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Ehrlichiosis believed on the rise; experts discuss prevalence, diagnosis, treatment

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Editor's Note: *This month, DVM Newsmagazine presents three experts in companion animal medicine discussing Ehrlichia in the dog population. The exclusive roundtable discussion, sponsored by IDEXX Laboratories, convened at the North American Veterinary Conference in Orlando this year.*

Panelists include: ***Richard B. Ford, DVM, MS,*** *a diplomate of the American College of Veterinary Internal Medicine (ACVIM), and a professor of medicine at North Carolina State University College of Veterinary Medicine. Dr. Ford also moderated the discussion.* ***Johnny Hoskins, DVM, Ph.D.,*** *is a diplomate of the ACVIM, and owner of DocuTech Services in Baton Rouge, La. He is a regular contributor to DVM Newsmagazine.* ***Roberta Relford, DVM, MS, Ph.D.*** *is chief of clinical pathology for IDEXX Veterinary Services. She is a diplomate of ACVIM and a diplomate of ACVP.*

In recent years, it has become increasingly apparent that ticks living in different regions of the world are capable of transmitting an ever-growing number of human and animal pathogens.

Molecular evidence suggests that simultaneous infection with multiple tick-borne pathogens may not be the unusual occurrence that it was once thought to be. Ehrlichiosis is a tick-borne disease caused by gram-negative pleomorphic, obligate intracellular bacteria of the family *Rickettsiaceae.* The infection, documented in both animals and humans, is recognized worldwide. It is among the most common tick-borne diseases recognized in dogs.

Two types of *Ehrlichia* infection are described: monocytic *Ehrlichia* (a disease infecting lymphocytes and monocytes) and granulocytic *Ehrlichia* (infecting neutrophils and eosinophils). However, the clinical manifestations of ehrlichiosis are highly variable and are now known to be caused by a variety of rickettsial species. At least 10 different ehrlichial species have been documented to infect dogs either naturally or under experimental conditions.

Geographic distribution, prevalence

Ford: **Considering the prevalence of ehrlichiosis within the U.S., do you believe that this truly is an emerging disease among dogs?**

Hoskins: Yes. It's emerging because of the wildlife population migrating across the country and becoming more evident. We know the deer tick associated with Lyme disease has migrated, too. Plus, people are often moving from one part of the country to the other and they're taking their dogs with them and probably some ticks, too. So, I think because of our mobile society, *Ehrlichia* is on the rise and becoming more evident in just about every state.

Relford: I agree and I think it is emerging in new areas. We have typically seen it in areas that have a high infestation of ticks, such as in Texas. In these areas it's been common for a long time. But now you are starting to see it in places where it has not routinely been seen in the past.

Hoskins: I think that the average incidence of canine ehrlichiosis is around 10 to 15 percent and only a small percent of those 10 to 15 percent are showing clinical signs of disease. There's more ehrlichiosis out there than most practitioners know, so there really is an important take-home message to practitioners that if you have any tick history you've got to consider tick-transmitted agents as part of your differential considerations.

Pathogenesis of infection

Ford: **The pathogenesis of canine ehrlichiosis is characteristically described in three phases: the acute phase, which may be up to four weeks followed by a brief subclinical phase, during which time the affected dog may appear normal. This is followed by a chronic phase lasting several weeks to months. What are the most characteristic clinical signs of dogs with acute disease vs. chronic disease?**

Hoskins: The classic, acute

presentation is what the veterinarian will not see. They honestly don't see it, or about the time that the animal is sick and the owner decides to take it to the veterinarian, then the animal has progressed into the subclinical phase. Thus, the animal showing oculonasal discharge, lymphadenopathy, not eating, vague GI upsets, fever and tick infestation many times are never seen by the practitioner. But really, what the practitioners are seeing in their veterinary offices more often is the chronic phase with oscillating clinical signs. The owner is saying to the practitioner that the animal is just not feeling right, which means that the animal is losing weight, variable in its appetite, and may or may not have a fever. It doesn't look like a healthy animal and doesn't act like a healthy animal.

