

***Ehrlichia canis* infections on the island of Curaçao**

An overview of the clinical picture and current
diagnostics & therapies



A research report by
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Abstract

During a ten week period the clinical picture caused by an *Ehrlichia canis* infection in fifty dogs was investigated and the clinical signs and current ideas about diagnostics and therapies were described on the island of Curaçao.

All the dogs were examined, a blood sample was taken, as many ticks as possible were removed and the dog owners filled in a questionnaire. The clinical signs had a wide variety and were often non-specific. Most common clinical signs were anorexia and lethargy, followed by a little more specific signs like pale mucous membranes, lymphadenopathy, fever and tick infestation.

Thirty-two of the fifty dogs were tested for hematological abnormalities of which 84% had thrombocytopenia, 69% had anemia and 3% had leucopenia.

The Snap[®] 3Dx[®] test was performed on 42 dogs, with an positive test result for 30 dogs and a negative test result for 12 dogs. Not all dogs diagnosed with ehrlichiosis showed antibody titers against *E. canis*.

Treatment for the infected dogs was started with doxycycline hyclate (5-10- mg/kg/day) for a period of one to three weeks. Additional treatment with Imidocarb dipropionate , 6.6 mg/kg (Imizol[®]) in combination with atropine-sulfate 0,03-0,1 mg/kg (Atropine[®]) against possible side effects, repeated after two weeks for a total of two treatments was given to two dogs and prednisolone-acetate 0,5-4 mg/kg/day (Prednisolon[®]) was given to one dog.

A clinical and hematological check-up was performed on ten out of the fifty dogs (22%), whereas all owners were requested to come back after treatment had finished.

Three out of the ten dogs needed prolonged treatment, because they were still suffering from anemia and/or severe thrombocytopenia.

Introduction

Canine monocytic ehrlichiosis is a life threatening infectious disease transmitted by ticks and is seen worldwide in dogs. The pathogen which causes ehrlichiosis is *Ehrlichia canis*, a small, obligatory intracellular gram negative bacteria, belonging to the genus Rickettsia.²²

The bacteria parasitizes monocytes in the bloodstream forming morulae and causing illness.

Incubation period for ehrlichiosis is 8 to 20 days, followed by an acute, subclinical and sometimes chronic phase.¹⁴

During the acute phase the parasite enters the bloodstream and invades the spleen, liver and lymph nodes for replication. After replication, the infected cells distribute to other organs in the body where they induce vasculitis by interacting with endothelial cells. Symptoms during the acute phase can be depression, fever, anorexia, lymphadenomegaly, splenomegaly and petechiae on the skin and mucous membranes and sometimes even epistaxis or mucous/purulent nose discharge. The acute phase lasts approximately 2 to 4 weeks and sometimes shows no symptoms at all.

After the acute phase, the subclinical phase always follows, which can last up to several years. It is during this phase of the disease that dogs can clear the bacteria from their blood. Dogs who not manage to do this will develop the chronic phase.

The chronic phase, which might be mild or severe, is characterized by depression, anorexia, weight loss, pale mucous membranes, fever, petechiae, peripheral edema, dyspnoea, nervous signs, splenomegaly and eye problems.

Dogs with canine monocytic ehrlichiosis may die due to hemorrhage and/or secondary infection.^{14,22}

Diagnosis of ehrlichiosis can be done on clinical signs, serological testing with the detection of antibodies against *E. canis*, PCR (Polymerase Chain Reaction) or demonstration of morulae in the monocytes.^{14,22,30}

Treatment for canine monocytic ehrlichiosis is most often doxycycline (10mg/kg/day) for a minimum of three to four weeks.^{14,22}

The bacteria *E. canis* is transmitted by ticks, it's vector being *Rhipicephalus sanguineus*, also called the brown dog tick.⁷

R. sanguineus is part of the Ixodidae family, genus Rhipicephalus.

The tick is the most widespread tick throughout the tropics and sub-tropics, being endemic in tropical and temperate climates where it is warm and moist. In these areas the tick is active all year round. *R. sanguineus* is endophilic and can thus be found inside houses and dog kennels.¹⁰

The primary host of *R. sanguineus* is the domestic dog, they are hosts for all the stages of development. The adult ticks are found primarily on the ears, neck and shoulders, nymphs are found on the ears and shoulders and larvae are found on the stomach and flanks.

Besides dogs as hosts, the *R. sanguineus* tick is sometimes also found on other animals, including humans.^{7,10}

The life cycle of *R. sanguineus* contains three developmental stages: eggs, larvae, nymphs and adults. The tick feeds on a host once, then falls off and develops into the next stage. This leads to the possibility of three hosts in one cycle. This plays an important role in transmitting the disease from host to host.⁷

Figure one reflects the cycle and the mean times for each stage. In optimal conditions a cycle is completed in 63-91 days.^{7,10}

The tick can transmit a disease through transstadial and transovarial passage. *E. canis* is transmitted transstadial (through successive life stages).⁷

