafety level P-3 research laboratory and cosupervised the Vector-borne Diseases Diagnostic Laboratory at North Carolina State University. A former associate editor for the *Journal of Veterinary Internal Medicine*, Dr. Breitschwerdt has published more than 150 articles in scientific journals.

Richard B. Ford, DVM, MS, DACVIM, DACVPM, graduated from The Ohio State University School of Veterinary Medicine. He completed an internal-medicine residency at Michigan State University. Dr. Ford is a professor of medicine at North Carolina State University's College of Veterinary Medicine in Raleigh, N.C., in the department of clinical sciences. He has published five books and numerous articles about the diagnosis and treatment of companion-animal diseases. His clinical interests include companion-animal infectious disease diagnosis and immunization. Dr. Ford is a brigadier general in the Air Force Reserve and is currently assigned to the Pentagon as the mobilization assistant to the surgeon general of the Air Force. He is the highest ranking veterinarian in the Department of Defense.

Steven A. Levy, VMD, is Hospital Director at Durham Veterinary Hospital in Durham, Conn. He received his VMD from University of Pennsylvania's School of Veterinary Medicine in 1977. In 1986, he diagnosed the first case of canine Lyme carditis, published in *Journal of Veterinary Internal Medicine* in 1988. He has published many other scientific articles, including a report on a safety and efficacy study of a canine Lyme disease bacterin in the *Journal of the American Veterinary Medical Association*. Dr. Levy received the AVMA's Practitioner Research Award and the AAHA's Outstanding Regional Practitioner Award.

Mario T. Philipp, PhD, is professor of microbiology and immunology at the Tulane University Health Sciences Center, and chair of the division of bacteriology and parasitology at the Tulane National Primate Research Center, in Covington, La. He has published more than 100 peer-reviewed articles on bacterial and parasitic diseases. Dr. Philipp's research interests currently encompass the pathogenesis, diagnosis, and chemotherapy of Lyme borreliosis, as well as the biology of B*orrelia burgdorferi*, the spirochete that causes the disease. Research conducted in Dr. Philipp's laboratory was instrumental to the development of the C6 diagnostic test for Lyme borreliosis.

Reinhard K. Straubinger, DVM, PhD, is currently head of the junior Molecular Medicine of Infectious Diseases research group, part of the Biotechnological-Biomedical Center and the College of Veterinary Medicine at the University of Leipzig, Germany. He received his veterinary degree in 1993 from the Ludwig-Maximilians University in Munich, Germany. The same year, Dr. Straubinger started his research at the James A. Baker Institute for Animal Health at Cornell University in Ithaca, N.Y., and focused primarily on the pathogenesis of Lyme disease in dogs. He received his DVM from the University of Munich in 1995, and two years later his PhD from Cornell University. Dr. Straubinger has authored or co-authored more than 20 papers on canine borreliosis, *Helicobacter* infection in dog and cats, and proinflammatory cytokines. He recently received two awards from the German Veterinary Medical Society in appreciation of his scientific achievements.

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A roundtable discussion

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In the past few years, several publications on tick-borne diseases in people and dogs have raised concern over the actual prevalence and clinical significance of these complex infections. With the introduction and widespread use of the Snap 3Dx test, a rapid ELISA-based test for in-clinic assessment of canine ehrlichiosis, Lyme disease, and heartworm disease, veterinarians now have an important testing platform to facilitate diagnostic and treatment decisions regarding these potentially fatal infections.

The following roundtable discussion is important because it not only addresses the clinical use and interpretation of the Snap 3Dx test, but it represents the opinions of internationally recognized experts on two of the most important tick-borne infections in the dog: ehrlichiosis and Lyme disease. Participants address several clinical aspects of these infections, ranging from prevalence and true geographic distribution, testing recommendations, and interpretation of the positive test result to treatment recommendations and prognosis.

—Richard B. Ford, DVM, MS, DACVIM

Disease — Prevalence —

Q. What changes have occurred in the prevalence of Lyme disease in the United States over the past decade?

Dr. Steven Levy: It's difficult to determine the changes in Lyme disease prevalence because there wasn't a good in-office test for infection with *Borrelia burgdorferi*, the Lyme agent, until 2001. But since the advent of the Canine Snap 3Dx test (IDEXX) that year, more and more veterinarians are realizing just how many of their canine patients are infected—that includes both sick dogs and infected dogs not showing signs of disease. The disease prevalence is more obvious because we now can recognize infection in dogs that have subclinical disease. Also, Lyme disease is moving geographically because its vectors are spreading and because people are traveling more with their pets.

Dr. Mario Philipp: The Centers for Disease Control and Prevention (CDC) offers incidence data on human Lyme disease

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HIGHLIGHTS

- When making a diagnosis of Lyme disease, you need your patient's travel history to find out whether the individual has been to an endemic area.
- In 2002, Lyme disease cases increased to 23,763. This is a 40% increase. If this increase is part of a trend, which will become clear in the near future, it certainly deserves attention.

through 2002. The number of cases reported to the CDC was 16,801 in 1998 and 17,092 in 2001. This is perhaps a low estimate of the overall incidence because it is possible that not all true disease cases were reported to the CDC. The number of reported cases remained fairly constant over those four years. Interestingly, in 2002 it increased to 23,763 in the United States. This is a 40% increase. If it is part of a trend, which will become clear in the near future, it certainly deserves attention.

Levy: And we know how ridiculously low that number is, because without an active surveillance system, Lyme disease is underreported in people.

Diagnostic — Criteria —

Q. What are the current CDC criteria for diagnosing Lyme disease in people?

Philipp: The current CDC criteria for a confirmed human Lyme disease case definition are as follows: a patient with erythema migrans (*i.e.*, a round skin lesion that typically begins as a red papule and expands over a period of days to weeks), provided that the patient is known to have been outdoors in an endemic area shortly before the onset of the lesion. If not, laboratory confirmation (*e.g.,* serology) is recommended. Lab confirmation is also required to confirm cases that present with late Lyme disease manifestations, such as arthritis. Unfortunately, erythema migrans is not detectable in dogs.