Clinical disease

Ford: **What are the predominant physical and laboratory abnormalities among dogs infected with *Ehrlichia canis (E. canis)*?**

Relford: I think the most common clinical signs seen that lead to *E. canis* testing are lameness or stiffness. Next, I think finding thrombocytopenia on the CBC is the most common laboratory abnormality that prompts testing for *E. canis*. Those are the two most commonly encountered clinical problems. Oftentimes, however, the clinical signs are vague, but can include a shifting leg lameness. Here again, I think the primary laboratory changes are going to be seen in the CBC. Thrombocytopenia is typically the earliest change and attributed to an immune mediated process. As the disease progresses, the *E. canis* affects the bone marrow, which eventually leads to alterations of other cell lines. Once this happens, any cell line can show a change. If all cell lines are affected, the animal can become pancytopenic, hence the name Tropical Canine Pancytopenia. You can also see mild to moderate elevations in ALT and/or the BUN. This is variable, but I have time and time again seen a totally normal chemical profile with very low platelets. It may be an anemia, it may be a white cell reduction if we're talking about bone marrow involvement. So what often clues me in first is seeing CBC abnormalities more so than biochemical changes. Although there may be biochemical alterations, there's nothing specific that points me to *Ehrlichia* on a biochemical panel. That has been my experience.

Ford: **In your opinion, is there an association between immune-mediated thrombocytopenia and/or hemolytic anemia and ehrlichiosis?**

Relford: I think so, because as I alluded to earlier, I think that the animal does have an overall stimulated immune system, and I think that a lot of times immune-mediated hemolytic anemia is a component of ehrlichiosis.

Ford: Considering the importance of performing platelet counts, as opposed to platelet estimates), there are some important laboratory considerations to take into account when collecting blood and performing the counts.

Relford: In my mind, there is no question that being able to do a platelet count within 20 minutes of collection from the animal is best. The platelet's job is to clump and, ideally, platelets do much better the quicker you can get them analyzed. And the other thing to remember is the coagulation process starts during collection of the sample from the animal. If it takes two or three sticks to get the sample, you have got enough tissue thromboplastin in that needle to clump the platelets. So if you're concerned with platelet counts, it really has to be a clean stick. Also, I always recommend that people make a good glass slide and send that in with the sample, because if there's any question regarding clumping and the effect on the platelet count, we can go back and look at that glass slide and perform an estimate.

Ford: In the assessment of serial platelet counts on individual patients, a count of, for example, 60,000 cells/cumm one day followed by a count of 70,000 cells/cumm the next day is *not* necessarily a positive prognostic sign.

Relford: That's correct. Documenting an increase of 10,000 platelets/cumm over 24 hours essentially represents no difference. You want to see at least a 25-30 percent change, which would represent a substantial change. If a patient goes from 60,000 to 80,000-90,000 that can be considered a significant increase.

Diagnostic confirmation

Ford: **We've come to the conclusion that hematologic changes are the hallmark laboratory findings that signal a possible diagnosis of ehrlichiosis. To confirm a diagnosis of *E. canis* infection, what tests are available?**

Relford: The tests most readily available are IFA and PCR. The IFA is reported with serum titers and is the most commonly used test.

Ford: **What about Western blot?**

Hoskins: The Western blot is primarily used as a research tool, and I really question the PCR technology that is available right now. And, the PCR results are only as good as the technology and the laboratory that is doing it. Unfortunately, the most reliable test a practitioner can use is the IFA test.

Relford: It's the most accessible. However, interpretation of IFA testing is very laboratory and technician dependent. By that I mean there are several variables that affect IFA testing. IFA testing measures the patient's antibody response by applying the patient's serum to an antigen-coated slide and adding an anti-antibody conjugate. Both the slide and the conjugate are made by several different manufacturers, which affects the test dramatically. Also different labs use different dilutional schemes for their titers. And finally interpretation of the test is technician dependent. So there are many things that can affect the quality of the test and contribute to the test results varying from lab to lab. As for PCR testing which detects the presence of the organisms, I believe as technology progresses, the PCR test will become a very important diagnostic tool.

Ford: **It's been published that an IFA titer of 1:80 or higher is diagnostic of ehrlichiosis. Is that an appropriate statement?**

Hoskins: No. If it's an IFA test that is done well, any IFA titer is a positive test for *Ehrlichia* infection. And, the practitioners don't have to use acute and convalescent titers like in canine leptospirosis or Rocky Mountain spotted fever to make a definitive diagnosis of *Ehrlichia* infection. A 1:40 can be just as positive of

an infection as one that has 1:8000. Titers don't equate into degree of infection.