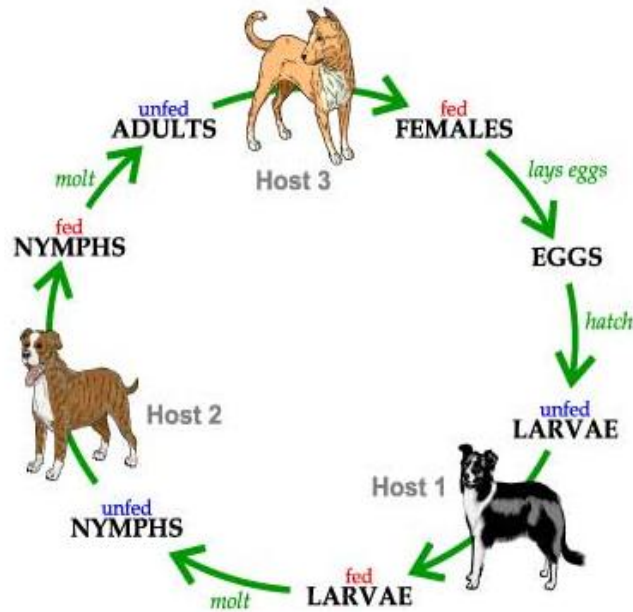


Figure 1. Life cycle of *Rhipicephalus sanguineus*

Source: <http://entnemdept.ufl.edu/creatures/urban/medical/browndog03.htm>

When the female tick has engorged and it is time to lay her eggs, she usually finds a place nearby the resting place of the host, e.g. in cracks in the wall or other nearby structures or under the bedding of the dog. This way, the larvae that come out of the eggs find their way to the host to feed easily. Moulting of the larvae and nymphs after feeding usually takes place in the same area as the female tick laid her eggs.^{7,10}

Apart from transmitting *E. canis*, *R. sanguineus* can also transmit other diseases, including *Babesia canis* (canine babesiosis), *Dipetalonema dracunculoides* (canine filariasis) and *Leishmania infantum* (canine leishmaniosis).⁷

Aim of the study

Curaçao (Dutch Antilles) is an island in the Caribbean with a tropical climate. According to the veterinarians working on the island, ticks and tick-borne diseases are a large problem amongst dogs. One of the main problems is canine monocytic ehrlichiosis, also called karpattenziekte.

Thus far, no scientific research has been done concerning ticks and tick-borne diseases on the island of Curaçao.

More recently, a similar research has been conducted on the island of Aruba. The prevalence of *E. canis* in stray and unwanted dogs was investigated. 58 % of the dogs tested positive for *E. canis* on the Snap® 3Dx® test and 14 % of the dogs had a positive PCR result for *E. canis*. Results like that emphasize once again that this disease is a big problem on the Dutch Antilles.²⁸

The aim of this study is to get a general view of the clinical picture caused by an *E. canis* infection on the island of Curaçao, to describe the clinical signs and the course of the disease and learn more about the current ideas about diagnostics and therapies as practiced by the local vets on the island. Hopefully, with this information and with further research on the subject, especially on the island of Curaçao, steps can be made towards eradication of ticks and tick-borne diseases.

As Curaçao is a part of the Dutch Antilles, controlling the situation on the island is also of great importance to the Netherlands. There is a lot of traffic from Curaçao to the Netherlands, including animals. This could potentially lead to the introduction of *R. sanguineus* ticks and possibly also *E. canis* in the Netherlands. Recent research has shown that the vector of *E. canis*, the *R. sanguineus* tick, is found more and more often in the Netherlands and the rest of Europe.^{2,3}

A retrospective study was performed by Zandvliet et al, which shows that from the 1st January 1992 and the 1st January 2001, 36 cases of canine ehrlichiosis or co-infection of canine ehrlichiosis/babesiosis were reported in the Netherlands. All the dogs with ehrlichiosis or co-infection with ehrlichiosis/babesiosis had a history of travelling to the Mediterranean Sea or the Dutch Antilles.

Materials & Methods

For a period of ten weeks, dogs presented at several veterinary clinics throughout Curaçao, were examined and tested for canine ehrlichiosis. At the end of the ten weeks, a total of 60 dogs were suspected of suffering from ehrlichiosis.

From these 60 dogs the description (breed, sex, age), foreign anamnesis, iatrotrope problem and clinical findings from the physical examination were noted and analyzed.

After this, as many ticks as possible, if present, were removed. The ticks were put in labeled sealable cups with 70% ethanol for later identification and PCR. The ticks were identified by development stage and species. Adult ticks were sexed.

A blood sample of 4ml, if necessary according to the local vet, was taken from the vena cephalica and stored in an EDTA vacutainer.

The blood was then analyzed by the IDEXX QBC Vet Autoread for hematological abnormalities and tested for *E. canis* by the Snap[®] 3 Dx[®] test following the instructions of the manufacturer.

The remaining blood was stored in EDTA vacutainers and kept refrigerated at 7 °C. Later it was fixated on filter-paper with methanol and stored at room temperature, all according to protocol. (see appendice 2).

In the near future these fixated blood spots will be examined by PCR for possible pathogens.

With the results from the IDEXX QBC Vet Autoread, the Snap[®] 3 Dx[®] test and the clinical signs, a dog was diagnosed with ehrlichiosis.

After the diagnosis, treatment was started immediately with doxycycline hyclate (5-10- mg/kg/day) for a period ranging between one to three weeks. In some cases additional treatment was given to the dog. This could either be Imidocard dipropionate 6,6 mg/kg (Imizol[®]), Intervet/Schering-Plough Animal Health, two injections given with a 14-day interval or prednisolone-acetate 0,5-4 mg/kg/day (Predisolone[®]), Alfasan.

Owners were requested to return to the clinic after three weeks of treatment for a check-up. A blood sample of 4 ml was taken from the vena cephalica and a physical examination was done. The blood was analyzed again by the IDEXX QBC Vet Autoread. The same procedures for storage and fixation of the blood were followed as mentioned above.