Dr. Edward Breitschwerdt: An epidemiology student in my lab spent several months studying the C6 peptide results with the 3Dx test in dogs from North Carolina, Virginia, Maryland, and Pennsylvania. I was amazed at the data. Out of the 987 dogs tested, we only found four dogs that were positive. Of those four dogs, two were born in New York and had moved to North Carolina. One dog, born in North Carolina, traveled frequently to the northeastern United States for hunting, and the other dog resided in North Carolina, but traveled to a farm in north central Pennsylvania once or twice a summer. Using the C6 peptide to test nearly 1,000 samples sent to the vector-borne disease diagnostic lab, we found that every dog that tested positive for antibodies to *B. burgdorferi* had traveled to or originated from an endemic northern state. The seroprevalence in dogs from Virginia was 8.7%. For Maryland dogs it was 14.4%, and for Pennsylvania dogs it was 25%. Again, the testing was done by the same individual using the 3Dx C6 peptide. I am amazed by the specificity of this peptide.

Philipp: That sounds like a good study, especially the North Carolina data with the rational explanation for positivity.

Breitschwerdt: The earlier tests—the immunofluorescence assay (IFA) and ELISA, which were done back in the early- to mid-1980s—always showed very low *B. burgdorferi* serum antibody prevalences. So the new data are impressive. They tell physicians and veterinarians, in North Carolina at least, that when making a diagnosis, you need your patient's travel history to find out whether the individual has been to an endemic area. If a dog indigenous to North Carolina was found to be C6 peptide positive and didn't have a suggestive travel history, we would confirm the result using Western blot, culture, or polymerase chain reaction (PCR).

Levy: We recently studied unvaccinated dogs in Middlesex County, Connecticut.[1] We found infection rates ranged from 41% to 73%. This gives some perspective on how intense the risk of infection is in this region compared with south of Virginia. I think once a dog has developed a positive C6 antibody, that dog is infected—and possibly infected for life—in spite of attempts at therapy. It takes four to six weeks for C6 antibodies to develop after a bite from an infected tick. Studies have demonstrated that *B. burgdorferi* infections will sequester and become chronic.

Q. How many of the dogs with a positive test result had clinical signs?

Levy: This study involved clinically normal dogs being tested with the 3Dx test for heartworm infection. How-

ever, the significance of a positive C6 ELISA is that it represents evidence of infection, and infection has been demonstrated to be persistent, even after antibiotic therapy.

Ford: That raises a question about disease prevalence in other locations. Texas is a particularly controversial state with respect to Lyme disease testing. Portions of southeastern Texas do have Lyme-positive dogs. But some clinicians deny that Lyme disease occurs there. I'm not sure that's the case.

Levy: I agree. If clinicians are finding positive C6 ELISA results on the Snap 3Dx test, then they are finding evidence of infection. If these tests are from local dogs with no history of travel, then the infection was acquired locally. I think the question about infection in unexpected areas comes from preconceived notions, a lack of good data from testing large numbers of dogs, and infection cycles that maintain and spread *B. burgdorferi* differently from the classic New England cycle.

Dr. Rick Alleman: We've had some referrals from Florida hospitals where Lyme disease was diagnosed, presumably by IFA testing. The dogs didn't respond to treatment and were referred to us. We questioned the diagnosis based on the patients' clinical presentation. Each time, they were negative with C6 peptide testing. As we've said, the test has a very high specificity.

Surveillance — Testing —

Q: Consider the role of surveillance testing, the routine testing of apparently healthy dogs for subclinical Lyme disease and ehrlichiosis. For example, do we really know today what the geographic distribution of borreliosis is in the United States, particularly in the southern states outside the classic endemic regions? Is it feasible for clinicians to perform surveillance testing in dogs living in areas not known to be endemic for either Lyme disease or ehrlichiosis?

Levy: If you're testing your patients for heartworm infection annually, you can also test for *Ehrlichia* and *Borrelia* infection with the 3Dx test. The only way we'll know if the disease is emerging in an area is to test for it. We've talked about how specific the test is—that's important. The false positive IFA patients referred to the University of Florida indicate that IFA testing is not as useful. We have a very specific and sensitive test that we can use for *Ehrlichia* and *Borrelia* surveillance testing while testing for heartworm infection.

Breitschwerdt: Somebody needs to look at the Texas story critically. As Dr. Levy said, we now have a test that can do just that. It would be important to screen populations of dogs with varying risks of tick infestation. As with North Carolina, Texas veterinarians also need to obtain a travel history. Many residents temporarily leave the Dallas, Fort Worth, and Houston areas for cooler northern climates, just like Floridians do. When there are positives in a Lyme nonendemic area, the travel history of the animal is important. As Dr. Levy alluded to—when you have a high prevalence of *B. burgdorferi* in *Ixodes scapularis* in northeastern states, such as Connecticut, it doesn't require exposure to too many ticks for a dog to become infected, develop a positive antibody response, and remain infected for months to years thereafter. I've wrestled with the concept of screening for chronic vector-borne infections, and I've come to the conclusion that screening for organisms that can induce chronic infection and remain quiescent for years before development of disease signs makes good clinical sense.

Alleman: Screening our pet population for diseases, such as *E. canis*, may be as important as vaccinating with regard to the practice of good preventive medicine. Heartworm disease, Lyme disease, and ehrlichiosis all have stages in which subclinical infections occur in the pet. In addition, if subclinical infections are untreated, they may develop into chronic disease. If we can detect animals with *E. canis* infection before chronic signs manifest, we can not only prevent the development of potentially serious disease, we also improve the probability of clearing the organisms from the infected animal.

HIGHLIGHTS

- Once a dog has developed a positive C6 antibody, that dog is infected—and possibly infected for life—in spite of attempts at therapy.
- Screening our pet population for diseases, such as *E. canis*, may be as important as vaccinating with regard to the practice of good preventive medicine.

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HIGHLIGHTS

- Certain dog breeds (*e.g.*, Labradors and Bernese mountain dogs) have dramatic antibody responses and may develop classic signs of Lyme disease and renal failure.
- Dogs with clinical signs of Lyme arthritis may have subclinical renal disease.

— Clinical Signs —

Q. Regarding Lyme disease testing, veterinarians using the 3Dx wonder when they should test. Probably no one disagrees that they should test when overt clinical signs are present. What are the clinical signs for Lyme disease?