Relford: I agree. I think there are two things that are important. One is that a 1:80 in one lab may not be a 1:80 in another lab due to all the variables I mentioned earlier. Also, he's very right that the titer value does not equate to clinical signs or degree. A study presented by Russell Greene a couple of years ago showed that antibody titers could stay high for a very long period of time after exposure and treatment. You don't know if these animals are getting re-exposed or are maintaining a high titer from just a single exposure. So just because a dog has a high antibody titer doesn't equate to clinical disease or exposure in the last couple of months. This is why I believe that a good quality PCR test that detects antigen will become a valuable diagnostic tool for diagnosing ehrlichiosis.

Ford: **An ELISA-based, in-hospital test for IgG antibody to *E. canis* was recently introduced to this market. What's your interpretation of a positive *E. canis* test by ELISA in the patient that has clinical signs consistent with ehrlichiosis versus in a patient without clinical signs?**

Relford: I think that if you have an animal that has clinical signs and a positive ELISA test, then you can interpret the test as that animal has the disease and treatment is warranted. It is basically giving you the same information as an IFA. The same is true for the patient without clinical signs and a positive ELISA. It would be the same as getting an IFA positive in that patient, regardless of the titer. The animal may have subclinical ehrlichiosis or may have another clinically similar disease with a lingering *E. canis* titer from a previous exposure.

Ford: **And the clinician is justified in prescribing treatment?**

Relford: Right. Treatment can be an option in both scenarios. If you have an animal with no clinical signs and a positive test, yes, you can empirically treat, however it is wise to continue with diagnostics. Monitoring the response of the animal to treatment can be difficult since antibody levels do not always drop with treatment.

Ford: **What if the patient is negative for clinical signs but has a positive ELISA test result?**

Hoskins: Well, the thing is, it's not going to be an uncommon observation where the animal is asymptomatic (has no clinical signs of being ill) and you derive a positive test result, either sent out to the outside lab or via a hospital test kit. We've realized with some of the existing PCR technology that once an animal gets infected with *Ehrlichia* and it goes into the cell, it can stay in those infected cells for who knows how long; hence, we see the chronic phase of infection. And what holds the *Ehrlichia* in the latent or quiet state, well it's probably the immune system. The animal's immune surveillance is possibly the reason why the animal will be positive on the serum antibody test but have no clinical signs and do fine. But all the sudden, something happens to that immune surveillance and then the *Ehrlichia* will come back up and express itself. So, it's very possible that animals can be completely asymptomatic for years and consistently be positive on an IFA test sent out to an outside laboratory or even on an in-house ELISA test. The positive test result really denotes exposure. If we have a positive titer what it says is that we have an organism within the body and need to monitor the animal. The animal is always the indicator system and not a positive test result.

Ford: **What about the polymerase chain reaction (also called PCR) tests to diagnose *E. canis* infection?**

Relford: Because animals exposed to *E. canis* can have long, lingering antibody titers even after treatment, there is a need to look for the organism with PCR. Therefore testing for antibodies will not always give the answer and PCR is helpful. However, the ELISA test is a good place to start.

Ford: **In the presence of a negative ELISA test result in a dog that has clinical signs compatible with ehrlichiosis, what action is indicated?**

Hoskins: A negative test truly doesn't rule out *E. canis* infection, and I say that in light of what test that you're doing and which reference laboratory you're using. A negative test could be true—the animal is absolutely negative and have no organisms in the body. Or, a negative test could be that the animal has organisms in its body, but, in essence, there's not enough there to create a positive test result. A positive serum test result may not necessarily be positive for *E. canis* infection but one of the other *Ehrlichia* species that could cause the same clinical signs that the animal is showing. To me, a negative test result does not say that we have completely ruled out *E. canis* infection. The case history is extremely important, because the history of tick exposure becomes such a key item when you think about tick transmitted disease. Did they actually pull a tick off the animal even though we come up with a negative test? We are still obligated to consider a tick-transmitted disease on the differential list.

Ford: A dog that has clinical signs consistent with ehrlichiosis, but has a negative ELISA test result for E. canis antibody, should undergo additional diagnostic testing aggressively. I would want to retest this patient in two weeks to determine if the antibody concentration (titer) will rise sufficiently to result in a positive test. Would you agree?

Hoskins: I agree. I've got faith in the medications we use today to be able to rescue the animal from an exasperation or acute infection and showing signs of disease. But, I don't have much faith that the medications that we use are clearing the animal completely of *Ehrlichia* organisms. I think the immune system is what really clears the body of organisms and not medications.