With the results from the IDEXX QBC Vet Autoread and the findings from the physical examination the decision was made whether to continue treatment or not.

If treatment was continued, owners were requested to return to the clinic again after three weeks and the same check-up procedure as mentioned above was done.

Results

A total of sixty dogs were presented at the veterinary clinic over a period of ten weeks with signs suspecting them of having an *Ehrlichia* infection. Of these sixty dogs presented at the clinic, fifty were eventually diagnosed with ehrlichiosis.

The owners of the dogs were not always familiar with the disease. Thirty-eight owners were familiar with the disease, seven owners were not familiar with the disease and it's unknown if the other five owners were familiar with it.

Of the dogs diagnosed by the vets with ehrlichiosis, 54% was female and 46% was male with a mean age of three years and four months (range 3 months-14 years). Breed and cross-breed dogs were represented equally.

Thirty-seven of the dogs had short hair, six had medium-long hair and seven had long hair.

22% of the dogs diagnosed with ehrlichiosis, had experienced an infection with *E. canis* before. Only four dogs had been off the island of Curaçao during their lifetime, of which three had been to Europe (Netherlands and Belgium).

The iatrotrope problems and the abnormalities found in the physical examination can be seen in table one and two.

Table 1. Overview of the iatrotrope problems of dogs (60) admitted to the veterinary clinic (Findings which only appeared once are not mentioned)

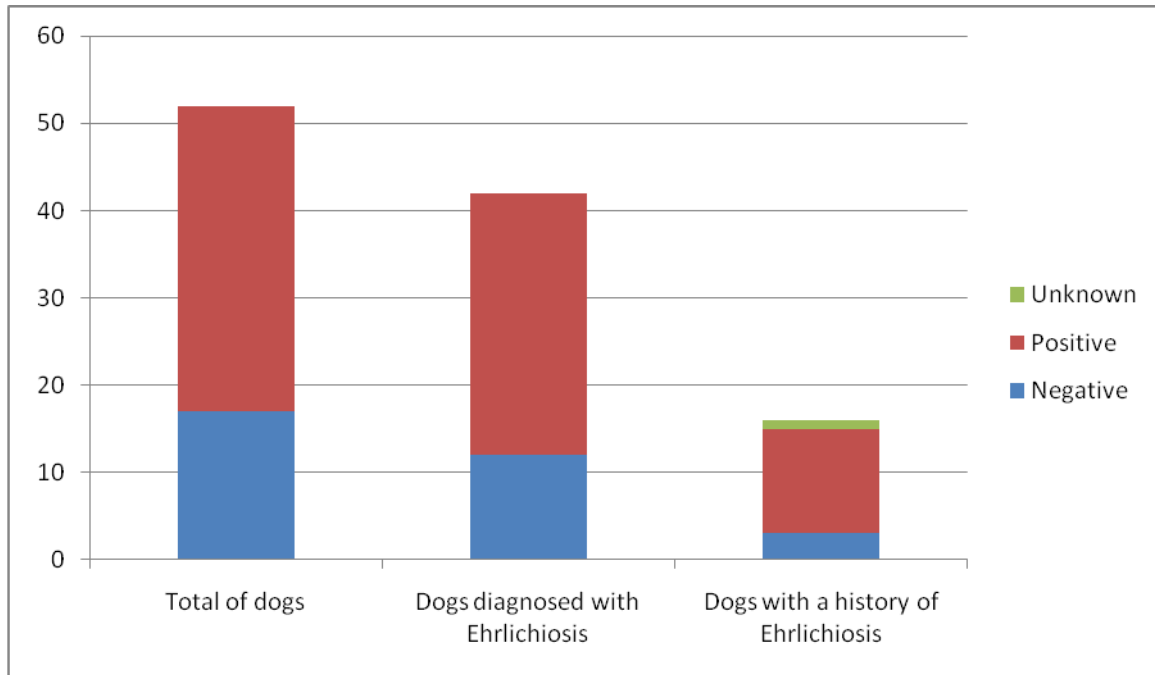
Symptoms	<i>Ehrlichia</i> infection	No <i>Ehrlichia</i> infection
Anorexia	31	7
Lethargy	31	6
Tick infestation	14	3
Unstable	7	2
Weight loss	6	3
Dirty eyes	5	-
Bleeding/Epistaxis	4	1
Fever ($\geq 39,0$ °C)	4	1
Dyspnoea	3	-

Table 2. Overview of the findings after physical examination of dogs (60) admitted to the veterinary clinic (Findings which only appeared once are not mentioned)

Symptoms	<i>Ehrlichia</i> infection	No <i>Ehrlichia</i> infection
Pale mucous membranes	17	2
Lymphadenopathy	15	3
Fever ($\geq 39,0$ °C)	15	2
Splenomegaly	11	3
Petechiae/Echymoses	6	1
Bleeding/Epistaxis	6	-
Tick infestation	5	-
Conjunctivitis	4	-

Blood was taken from forty-two dogs and analyzed by the Vet IDEXX QBC autoread. After analysis of the blood, only thirty-two dogs were diagnosed with ehrlichiosis, based on hematological abnormalities and clinical signs. Out of these thirty-two dogs treated for ehrlichiosis, twenty-two dogs had anemia (69%), twenty-seven dogs had thrombocytopenia (84%) and one dog had leucopenia (3%). Dogs were considered to have anemia if the hematocrit is lower than 37,0 %, thrombocytopenia if the platelet count is lower than $175 \times 10^3 \mu\text{L}$ and leucopenia if the WBC (white blood cell count) is lower than $6,0 \times 10^3 \mu\text{L}$.