Levy: I have eliminated most of the Lyme disease in my practice through aggressive vaccination since 1990, but the most common sign was joint disease. A dog would present with a sudden onset of fever, lameness, lymphadenopathy, swollen joints, and a reluctance to move. Clinically, we saw a very sick dog with joint problems indicating Lyme arthritis or synovitis. The fever could be 105 F or higher. Many dogs responded rapidly to antibiotic therapy. Another manifestation was dogs with severe renal disease—dogs with uremia, hyperphosphatemia, and hypoproteinemia. Sometimes the protein loss was so bad that they had peripheral edema. It generally happens in young dogs, and they do not respond well to therapy, as opposed to old dogs that have chronic renal disease not associated with *B. burgdorferi*.

Breitschwerdt: Dr. Straubinger, would you comment on the severity of the Lyme disease-associated polyarthritis that you found in experimental dogs? I'd also like to hear about Labrador retrievers—and Labrador crosses, in particular—with the acute renal failure syndrome associated with *B. burgdorferi*. I understand that no one has seen evidence of renal lesions in any experimental animals or in association with human Lyme disease. That doesn't mean that acute renal failure caused by *B. burgdorferi* can't occur in a genetically predisposed group of dogs.

Dr. Reinhard Straubinger: In the experimental dogs, we noticed monarthritis or oligoarthritis after exposure to ticks. The dogs developed it about 60 days after exposure. We did synovial taps to investigate the cases and show the effects in the joint. The synovial fluid increased in volume and contained neutrophils, which prompted us to look into the cytokine network. We found that a chemoattractant was drawing the neutrophils into the joints.

In regard to renal failure, there are several publications on this topic.[2,3] Certain dog breeds (*e.g.*, Labradors and Bernese mountain dogs) have dramatic antibody responses and may develop classic signs of Lyme disease and renal failure. A Netherlands study[4] found that affected dogs have severe clinical signs (*e.g.*, lameness and nervous system and urinary tract abnormalities) and high antibody titers during the phase of acute arthritis. We don't know the real cause for renal failure in these breeds. There is no animal model that can show us what happens. In the Bernese mountain dog, you find high antibody titers and possible immune complexes in the kidney, which you will probably not find in beagles or other breeds.

Levy: In these European studies, do we know that the antigen in cases of immune complex formation and glomerulonephritis actually is a *B. burgdorferi* antigen?

Straubinger: In the studies mentioned above, antibody testing was done for *B. burgdorferi* antibodies. Antibody titers correlated to the renal disease. So there is evidence that the dogs presenting with renal failure were infected with *B. burgdorferi*.

Levy: The gateway to renal borreliosis is infection with the organism. It sounds very simplistic, but I think if the patient isn't infected it won't get the disease. We have virtually eliminated renal borreliosis in our practice through aggressive vaccination.

Laboratory — Findings —

Q: Are there certain laboratory findings that might cause you to test for Lyme disease in an apparently healthy dog?

Levy: You may find protein, inflammatory cells, and casts in the urine of a dog that has signs of lameness. These dogs are acting ill—they don't want to move. I did complete blood counts (CBCs), serum chemistry analyses, and immune profiles on a large number of dogs with Lyme arthritis back in the early 1990s, and I didn't find any trend toward specific abnormalities. In fact, the CBCs, immune profiles, and serum chemistries were all within

normal limits unless another disease was present, such as renal failure. Data have been published[5] on the synovial fluid of experimental dogs with Lyme arthritis demonstrating the presence of neutrophils and other changes. I think the important message here is that dogs with clinical signs of Lyme arthritis may have subclinical renal disease.

Q. Dr. Alleman, what are your predominant findings with respect to clinical signs and laboratory findings for ehrlichiosis?

Alleman: Usually, the first indication is an animal with generalized malaise or sometimes a lymphadenopathy. When we see thrombocytopenia on the minimum database, that's usually the first key that it's a tick-borne disease. These animals will often have a nonregenerative anemia and hyperglobulinemia as well.

— Test Methods —

Q. Let's move on to testing platforms. Various laboratory methods are used to test dogs for both borreliosis and ehrlichiosis (*e.g.*, IFA, Western blot, ELISA, and PCR). Which tests are being used most commonly?

Alleman: Most clinicians are still using the IFA for serologic diagnosis of canine ehrlichiosis. I'll tell you how we use it. If an animal presents with clinical signs or laboratory findings consistent with ehrlichiosis, we'll do a Snap test. If that's positive, then we'll use IFA to quantitate the antibody titer. We do that to find out what the patient's titer is initially and to recheck the titer after the patient has been treated. In some cases, the Snap test may stay positive for a long time, and practitioners question the effectiveness of the treatment. Then we have to quantitate what is happening with the antibody titer. As you know, some of these animals will have titers so high that they can take a year or so to become negative. However, because of the subjectivity of the IFA and potential for false positive results, we routinely use the Snap test for our first line of testing. Testing animals both experimentally infected and naturally infected in our laboratory, we found that false-positives with the Snap assay were extremely rare. In addition, in speaking to veterinarians around the country, more and more are now using the 3Dx assay because it's so convenient.

Q. Any comments on quantitative antibody testing?

Levy: In fact, we have been studying C6-positive dogs. First, we identified the infected dogs with the 3Dx test, then they were treated with antibiotics and vaccinated against Lyme disease. Samples from these dogs were followed for a year using a developmental C6 quantitative test. The antibody titers did decrease after therapy. The test is being perfected, but it will eventually become available to practitioners.

Q. Dr. Levy, do you think veterinarians are still using an IFA test to diagnose Lyme disease?

Levy: If they are, I wish they would not. I see no reason to use any other test in veterinary practice for a dog than the 3Dx. The IFA is subject to reader interpretation and is less specific than the C6. Both the IFA and whole-cell ELISA are affected by the vaccination status of the dog; vaccine-induced antibodies cross-react in these tests. Research findings presented at the ACVIM forum[6] showed that samples from Lyme-vaccinated dogs can confuse laboratories using the Western blot—the samples are read as positive, incorrectly indicating infection. The 3Dx test answers the question: Is this dog infected or not? The C6 ELISA is just a better test: It is specific, sensitive, and inexpensive, and you get results in eight minutes in your own office.

— Test Interpretation —

Q. Considering the interpretation of Snap 3Dx test results, a practitioner is faced with a dilemma—for example, performing surveillance testing on healthy animals will occasionally produce positive test results. The question then becomes: Based on the test result alone, do I treat or do I not treat? Is the patient infected, or, in fact, does the positive test simply represent previous exposure?