Relford: I also agree that with a negative ELISA and concurrent clinical signs, IFA would be the next step to determine if low antibody titer is present. If the IFA is negative then retesting in two weeks to check for a rising titer would be needed. If the IFA remains negative, then another diagnosis or PCR should be considered.

Hoskins: A single test result doesn't say you do not have a problem because what's very interesting about *Ehrlichia* is that you can have a negative titer, put them on appropriate medication, and you can see a rise in serum titer while they're on the medication. So, I guess my point is, if it

looks and smells like ehrlichiosis and you get a negative test, it says Doctor, let's come along in three or six months down the road and let's retest. In other words, if a practitioner is suspicious of *Ehrlichia* infection in the animal, it just says to retest several times before saying this animal is free or is not free of *E. canis* infection.

Relford: I think you hit on a very good point in that you have to consider the history, the clinical signs, any hematological parameters and the serum antibody levels when diagnosing and monitoring a patient for ehrlichiosis. A single test should not be isolated and interpreted. You have to take the whole picture, like Dr. Hoskins is saying.

Ford: **Would you agree that finding *E. canis* morulae in cells from the peripheral blood or bone marrow is diagnostic of ehrlichiosis?**

Hoskins: Oh, no question by definition, morulae are nothing more than a cluster of organisms contained within vacuoles of the cytoplasm of a cell. Anytime you see morulae on whatever sample you are looking at, be it bone marrow, peripheral blood, buffy coat smears, it really makes no difference, seeing the morulae is always a definitive diagnosis.

Ford: **How often do you see morulae in peripheral blood smears or in buffy coat smears?**

Relford: It's not common. We do see it, but it's not common. It is not a reliable way to always diagnose *E. canis*. You're going to miss a lot of cases.

Treatment

Ford: **What's your recommendation for treatment in a dog with acute ehrlichiosis? And what response will the owner most likely observe first?**

Hoskins: I expect that once I start the doxycycline at adequate dose and administration (5 mg/kg PO BID for three to four weeks), we should see a clinical response from the animal within 48 to 72 hours. Now, complete clinical recovery may take several weeks.

Ford: To me, the most striking change is behavior, their attitude improves.

Ford: **How quickly do you expect platelet counts to rise?**

Relford: Usually within 48 to 72 hours. There should be significant increase in platelets. Again, at least a 30 percent increase should be interpreted as significant and not just a 10 percent increase.

Ford: **Empiric administration of both a corticosteroid and doxycycline has become commonplace in patients with clinical signs compatible with either ehrlichiosis or immune-mediated hemolytic anemia/thrombocytopenia. Is this appropriate?**

Hoskins: It all depends on the animal's clinical presentation. Animals with weight-bearing lameness or severe bleeding problems probably do benefit from steroid administration, at least until the serum test results for *Ehrlichia* are known.

Ford: **What about supportive treatments such as granulocyte colony stimulating factors, or human recombinant erythropoietin. Are these products of any value in managing these patients?**

Hoskins: When we're talking about pancytopenia from ehrlichiosis, I really don't see any benefit in using these recombinant products. The recombinant granulocyte colony stimulating factors products are very short-lived in the host, generally last only for three or four days. If you're trying to get the animal out of an emergency situation, then they could be used. They're so costly that the average person out there honestly can't afford to use them. They are too expensive. So, even in light of pancytopenia, I'd go ahead and do supportive care and administer doxycycline. Doxycycline can be given as an injectable as well as oral. So in those animals that are so sick that they can't take the oral form of the medication, then we'll do the parenteral routes.

Long-term prognosis

Ford: **If treatment is continued for the standard two weeks, and the patient improves, has the infection actually been eliminated?**

Hoskins: Well, I guess I would rather say that because affected animals are often living in such contaminated environments, I have a tendency to say once infected always infected. Infected being that they may have not gotten rid of the organisms completely through appropriate medical therapy, or they're in such a strong environment of tick problems that exposure occurs over and over. Once the animal recovered from ehrlichiosis, and they get re-exposed to organisms, it doesn't take many organisms to have recurrence of disease. There's no question, it's probably the exceptional animal that has cleared completely of *E. canis* organisms, because once they're infected you can get them doing clinically better at home as far as the owner is concerned. But, if we do PCR technology, you would be amazed with the test results. There's no question that tick prevention is a must because we have no vaccine. And, just because we're using a very effective tick infestation control product, does not necessarily mean we are out of the woods as far as *E. canis* is concerned.

Relford: I agree, I think we are going to find that there's always going to be low numbers of the organism present even if the patient is clinically improved. □