Graph 1. Test results for the Snap® 3Dx® test *E. canis*



The Snap® 3Dx® (ELISA) detects IgG antibodies against *E. canis*. In this study, a Snap® 3Dx® test was performed on fifty-two out of the sixty dogs, with a positive test result for *E. canis* for thirty-five dogs and a negative test result for seventeen dogs. Three dogs were positive for heartworm (*Dirofilaria immitis*) and one was positive for anaplasmosis (*Anaplasma phagocytophilum*). From the dogs that were eventually diagnosed with ehrlichiosis, thirty out of the forty-two tested, had a positive result, the rest had a negative result.

Treatment was started for all the dogs diagnosed with ehrlichiosis, except one, who died shortly after being admitted to the clinic.

The remaining forty-nine dogs were treated with doxycycline hyclate (5-10 mg/kg/day) for a period ranging between one to three weeks.

No other type of antibiotics was used to treat ehrlichiosis. In some cases additional treatment was given to the dog. In two cases an Imidocarb dipropionate, 6.6 mg/kg (Imizol®) injection was given in combination with atropine-sulfate 0,03-0,1 mg/kg (Atropine®) against possible side effects, repeated after two weeks for a total of two treatments.

In one case prednisolone-acetate 0,5-4 mg/kg/day (Prednisolon®) was given in addition to the doxycycline.

Ten of the fifty owners (20%) came back with their dog for a check-up after three weeks of treatment. For all ten dogs, the clinical signs had resolved after three weeks. Even though, treatment with doxycycline was prolonged for three out of the ten dogs, because they were still suffering from anemia and/or severe thrombocytopenia.

For those dogs who had prolonged treatment, the owners were requested to come back for check-up again after three weeks. Two owners returned with their dog, one needed another additional three weeks of treatment because the dog was still suffering from anemia and thrombocytopenia.

A total of five dogs died during the course of the 10-week study. One died several hours after being admitted to the clinic, the other four dogs died during the three weeks of treatment.

The ticks collected from the dogs were all identified as *R. sanguineus*. The prevalence of ticks on the dogs was 89%. From fifty-six dogs a total of 661 ticks were collected, with a mean of twelve ticks per dog and the highest amount of ticks found on a dog being 108. The ratio of adult male/female ticks is 1.16:1. Looking at the dogs diagnosed with ehrlichiosis, a total of 573 ticks from fifty dogs were collected. Mean amount of ticks per dog is eleven. On ten of the fifty-six dogs no ticks were found.

To get a better idea about ways to improve the current situation on the island of Curaçao, an overview of the general work-up of dogs admitted to the veterinary clinic, who are suspected of suffering from an infection with *E.canis*, is presented in figure 2.