Generally, there are four patient categories to consider: With *positive* test results, there are those with clinical and laboratory signs, and

HIGHLIGHTS

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- Because of the subjectivity of the IFA and potential for false positive results, we routinely use the Snap test for our first line of testing.

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HIGHLIGHTS

- By the time animals show signs of Lyme disease, they're usually antibody titer positive. That may not hold true for ehrlichiosis.
- The diagnosis of Lyme disease is not a serologic diagnosis—it's a clinical diagnosis for which proof of infection with the C6 test is a useful component.

those that are clinically normal. Likewise, among dogs with a *negative* test result, there are those with clinical and laboratory signs consistent with infection, and those that are clinically normal. How do you interpret the Snap 3Dx test for *E. canis* antibody and the Lyme disease C6 peptide antibody?

Levy: For Lyme disease, if the dog has a negative test result, but is clinically sick, I would conclude that it is probably not from *Borrelia* infection. I'd do more testing to find out what was making the patient sick. If I suspected tick-borne disease, I'd probably start a therapeutic trial of doxycycline because it is a good broad-spectrum tick-borne disease drug. I would treat the dog, but I would not think I was treating Lyme disease in the absence of a positive test.

Alleman: I agree with Dr. Levy about Lyme disease—by the time animals show signs of the disease, they're usually antibody titer positive. That may not hold true for ehrlichiosis.

Breitschwerdt: As Dr. Philipp suggested, if practitioners suspect tick-borne infections in animals with acute presentations, then doing acute and convalescent serology would be highly recommended.

Levy: Another point on Lyme disease. The diagnosis is not a serologic diagnosis—it's a clinical diagnosis for which proof of infection with the C6 test is a useful component.

Straubinger: I agree with Dr. Levy that Lyme disease is a clinical diagnosis. Therefore, treatment should be initiated in cases that present with clinical signs of disease. But keep in mind that not all *B. burgdorferi* organisms in the host tissue are eliminated by antibiotic therapy. Duration of the infection and disease may influence the outcome of therapy in that chronic cases are more difficult to treat. However, dogs that were infected experimentally and treated with antibiotics starting at 50 days after tick exposure were more likely to harbor viable, culturable *Borrelia* organisms than dogs that were treated starting at 120 days after tick exposure, although the latter dogs were positive for *B. burgdorferi* DNA by PCR testing.

Levy: Dr. Philipp, do you consider a positive C6 in a field-exposed dog an indication that the dog is infected with *Borrelia*—that *Borrelia* organisms are present in the dog?

Philipp: The only way you can assess the C6 antibody and its possible relation to infection status is to see if antibody titer, or some other measure of antibody concentration, declines in response to treatment. Our experience with human patients is that the C6 antibody titer decreases by a factor of four or more in a statistically significant majority of treated patients whose symptoms disappear. The decline is more significant in patients with early infection than it is in patients with late (disseminated) infection. Such a detailed study has not as yet been done in dogs, but there is evidence to suggest that a similar paradigm may apply to dogs as well.

Levy: I'd like to comment on the difference between dogs and people. I do not think we see many dogs with early infections. We know it takes four to six weeks for the C6 antibody to develop and produce a positive 3Dx result. Dr. Straubinger's work supports the notion of chronic infection, even after antibiotic therapy. Our study in progress using the developmental quantitative C6 test supports the finding that titers decrease after treatment. Whether a dog's C6 antibody titer will ever reach zero or an infection will be eliminated hasn't been determined. Prevention of infection is imperative. Ideally, we should be using the C6 ELISA to verify that our prevention programs are effective. In the face of infection we will use the quantitative test to evaluate response to therapy.

Breitschwerdt: Dr. Straubinger, my impression is that your research may lead us to question whether we put people into a state of remission when we treat them, particularly those with more chronic *Borrelia* infections, or whether we actually eliminate the infection. My other concern is that although the C6 peptide is an outstanding diagnostic peptide, we haven't proved therapeutic elimination of infection, even if it

decreases over time. To prove that, we'd need to culture tissues and do PCR testing at a later date, preferably following a corticosteroid challenge.

Straubinger: We and others have shown that despite long-term therapy with antibiotics commonly used to treat patients with Lyme disease, *B. burgdorferi* organisms are able to survive for prolonged time in host tissues. For direct detection, culture and PCR are the methods that are regularly used for research and diagnostic purposes. However, after antibiotic therapy, testing of multiple samples from a single patient is often necessary to detect persisting *B. burgdorferi* organisms.

Breitschwerdt: An article in the January 2004 issue of *Arthritis & Rheumatism*[7] detailed the case of a young woman who had a very successful anterior cruciate and chondrocyte transplantation surgery, and five weeks later her knee was severely swollen and inflamed. Not only did her physicians detect serum *B. burgdorferi* antibodies and *B. burgdorferi* DNA (by PCR) in her joint effusion and chondrocyte implant, but the authors also cultured *B. burgdorferi* from the joint effusion. I mention this case because the young woman had an erythema chronicum migrans-like lesion 15 years earlier. This case report appears to illustrate how effectively *B. burgdorferi* can hide in the human body. Whether the *B. burgdorferi* contributed to her cruciate disease, or whether the injury was related to athletic activities would be anyone's guess, but *B. burgdorferi* infection certainly contributed to her severe knee inflammation a month after surgery.

Philipp: What was the C6 test result?

Breitschwerdt: They did not do it, unfortunately. But that does raise another question. What's the status of C6 testing in human medicine? When someone contacts me and suggests he or she has Lyme disease, I tell the person that if he or she were a dog, I wouldn't believe the *B. burgdorferi* infection status unless supported by a positive C6 peptide test. I base this opinion on the specificity that we observed when testing dogs from nonLyme endemic regions.

Q. Let's concentrate now on ehrlichiosis, and, in particular, a comparison of 3Dx testing vs. IFA testing for *Ehrlichia* infection.

Alleman: Because IFA testing incorporates a whole organism and multiple antigens, you will detect samples with lower titers than you would with the 3Dx assay. However, the more antigens present, the greater likelihood you will have cross-reactivity with closely related organisms, such as *Ehrlichia* and *Anaplasma* species and other rickettsiae.