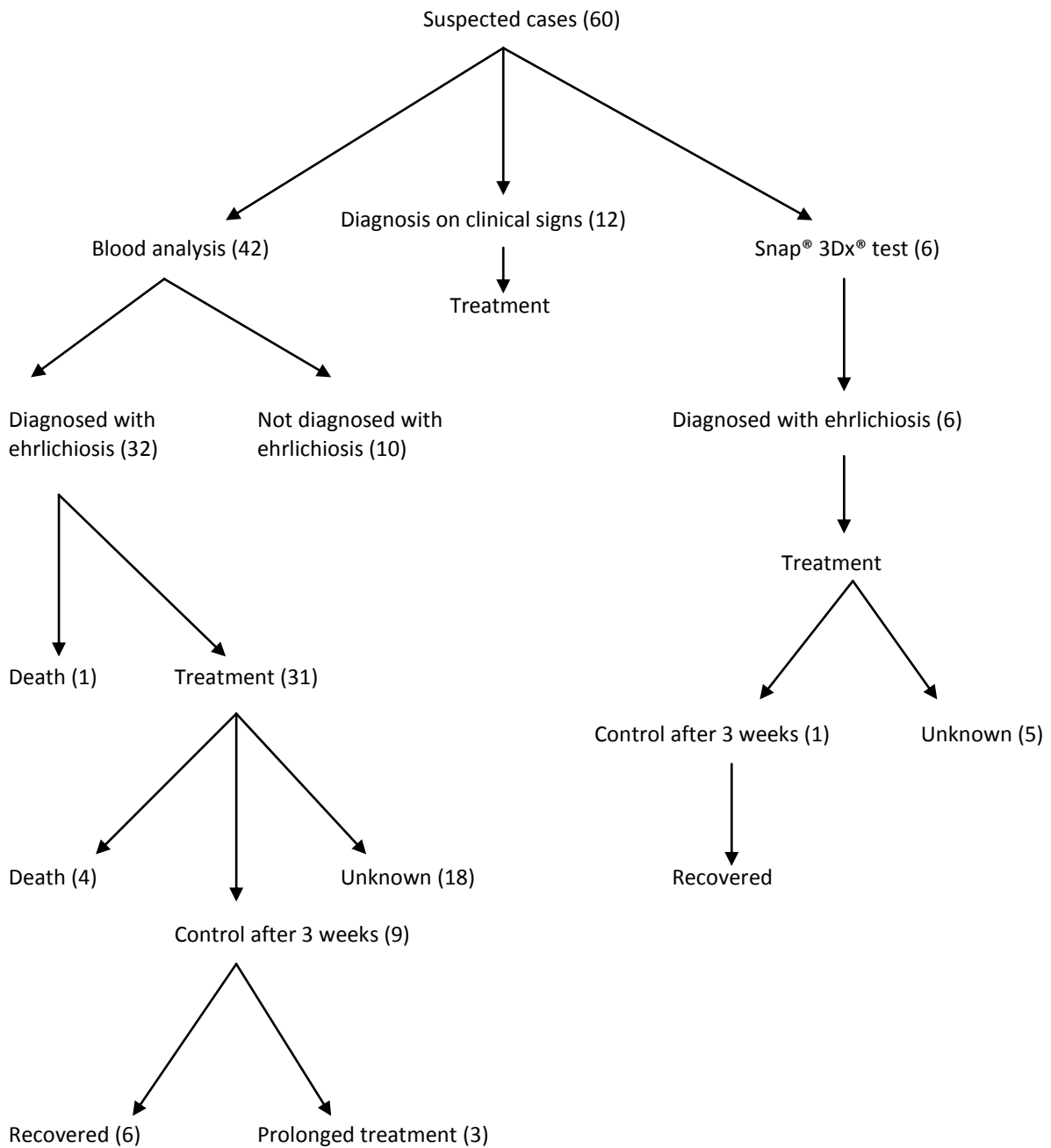


Figure 2. Work-up of suspected dogs admitted to the veterinary clinic

Discussion

This study gives no reason to believe that there is a sex or age predisposition, as well as a breed predisposition. Breed and cross-breed were equally divided. In the breed group, Rottweiler's were overrepresented. Reason for this can be that the Rottweiler is a very popular breed on the island of Curaçao.

In previous studies, German shepherds were reported to be more susceptible to ehrlichiosis.¹⁴ In this study, the German shepherds were not overrepresented.

Nearly a quarter of the dogs diagnosed with ehrlichiosis had previously been infected with *E. canis*. Re-infection of a dog is possible, as permanent immunity after recovery from a previous infection is not necessarily the case.²¹

Travels from the Dutch Antilles to Europe are a big risk for introducing *R. sanguineus* and *E. canis* in Europe. Three out of the four dogs with ehrlichiosis who had travelled abroad, had been to Europe (the Netherlands and Belgium). These cases are exactly the problem when it comes to the introduction of *R. sanguineus* and *E. canis* in Europe. Even though dogs travelling abroad are tested for *E. canis* by the Snap® 3Dx® test, not all dogs with a current infection of *E. canis* will be caught this way, because the sensitivity of the Snap® 3Dx® test is not equal to one.¹⁸

Dogs infested with ticks will not be allowed to travel, but one cannot guarantee that a seemingly clean dog doesn't carry some ticks with it while travelling abroad.

Diagnosis of canine ehrlichiosis on the island of Curaçao is done on clinical signs, hematology and/or the Snap® 3Dx® test.

Diagnosis on clinical signs is done when the clinical signs alone give enough indication for an infection with *E. canis*. Unfortunately, canine monocytic ehrlichiosis has a wide variety of clinical signs that are very non-specific, as can be seen in table 1 and 2. The different phases of the disease manifest itself differently with usually mild and non-specific signs in the acute and subclinical phase and more severe and specific signs during the chronic phase.¹⁴ The patients that were diagnosed on clinical signs only came in with non-specific problems like anorexia and lethargy, according to the owner. After physical examination by the vet, clinical signs most frequently added to that were lymphadenomegaly, splenomegaly, bleeding and petechiae. These clinical signs together were enough reason to diagnose a dog with ehrlichiosis and start treatment.

Besides clinical signs that are obvious, sometimes owners don't have enough money to pay for further examination and the local vet has no other choice than to diagnose on clinical signs only.

It depends on the range of clinical signs that a patient is presented with, the age of the dog and whether or not the dog has suffered from ehrlichiosis before, if blood is analyzed by the Vet IDEXX QBC autoread.

The age of the dog is important, because a young dog is less likely to have had an infection with *E. canis* during its lifetime than an older dog. For these younger dogs, the disease can be diagnosed by the Snap® 3Dx® test.

The Snap® 3Dx® test (ELISA) detects IgG antibodies against *E. canis*. A positive test result indicates that the dog has had an *E. canis* infection during its lifetime, but it is not hard evidence that the dog is currently infected with *E. canis*.⁷ It can take several years for antibody titers to return to normal after infection (and treatment), especially if initial titers at the start of infection were very high. Dogs with persistent elevated antibody titers might represent a subclinical infected population.^{1,17}

On the other hand, negative test results don't rule out the possibility that the dog is in fact suffering from an *E. canis* infection at the moment. Several reasons can be found for this.

Sensitivity of the test is between 0.71 and 0.99^{18, IDEXX} which leaves room for false negatives. Another *Ehrlichia spp.* could also be the cause of the clinical signs of ehrlichiosis but this species is not detected by the test. It's also possible that the dogs were in the acute phase of infection with antibody titers not yet high enough to give a positive test result.^{18, 23}

Three dogs who were tested for *E. canis* with the Snap® 3Dx® test also had a positive result for heartworm. Roughly said, this gives a prevalence of 6%. Previous studies performed on the island of Curaçao by Hesselink et al and Saleh et al report a prevalence of 10% and 13,5% of heartworm in (pet)dogs.

Hematological findings often include thrombocytopenia, leucopenia and anemia in the acute phase^{4,14}, mild thrombocytopenia in the subclinical phase and pancytopenia in the chronic phase.¹⁴ This is in concurrence with this study, where 69% of the dogs had anemia and 84% of the dogs had thrombocytopenia.

The pathogenesis of thrombocytopenia during the infection remains unclear. Several mechanisms have been proposed. The reduced platelet counts can be explained by vasculitis (increased consumption), sequestration due to a platelet migration inhibition factor, splenic pooling, suppression of platelet production and increased platelet destruction by anti-platelet antibodies.¹⁴ This last method suggests an immunological component in the pathogenesis of the disease.^{14,17} It is thought that besides thrombocytopenia, platelet dysfunction also plays a role in the bleeding tendencies seen by dogs with ehrlichiosis. Platelet aggregation is inhibited when sera of *E.canis* positive dogs were incubated with platelets from healthy dogs.^{12,13}

As previously said, diagnosis on the island of Curaçao is done on clinical signs and/or hematological abnormalities and/or the Snap® 3Dx® test. These diagnostic methods are not ideal, because the causative agent is not detected and the antibodies cannot always be detected by the Snap® 3Dx® test.

If you want to detect the causative agent, *E. canis*, there are a few specific diagnostic tests. These are demonstration of *E. canis* morulae in the monocytes, culturing the bacteria and PCR.¹⁸

The immunofluorescence antibody test (IFA) is said to be the gold standard for the detection of *E.canis* antibodies.³⁰ It is often used as a reference. Unfortunately, this test is not available everywhere. Other methods of detecting antibodies against *E. canis* are the commercially available testkits; the Immunocomb® and the previously mentioned Snap® 3Dx® test. The sensitivities and specificities for these tests are 0.86 and 0.98 for Immunocomb® and 0.71 and 1.00 for the Snap® 3Dx® test. The main problem with these tests is that their sensitivity declines when antibody titers are low.¹⁸

Dogs on the island of Curaçao are treated with Doxycycline hyclate (5-10 mg/kg/day) for a minimum of three weeks. According to other researches, this treatment can be successful, depending on the phase of the disease the dog is in. Doxycycline (6 mg/kg/day, twice daily) gives 100% clearance of *E.canis* after two weeks of treatment in the acute phase.⁶ In the subclinical phase, longer treatment is needed. Doxycycline (10 mg/kg/day) gives 75% clearance of *E. canis* after six weeks of treatment in the subclinical phase.¹⁶ During the chronic phase of the disease, doxycycline (5 mg/kg/day, twice daily) gives 100% clearance of *E. canis* after three weeks of treatment.⁹

The clearance of *E. canis* in the above mentioned studies was investigated by PCR.

Summarized, this means that the treatment as given on the island of Curaçao is sufficient for dogs who are in the acute or chronic phase of the disease, but not always for dogs in the subclinical phase of the disease.

Besides doxycycline hyclate treatment, two dogs received imidocarb dipropionate (Imizol®) injections. Imidocarb dipropionate alone does not clear *E. canis* from the blood. Eddlestone et al. revealed a 0% clearance of *E. canis* after two injections being given two weeks apart.

The injections with imidocarb dipropionate are given against a possible co-infection with *Babesia spp.* According to the literature, two doses of 7 mg/kg imidocard dipropionaat with a 14-day interval sterilizes the infection with *Babesia spp.*⁵

The 3-week time interval between the first diagnosis and check-up seems reasonable. If treatment is successful, platelets counts will increase after 24-48 hours and will return to normal within 14 days.²¹ Blood analysis can give an indication if treatment is successful. It is very important that all dogs get checked after three weeks of treatment, because clearance of the infection after three weeks of treatment is not a given thing. In the subclinical phase, it might take longer to clear the bacteria, which might as well be the case for some dogs in other phases of the disease.

As expected, all the ticks collected from the dogs were of the species *R. sanguineus*. The number of ticks found per dog ranged from zero to 108. This big range can be explained by several variables, for example the use of tick-control products on the dog and in the environment⁷ and the living environment of the dog (see research report of Drs. M.M. Klarenbeek, 2010).

As can be seen in the work-up in figure 2 only 22 % of the owners return for the 3-week check-up. This is alarming, when you face the fact that of the ten dogs who were checked after three weeks, three dogs needed prolonged treatment with doxycycline. This could potentially mean that dogs that do not return for a check-up, or at least some of them, remain infected after three weeks of treatment.

This aspect of the work-up allows for improvement.

Some of the material collected during this ten week study is not analyzed yet. This includes the blood spots fixated on filter paper and the ticks. On both these materials, PCR and Reverse Line Blot techniques will be performed which will give information about the pathogens carried by the dogs and the ticks.

With this information, diagnosis's made by the veterinarians on Curaçao can be verified and the effect of the treatment protocols can be tested.

Further research is needed to get a better understanding of ehrlichiosis on the island of Curaçao. This research has hopefully made the first step towards better control of the disease.

First of all the situation concerning the dogs should be looked into. My colleague Drs. M.M. Klarenbeek has made the first steps in that field of the research. What are the best methods for keeping your dog free of ticks? It is already clear that tick prevention products are necessary to control ticks on your dog and in the environment, but what is the best way to do this on the island of Curaçao.

Second of all, the treatment protocol should be tested. Can 3 weeks of doxycycline treatment eradicate the pathogen from the blood of the patient? If not so, initial treatment could be prolonged by, for example, a week.

It is very important to make the local population aware of the fact that ehrlichiosis is a serious problem threatening the dog population on the island. In this research 12% of the dog owners were not familiar with ehrlichiosis. They should be informed about what products to use against ticks (and what not) and how to recognize the first symptoms of the disease.