We found that some laboratories are a lot more experienced in running IFAs than others. It certainly depends on operator experience in evaluating what is truly positive. So we get nebulous titers on patients that we try to address. It may not be a positive titer at all. We don't use IFA as a first-round test for *Ehrlichia* infection—we use the Snap test first. If the patient is positive with the Snap test, then we use the IFA as a quantitative test.

Q. Let's discuss interpretation of the 3Dx test results in more detail, specifically Lyme disease. The approach to patients with positive test results, but without clinical or laboratory signs of the disease, is a much-discussed issue. That is the patient that is apparently healthy, but surveillance testing produces a positive result with C6 peptide antibodies. Dr. Levy, please validate this, but I think there's a tendency to do less questioning and just treat in the Northeast. But outside the northeastern United States, there is a tendency to do less treating and just wait and see.

Levy: That may be true. Clearly I'm a strong advocate for testing and treating. I have a protocol in my practice—every dog gets Snap 3Dx tested. If the dog is positive, I prescribe doxycycline and vaccinate that dog with a Lyme disease vaccine. I've followed 160 dogs on this protocol from 2001 through 2004 and none have developed Lyme disease. I have no control group because who wants their dog to be in the control group in Durham, Conn.? My earlier studies

HIGHLIGHTS

- Despite long-term therapy with antibiotics commonly used to treat patients with Lyme disease, *B. burgdorferi* organisms are able to survive for a prolonged time in host tissues.
- We don't use IFA as a first-round test for *Ehrlichia* infection—we use the Snap test first. If the patient is positive with the Snap test, then we use the IFA as a quantitative test.

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HIGHLIGHTS

- It is difficult to say what the outcome will be years after infection with or without antibiotic treatment in a Lyme disease case.
- Positive animals that are clinically and hematologically normal should be treated because of the occurrence of subclinical infections and the relatively inexpensive cost of therapy. Also, you may be preventing the development of the chronic phase of infection.

involved infected dogs or dogs that became infected after we tested them and followed them for two years. These dogs were not treated, and, because the study predated the release of the first Lyme disease vaccine, none were vaccinated. Lyme disease developed in 5% of these untreated and unvaccinated dogs. With my new protocol, to date there is no disease if I treat and vaccinate them.

Q: Let's discuss the *B. burgdorferi*-infected dog that does not manifest obvious clinical signs, such as lameness. Is there a true subclinical form of the disease? Is there a risk of development of chronic arthritis later in life? Dr. Straubinger, would you comment on this?

Straubinger: Regarding long-term infection, we had control dogs that were infected by ticks and kept up to 580 days. We were able to reactivate Lyme disease through immunosuppression by administering oral corticosteriods for 14 days in dogs that weren't treated otherwise. However, corticosteroid treatment did not reactivate Lyme disease in dogs that were treated with antibiotics 120 days after tick exposure. It is very difficult to say what the outcome will be years after infection with or without antibiotic treatment in a Lyme disease case. I think it is very likely that persistent infection can be reactivated later on; the surgery we discussed before is a good example.

But I don't think every C6-positive dog should be treated with antibiotics. As Dr. Levy mentioned earlier, only a fraction of infected dogs will show clinical signs that relate to Lyme disease after tick exposure. Taking into account a large proportion of the canine population may have had contact with *B. burgdorferi* in certain endemic areas, this would mean that hundreds of thousands of dogs would need treatment. Antibiotic therapy can have side effects in some patients.

Levy: I disagree. I think it's important to be very aggressive with dogs that test positive. I use antibiotic therapy to decrease the number of spirochetes and vaccination to prevent new infections and possibly manage recrudescent infections if a dog is immunosuppressed or stressed, like the human surgery patient.

Alleman: With regard to ehrlichiosis, I'd like to discuss the positive animal detected through routine screening. Positive animals that are clinically healthy will often have some hematologic abnormalities. It is not a consistent finding but occurs frequently enough to assist us with some of these cases. Typically, it's a nonregenerative anemia and a mild thrombocytopenia that is often not sever enough to cause clinical bleeding. In those cases, the abnormal laboratory parameters support your decision to treat the animal. The harder question is the positive animal that is clinically and hematologically normal—what do you do with that patient? We've treated those patients after we quantitate the titer, then we recheck the titer to see if it's changed. These animals are given doxycycline for the required three-week duration and IFA titers are rechecked three months after completion of therapy. We look for a 50% reduction in titer at that time to indicate effective treatment. I think these animals should be treated because of the occurrence of subclinical infections and the relatively inexpensive cost of therapy. In addition, you may be preventing the development of the chronic phase of infection, which can result in severe disease or death. But I'm not sure what we should do with these patients. I'd like your input.

Ford: I think patients positive for *E. canis* antibody but showing no clinical signs have been exposed. We retest them later but the antibody can persist for a long time.

Alleman: Do you consider these animals to be exposed but not necessarily persistently infected?

Q. That's a good question. Dr. Breitschwerdt, how would you address that?

Breitschwerdt: I think that some dogs exposed to *Ehrlichia* species are capable of developing both a strong cell-mediated and humoral immune response, and most of these dogs do not become persistently infected. But we don't know the extent to which this occurs following natural infection. I tell veterinarians that if I were to get

a positive *E. canis* result on a healthy animal using the 3Dx test, the first thing I would do is a CBC. As Dr. Alleman mentioned, if there are any hematologic abnormalities consistent with ehrlichiosis, I'd stop spending the client's money at that point and treat the dog. Until we sort out screening healthy dog populations for *Ehrlichia* infection, getting an IFA titer when you initiate treatment is helpful. Some infected dogs will have titers of 1:20,000 or greater, whereas others will have a titer of 1:512 or less. Obviously, it will take much longer for dogs with high *E. canis* antibody titers to become antibody-negative following effective treatment. The problem that we now recognize relates to veterinarians repeating the 3Dx test on an annual basis and still detecting a positive *E. canis* titer a year after treatment. There is no protective immunity following infection with *Ehrlichia*, and therefore, the dog could have been reexposed and reinfected. Alternatively, the dog could have had a very high antibody titer that has dropped substantially in magnitude, but is still detectable one year later. So it helps to know the IFA titer, particularly in the subgroup of *Ehrlichia*-infected dogs that we refer to as nonresponders. These are dogs that do not respond appropriately following therapy with doxycycline. Some of those dogs are "nonresponders" because their *E. canis* infection has been therapeutically eliminated, but they are concurrently infected with *Babesia* or *Bartonella* species. In these dogs, persistent clinical or hematologic abnormalities can be eliminated by more directed therapy for babesiosis or bartonellosis.

Q. Dr. Alleman, what about patients with laboratory signs consistent with ehrlichiosis, such as thrombocytopenia, that test negative with the 3Dx test? Do you treat them, or do you do other diagnostics?

Alleman: If the animal presents with thrombocytopenia and nonregenerative anemia and is Snap-test negative, I'd be reluctant to treat unless the patient has had a history of tick exposure, because so many diseases can cause those lab abnormalities. But if the patient has a history of tick exposure or a tick is found on the animal, that's a different situation.

Levy: Signs of ehrlichiosis can precede antibody detection, and history of tick exposure can be so unreliable that I am inclined to treat a dog with signs and likelihood of tick exposure. If the dog is from an area with *Rhipicephalus sanguineus*, I would suspect tick exposure.

I want to comment on the issue of treating a healthy dog with a positive Snap test, whether it is for *E. canis* or *B. burgdorferi*. I have read articles stating there is no indication for using antibiotics to treat the *B. burgdorferi*-positive animal with no signs. I think the correct statement should be that there is currently no peer-reviewed data on *B. burgdorferi* infection from natural exposure to indicate if treating a dog with a positive test and no signs is beneficial. However, Dr. Straubinger's experimental data certainly support use of antibiotics in infected dogs; spirochete numbers decreased after therapy, and recrudescence of disease was prevented after immune suppression. My unpublished data suggest that infection from natural exposure responds in the same fashion. I also wonder why someone isn't collecting solid data on the value of using the 3Dx test as a screening test for *E. canis* infection, quantitating the response to therapy using the IFA, and preventing patients from getting reinfected through intense tick control. Again, I think not being infected is far better than being infected.

Alleman: I wouldn't disagree with that at all. One of the things that we have noticed in our work with the 3Dx is that the specificity of *Ehrlichia* testing is extremely high. We haven't found any false positive results. From our experience, when you get a positive, the patient has been exposed. I certainly would not fault anyone for treating an animal that was positive, regardless of whether it had hematologic abnormalities.

Treatment – Recommendations –

Q. What are your treatment recommendations with respect to Lyme disease?

HIGHLIGHTS

- It helps to know the IFA titer, particularly in *Ehrlichia*-infected dogs referred to as nonresponders.
- If a dog presents with thrombocytopenia and nonregenerative anemia and is Snap-test negative, I'd be reluctant to treat unless the patient has had a history of tick exposure, because so many diseases can cause those lab abnormalities.

Managing Lyme Disease and Ehrlichiosis

HIGHLIGHTS

- It's prudent to avoid corticosteroids if at all possible for these infectious diseases to see whether the patient responds to antibiotics alone.
- In cases in which the animal had ehrlichiosis and was treated appropriately, clients may be content waiting until the next checkup to see whether the animal has attained a negative status.

Specifically, this refers to the patient with clinical signs that are consistent with Lyme disease and a positive test for C6 peptide antibody. Which treatment protocol would you initially select for the patient?

Levy: My drug of choice is doxycycline because of the possibility of coinfection. I know that many of my Lyme-positive dogs are also positive for *Anaplasma phagocytophilum*.

Q. What dosage do you use?

Levy: I use a high dose (5 mg/lb b.i.d. for 28 days). I make sure the drug is given with food and I round my dosage down to whole tablets. I decrease the dosage to no lower than 2.5 mg/lb twice a day if the dog is not tolerating it well. If the dog just can't tolerate doxycycline, I use amoxicillin at 10 mg/lb twice a day for 28 days.

Q. Dr. Alleman, what are your initial treatment recommendations for the dog with clinical signs of ehrlichiosis and a positive test for *E. canis* antibody?

Alleman: We use a similar dosage of doxycycline for ehrlichiosis (2.5 to 5 mg/lb b.i.d. for a minimum of 21 days). It's prudent to avoid corticosteroids if at all possible for these infectious diseases to see if the patient responds to antibiotics alone. In some cases, though, the thrombocytopenia may not respond well to just antibiotics if there is an immune component. Then we try anti-inflammatory doses of corticosteroids.

Q. After a 28-day course of treatment in a positive case, when would you retest the patient, and what test results would be expected?

Levy: For Lyme disease, I'd retest when the dog is due for its next Snap test for heartworm. When there is a quantitative test available, I'll probably test at six months.

Alleman: With regard to ehrlichiosis, we usually wait three to six months for titer testing. If the animal has severe thrombocytopenia, we would not wait that long to recheck the patient. We'd monitor that patient's hemogram very closely for a response to therapy. Animals with severe thrombocytopenia (<50,000 cells/μl) should be monitored closely (daily or every other day) until platelet counts climb to acceptable levels (>150,000 cells/μl). Then sometime between three and six months later, a second IFA can be done to determine if there has been a change in titer.

Response — to Therapy —

Q. Are you using a Snap 3Dx test to monitor response to therapy?

Alleman: No. If we have a clinical case that we're treating, we'd monitor antibody titers using a quantitative assay, such as an IFA.

Levy: I second that—the Snap test is not indicated for following the short-term response to treatment, for checking whether the animal is still infected. I've seen dogs test negative in one year. I've seen dogs test negative in three years. And I've seen them stay positive for three years and remain healthy.

Alleman: In cases in which the animal had ehrlichiosis and was treated appropriately, clients may be content waiting until the next checkup to see whether the animal has attained a negative status.

Levy: As a clinician there is nothing better than a healthy patient, no matter what its tests results are.

Q. With respect to response to therapy, how soon do you expect a patient to show clinical improvement?

Levy: In my experience, 85% of dogs with Lyme arthritis respond well, within one to five days of starting therapy. About 15% of dogs had less than a complete response or had recurrent disease over the course of weeks, months, or years. If the dog does not respond well or if the signs exacerbate within five days, ask yourself whether the dog could have a condition other than Lyme arthritis.