Apart from *E. canis*, other diseases might be of significance too on the island of Curaçao. Babesiosis is one of them. *R. sanguineus* is believed to transmit the protozoa *Babesia vogeli*.²⁶

So far, the actual protozoa, *B. vogeli*, has not yet been detected on Curaçao. Still, veterinarians sometimes treat dogs with ehrlichiosis for babesiosis as well, when treatment for ehrlichiosis alone isn't successful.

A co-infection of *Ehrlichia spp.* and *Babesia spp.* is often found.²⁶ It would be useful to establish the presence of *B. vogeli* on Curaçao, that way, local veterinarians can take into account the possibility of a dog being infected with *B. vogeli*, with or without co-infection with *E. canis*.

Conclusions

Canine monocytic ehrlichiosis is a serious problem threatening the dog population on the island of Curaçao.

The disease manifests itself as non-specific, with symptoms like anorexia and lethargy being one of the first to be seen in the dog. With the profound experience of the vets on Curaçao concerning ehrlichiosis, sometimes dogs can be diagnosed on clinical signs, but often further diagnostics are needed to be sure.

Hematological abnormalities often found are thrombocytopenia (84%) and anemia (69%), but these are not pathognomonic. Even with blood analysis one cannot be sure whether the dog has an *E. canis* infection or not.

The methods used for further diagnosis are a step towards getting more certainty about the diagnosis. Actual detection of the causative agent *E. canis* does not happen on Curaçao.

Detecting of antibodies can be done by the Snap® 3Dx® test, but the sensitivity for this test, which is used on Curaçao, is 0.71.¹⁸ Meaning that not all dogs infected with *E. canis* will be caught with this test.

Use of the IFA test for the diagnosis would be ideal. This test is said to be the golden standard for the detection of antibodies. Unfortunately this test is not always available everywhere.

Ehrlichiosis is treated with doxycycline hyclate (5-10 mg/kg/day), for a minimal period of three weeks.

A certain number of dogs will be able to clear the bacteria from its blood during these first three weeks, but not all. That is why the check-up after three weeks of treatment is so important.

Dogs that do not recover in those 3 weeks can undergo prolonged treatment, for as long as it takes to clear the bacteria.

Unfortunately, the return rate of owners is not very high (22%). A lot of progress can be made in this area of the work-up.

Travels abroad form a big risk for export of *E. canis* and *R. sanguineus* to Europe and/or the Netherlands. According to the questionnaire, 8 % of the dogs with ehrlichiosis had travelled abroad, of which 75% to the Netherlands and Belgium.

As *E. canis* infections have already been reported in the Netherlands for several years and travels to endemic regions are thought to be the cause of this³¹, the measurements taken when dogs travel from the island of Curaçao need to be relooked at.

Finally, more research is needed on tick control and treatment strategies to optimize control of the disease on the island. This includes educating the local population on tick control and awareness of the disease.

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Appendices 1

Package insert Snap® 3Dx® test by IDEXX Laboratories, 2008

Canine Heartworm Antigen/Borrelia Burgdorferi/
Ehrlichia Canis Antibody Test Kit

For veterinary use only.



SNAP® 3Dx®

In-vitro diagnostic for the detection of *Dirofilaria immitis* antigen, antibody to *Borrelia burgdorferi*, and antibody to *Ehrlichia canis* in canine whole blood, serum or plasma.

Precautions and Warnings

- All wastes should be properly decontaminated prior to disposal.
- Do not mix components from kits with different lot numbers.
- Do not use a SNAP device that has been activated before the addition of sample.

Storage

- Store at 2°–7°C (35°–45°F) until expiration date.
- SNAP devices and reagents can be stored at room temperature (15°–25°C/59°–77°F) for 90 days or until the expiration date, whichever occurs first.
- After SNAP devices and reagents are removed from 2°–7°C (35°–45°F) for more than 24 hours, the expiration date is 90 days or the printed expiration date, whichever occurs first.
- If the 90-day expiration date occurs before the printed expiration date, record the new date in the space provided on the kit.




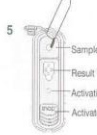
Kit Components

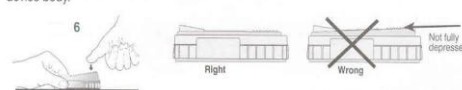
Each kit contains one 8-mL bottle of anti-HW/LY/EC:HRPO conjugate and one reagent rack as well as 5, 15 or 30; transfer pipettes, sample tubes and SNAP devices. Each SNAP device contains 0.4 mL of wash solution and 0.6 mL of substrate solution.

Sample Information

- Samples must be at room temperature (15°–25°C/59°–77°F) before beginning the test procedure.
- Serum, plasma or anti-coagulated whole blood (e.g., EDTA, heparin), either fresh or stored at 2°–7°C (35°–45°F) for up to one week, can be used.
- For longer storage, serum or plasma can be frozen (-20°C/-4°F or colder) and then centrifuged before use.
- Hemolyzed or lipemic samples will not affect test results.

Test Procedure

- If stored in a refrigerator, allow all components to equilibrate at room temperature (15°–25°C/59°–77°F) for 30 minutes. **Do not heat.**
- Using the pipette provided, dispense **3 drops of sample** into a new sample tube.
 
- Holding the bottle vertical, add **4 drops of conjugate** to the sample tube.
 
- Cap the sample tube and mix it thoroughly by inverting it 3–5 times.
 
- Place the device on a horizontal surface. Add the entire contents of the sample tube to the sample well, being careful not to splash the contents outside the sample well.
 

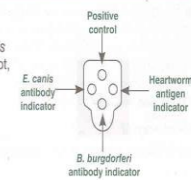
The sample will flow across the result window, reaching the activation circle in 30–60 seconds. Some sample may remain in the sample well.
- When color **FIRST** appears in the activation circle, push the activator firmly until it is flush with the device body.
 

NOTE: Some samples may not flow to the activation circle within 60 seconds, and, therefore, the circle may not turn color. In this case, press the activator after the sample has flowed across the result window.


7. Read test result at **eight minutes.**

Interpreting Test Results

To determine the test result, read the reaction spots in the result window. Any color development in the sample spots indicates the presence of the heartworm antigen, *B. burgdorferi* antibody or *E. canis* antibody in the sample. If no color develops in the positive control spot, repeat the test.

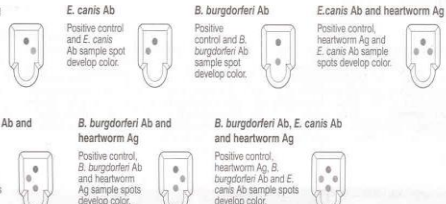


Negative Results
Only the positive control spot develops color.



Positive Results

- Heartworm Ag:** Positive control and heartworm Ag sample spot develop color.
- E. canis Ab:** Positive control and E. canis Ab sample spot develop color.
- B. burgdorferi Ab:** Positive control and B. burgdorferi Ab sample spot develop color.
- E. canis Ab and heartworm Ag:** Positive control, heartworm Ag and E. canis Ab sample spots develop color.
- B. burgdorferi Ab and E. canis Ab:** Positive control, B. burgdorferi Ab and E. canis Ab sample spots develop color.
- B. burgdorferi Ab, E. canis Ab and heartworm Ag:** Positive control, heartworm Ag, B. burgdorferi Ab and E. canis Ab sample spots develop color.



Invalid Results

- Background**—If the sample is allowed to flow past the activation circle, background color may result. Some background color is normal. However, if a colored background obscures the test result, repeat the test.
- No Color Development**—If the positive control does not develop color, repeat the test.

Sensitivity and Specificity of SNAP 3Dx

Comparison Test	Sample Size					Sample Type	Relative Sensitivity and Specificity 95% Confidence Limit	Kappa Statistic
	+/+	-/+	+/-	-/-	Total			
PetCheck Heartworm	152	3	0	157	312	Serum/Plasma/Whole Blood	Sen., 98% (95% CL 94%–100%) Spec., 100% (95% CL 97%–100%)	0.98
Heartworm Necropsy	54	8	0	0	62	Serum/Plasma	Sen., 87% (95% CL 76%–93%)	N/A
E. canis IFA/Western blot	79	1	0	164	244	Serum	Sen., 99% (95% CL 92%–100%) Spec., 100% (95% CL 97%–100%)	0.99
B. burgdorferi IFA/Western blot	171	14	0	170	355	Serum	Sen., 92% (95% CL 86%–96%) Spec., 100% (95% CL 97%–100%)	0.92

The heartworm sensitivity values in the table above were generated from mixed worm-burden populations. Sensitivities calculated for samples from dogs with very low worm-burdens (<2 female worms) were 92.9% (Study 1, n=28) and 79.2% (Study 2, n=24). Sensitivities calculated for samples from dogs with >2 female worms were 99.2% (Study 1) and 97.4% (Study 2).


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Appendices 2

Protocol for sampling blood on filter paper

1. Spot untreated/treated with anticoagulants blood on a filter paper.
2. Dry blood spot for 3 hours at room temperature.
3. Fix blood spot with 100 µl methanol.
4. Dry blood spot for 1 hour at room temperature.
5. Store blood spot in a dry place at room temperature.

Appendices 3

Questionnaire 'Karpattenziekte'

Date: _____
Dog number: _____
Owner: _____
Telephone number owner: _____
Address: _____
Name of dog: _____
Sex: M / F
Age: _____
Breed: _____
Furtype: Long haired / Medium-long haired / Short haired
Furcolor: DARK / INTERMEDIATE / LIGHT / SPOTTED

Are you familiar with the Karpattenziekte? Yes/No
Has your dog had this disease before? Yes/No

Complaints owner: _____

Findings veterinarian: _____

Do you have more dogs, if yes, how many?
Have your other dogs ever had Karpattenziekte? Yes/No

Do you use tick prevention on your dog? Yes/No

Vet	(Pet) Shop				
Frontline plus	Frontline spray	Ovitrol	Preventic collar	Paramite	
Ivomec injectable	Shampoo	Other;			

How often? _____

Environment What, how often? _____

Are you satisfied with these products? Yes/No

Do you remove ticks from your dog? Yes/No

How often?

Can you give an estimate of the amount of ticks?

Does your dog live inside or outside?

INSIDE / PART INSIDE, PART OUTSIDE / OUTSIDE
KENNEL / CHAIN / LOSE

Have you ever taken your dog abroad?

Result Snap® 3Dx® test *E. canis* : Positive/ Negative

Blood sample taken for Vet IDEXX QBC autoread? Yes/No

Treatment:

Appendices 4

Results Vet IDEXX QBC autoread

	Result	Difference
Hematocrit		
Hemoglobin		
WBC		
Granulocytes		
% granulocytes		
Neutrofil		
Eosinofil		
Trombocytes		
Reticulocytes		
Lymfocytes/monocytes		
% Lymfo's/monocytes		
MCHC (hemoglobin)		