Q. Dr. Straubinger, does that compare with your experience?

Straubinger: In our studies, we didn't treat the dogs until they showed clinical signs of arthritis. Within days of the start of treatment, the dogs responded.

In our experience, dogs with acute Lyme disease arthritis will respond in a few days.

Q. Dr. Breitschwerdt, what about cases of ehrlichiosis? What is your expectation?

Breitschwerdt: Based on our research findings and clinical experience treating referral cases of canine ehrlichiosis, we administer doxycycline (5 mg/kg b.i.d.) for 3 weeks. I do agree with what Dr. Levy said earlier about canine ehrlichiosis. It is wise to treat *E. canis* infections at the time the diagnosis is established, whether the dog is symptomatic or asymptomatic. For chronic intracellular infections, aggressive treatment using an efficacious dose for a long duration is required to eliminate, rather than suppress the infectious agent. The evidence now suggests that most dogs treated with doxycycline at an appropriate dose and duration will clear the *Ehrlichia* infection. If they do not clear their infections, they are probably immunosuppressed or coinfected.

— Coinfection —

Q. Over the last two years, several articles have expounded on people who are coinfected with multiple tick-borne agents in the Northeast. Dr. Philipp, what is your experience with coinfections in people?

Philipp: Many more articles are being published about coinfections. However, these papers pertain mostly to coinfections in ticks. Whether that translates into the human population I do not know.

Q. Recently published articles discuss individual human patients living in the northeastern United States who have antibodies to a variety of different *Ehrlichia* species, and, in some cases, to *Ehrlichia* and *B. burgdorferi*. Dr. Levy, what is your experience in the Northeast with regard to coinfections in dogs?

Levy: An article published in 1997 involved frozen samples from a Lyme study done in the mid-1980s.[8-10] From our area, 10% of Lyme-positive dogs were also found to be positive for *A. phagocytophilum*. In 2001, we found that 40% of dogs from our practice that had positive C6 ELISA results were coinfected with *A. phagocytophilum*. This shows that dogs are being bitten by ticks and the ticks are carrying multiple infections. I think this is just the beginning of our investigation of coinfection. The issue is if you treat a dog for tick-borne infection and the dog does not improve, it may have more than one infection, or you may not be using the right drug.

Q. Would you agree with the statement that the clinical signs of a dog simultaneously infected with more than one tick-borne pathogen may not be characteristic for either infection, and that this argues for testing for *E. canis* and *B. burgdorferi*?

Alleman: I am a proponent of screening for tick-borne diseases for a number of reasons. First, these diseases are notorious for causing subclinical infections—we need to detect these animals to reduce the infected population and prevent the development of the chronic forms of the disease.

Second, these diseases are increasing in number in both veterinary and human medicine, and we need to monitor prevalence in different areas of the country for both pet health reasons and zoonotic potential.

Levy: I am looking for *B. burgdorferi* now, but the day will come when I detect *E. canis* infection, just as some fellow down in Florida will detect a locally acquired *B. burgdorferi* infection. I am looking forward to the development of tests for other tick-borne organisms because surveillance is incredibly important.

Q. Dr. Alleman, have you seen multiple *Ehrlichia* species infecting a single dog in Florida?

Alleman: We mostly see *E. canis* infection, but we've detected *Ehrlichia chaffeensis* infections as well in the state of Florida. However, these were not in the same patient. We have also found *Ehrlichia platys* infection in the canine population; infected dogs are sometimes coinfected with other tick-transmitted agents, such as *Babesia canis*. We don't see coinfections very frequently, but we are not actively testing for coinfections in our canine population, so we really don't know how frequently it is occurring.

HIGHLIGHTS

- Screening for tick-borne diseases is a good idea because 1) these diseases are notorious for causing subclinical infections, and these diseases are increasing in number in both veterinary and human medicine, and 2) we need to monitor prevalence in different areas of the country for both pet health reasons and zoonotic potential.

Managing Lyme Disease and Ehrlichiosis

HIGHLIGHTS

- Adding the *Anaplasma* species to the testing panel would certainly make sense—determining how many dogs are infected with *Anaplasma phagocytophilum*.
- *Mycoplasma haemofelis* is so problematic from a clinical standpoint that a serologic assay would be very useful in clinicians' hands.

However, I know Dr. Breitschwerdt has seen coinfection in the dogs in the North Carolina area.

Q. Dr. Breitschwerdt, can you summarize your clinical experience with coinfection in dogs?

Breitschwerdt: Dogs with extensive vector exposure can develop simultaneous infections (based on detection of organism-specific DNA) with multiple tick-borne organisms. Our best example of this scenario was a Walker hound kennel located in rural North Carolina.[11] While investigating unexplained deaths in this kennel, we amplified the DNA of up to six different tick-borne organisms *(i.e.,* six different species from four different genera: *Ehrlichia*, *Anaplasma*, *Bartonella*, and *Rickettsia*) from an EDTA anticoagulated blood sample obtained at a single point in time. Part of the message to veterinarians is that the adaptation of these organisms to persist in our patients for long periods is extremely good. I also think that polymicrobial infections will help us explain some of the people who have had Lyme disease but remain ill after intensive antimicrobial treatment. Clearly, recent observations suggest that some of these individuals are infected with *Babesia microti*, which would be suppressed, but not therapeutically eliminated, by many antibiotics.

Future Diagnostic — Needs —

Q. Given the fact that we have a test for Lyme C6 peptide antibody and *E. canis* antibody, what is the next round of rapid, in-clinic diagnostics for infectious disease that we need? Should we be testing for *Ehrlichia* species? What about *Babesia*?

Breitschwerdt: Adding the *Anaplasma* species to the testing panel would certainly make sense—determining how many dogs are infected with *A. phagocytophilum*. I've also been a strong proponent of *Bartonella* species as important evolving pathogens in both human and veterinary medicine. Recent research suggests that *Bartonella* species are complicating our lives as clinicians on a daily basis. These are some of the pathogens that we should consider for annual screening in the future. If you have a sensitive and specific testing platform that screens for exposure to vector-borne organisms, you should only add organisms that can induce chronic occult infection, such as *A. phagocytophilum*, *Babesia canis*, *Babesia gibsoni*, and *Bartonella vinsonii* subspecies *berkhoffii*. Detection of serologic evidence of occult infection would be the basis for me to screen healthy dogs on an annual basis. Clearly, more research is needed to establish the correlation between detection of antibody and persistence of infection for most of these organisms.

Ford: Dr. Alleman, representing the southeastern kingdom of ticks and fleas, what other organisms should we be testing for?

Alleman: I agree with Dr. Breitschwerdt on the three groups of organisms he included as potential candidates for the Snap assay. The one that I'm most interested in is *A. phagocytophilum*—we are missing an important tick-transmitted disease with current testing. And I'd add just one other organism to that list, and that is *Haemobartonella felis*, now known as *Mycoplasma haemofelis*. That organism is so problematic from a clinical standpoint that I think a serologic assay would be very useful in clinicians' hands.

Ford: And Dr. Levy, as the guy in the trenches of clinical practice, what new tests do you want?

Levy: I echo what you have all said. My first choice is *A. phagocytophilum*. My next choice is for equine practitioners—they would love a Snap test for Lyme disease.

Philipp: I'd like to see a quantitative test developed to determine response to treatment in Lyme disease cases.

Levy: The 3Dx platform, as Dr. Alleman said, is the test for screening for *E. canis* infection; IFA is used to determine response to treatment. Similarly, the 3Dx platform is the screen for Lyme C6 antibodies and then a quantitative test sent off to a reference lab will be the real creme de la creme for following the response in these dogs. I am looking forward to the quantitative C6 test becoming available.

I have found that my protocol of test, treat, and vaccinate has been very successful in eliminating Lyme disease in my practice. The C6 test is the tool that indicates that not only am I preventing signs of Lyme disease, but I am also preventing infection.

— Conclusion —

From the preceding discussion, it is apparent that Lyme disease and ehrlichiosis represent two of the most clinically significant tick-borne diseases affecting dogs in the United States. However, the complex pathogenesis, the variation in clinical and laboratory findings, and even the regional prevalence of each disease continue to complicate identification and management of the infected patient. Introduction of the Snap 3Dx test, while not a definitive diagnostic test for either infection, serves two important roles: 1) clinical surveillance of exposure and infection in populations of healthy dogs considered at risk of exposure and, 2) an aid to the assessment of patients with clinical or laboratory findings consistent with infection.

Considerable research into diagnostic strategies and medical management of tick-borne infections in dogs continues. As new information becomes available, it is our hope that this roundtable will be reconvened to update practitioners on the latest findings.

—Richard B. Ford

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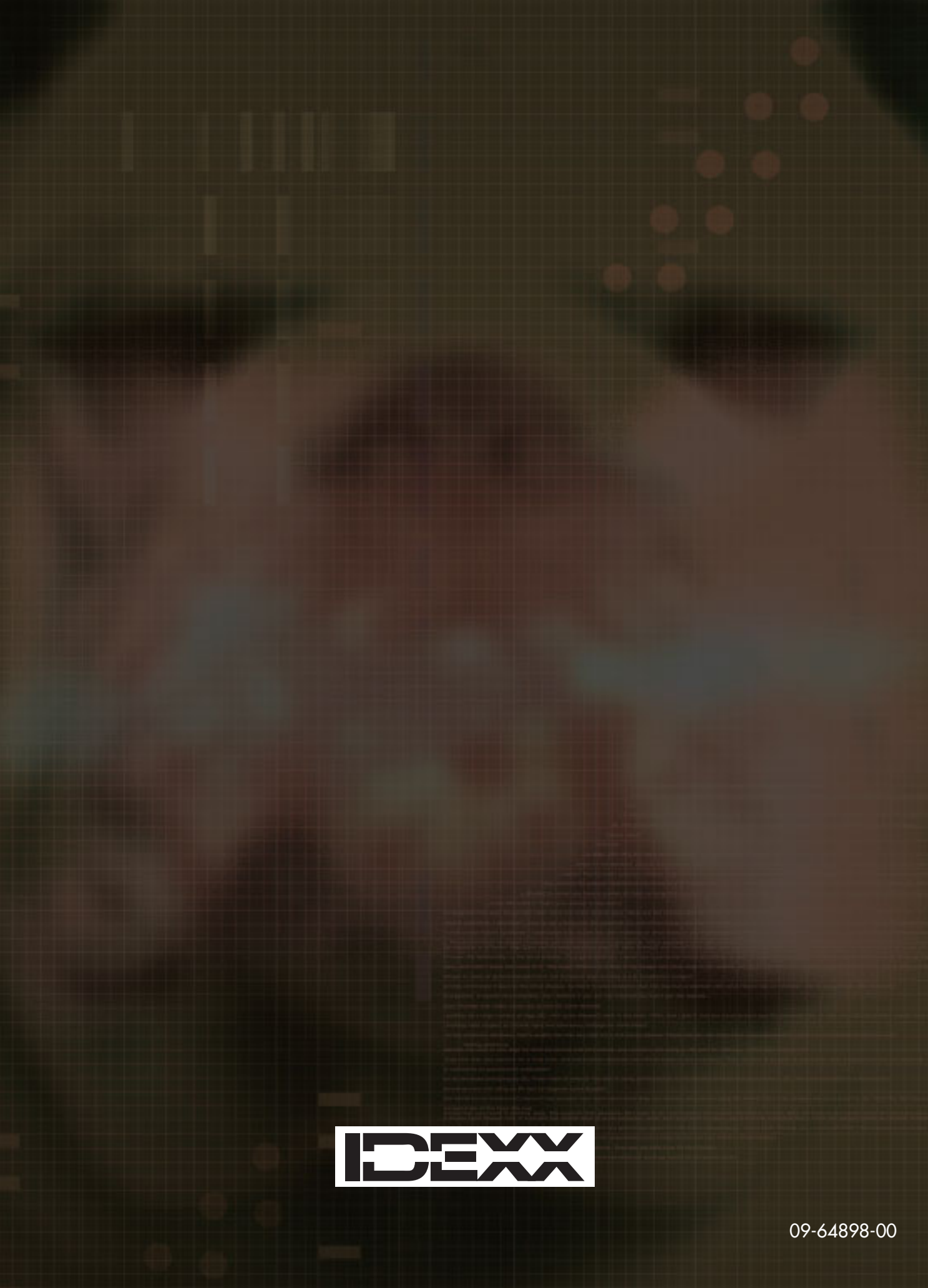

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