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Research Article

Clinical and Serological Evidence of Canine Anaplasmosis and Ehrlichiosis in Urban and Rural Panama

Joao Varela Petrucelli[1]* and Sergio Bermúdez[2]

[1]*Fundación San Francisco de Asís, Clínica Veterinaria Happy Pets, Panamá*
[2]*Departamento de Entomología Médica, Instituto Conmemorativo Gorgas de Estudios de la Salud, Panamá*

***Corresponding author**
Joao Varela Petrucelli, Fundación San Francisco de Asís, Clínica Veterinaria Happy Pets, Panamá, Panamá, Email: joaovarelap@gmail.com

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Abstract

Canine tick borne disease, such as anaplasmosis and ehrlichiosis are common diseases in veterinary practices. Most patients are diagnosed using clinical findings, history of tick bites and hematological findings; however, proper approach to diagnostic is not always feasible. Blood from 104 animal patients from urban and rural areas were analyzed finding 53.84% (n=56) having parameters compatible with tick borne disease, 18 from rural settlements and 38 from urban. Immunochromatographic test was performed, finding two animals positive to anaplasmosis, 14 to ehrlichiosis, three with co-infection, and three with negative antibody test. Patients from rural areas were the only to test positive to anaplasmosis or to be co infected. Our results demonstrate a high incidence of ehrlichiosis in both urban and rural areas of Panama.

INTRODUCTION

Tick-borne pathogens are among the principal concern to world public health, both in humans and animals [1]. In veterinary medicine their impact is reflected by economic loss in livestock animals at major scale and home economies, not only due to health problems, but also due to control and treatments [2,3]. In companion animals, specifically dogs, the main health associated problems with ticks bites are skin problems (allergic reactions) and transmission of diseases such as tropical canine pancytopenia (caused by *Ehrlichiacanis*), canine cyclic trombocytopenia (caused by *Anaplasma platys*), canine babesiosis (caused by *Babesiacanis*), hepatozoonosis (caused by *Hepatozoon* spp.), rickettsiosis and borreliosis [4].

In the veterinary practice, reports of tick bites in the anamnesis present a first step for the diagnostic of these diseases, which are complemented to non-specific clinical signs that vary from fever, depression and anorexia to more severe as such vomit, diarrhea, lymphadenopathy, splenomegaly, clotting disturbs, and bone marrow suppression [5]. Laboratory tests that can be performed to confirm these diseases include a complete blood count showing alterations such as anemia, thrombocytopenia for *A. platys* and leukopenia included for *E. canis* [5,6].

In Panama the presence of *E. canis* and *A. platys* have been reported in metropolitan area of City of Panama, by observation in blood smears [7] and also PCR [8] from clinically ill dogs. Even so, it is possible that many cases are not diagnosed. This fact shows the relevance of these pathogens in the most important urban area in Panama, where its vector, the *Rhipicephalus sanguineus* tick represent a potential caveat to human health as well [9,10]. The aim of this work is to present recent clinical finding, captured at a veterinary clinic in City of Panama, during a period of fifteen days of veterinary care from animals suspected to have tick borne disease clinic and hematological findings.

MATERIALS AND METHODS

During November 2016, blood tests were performed from 104 canine patients for routine checkup, pre-operatory screening or clinical illness. Patients came from rural and urban areas of the provinces of Panamá, Panamá Oeste and Colón (Table 1). Complete blood count was performed on ProCyteDx Hematology Analyzer – Idexx (Idexx, United States). Results for red blood cells and white blood cells were confirmed using a hemacytometer (Marienfeld, Germany); references for normal hematological values were taken from a highly cited textbook [11].

Morphology of erythrocytes, white blood cells differential and platelets morphology were evaluated by direct blood smears stained with PanoticoRapido (LaborclinProdutos Para

Table 1: Distribution of patients with positive clinical signs and hematological findings for blood-borne parasites.

Province (Settlement)	Number of Animals
Panama	47
Panama Oeste	6
Colon	3

Laboratórios, Brasil) under a Leica microscope model Moticam S2 (Leica, China). In addition, the blood smears were revised for hemoparasites inclusions. Owners of ill dogs were offered the use of a commercial immunochromatographic test for detection of *E. canis/E. chaffeensis* and *A. platys/A. phagocytophilum* antibodies (Idexx SNAP 4Dx Plus), however not all accepted the use of this test. Samples from owners that accepted the use of immunochromatographic assays were processed according to manufacturer's instructions. All patients suspected for anaplasmosis or ehrlichiosis were treated with doxycycline at dose of 10 mg/kg and prednisone at 0,5 mg/kg every 24 hours for 21 and 5 days, respectively. In addition, a complete blood count follow up was performed at 15 days to assess the effectively of the treatment which would be expected by an increase in red blood cell count, white blood cell and platelet count.

RESULTS AND DISCUSSION

Out of the 104 dogs, a total of 56 (53.8%) showed hematological findings compatible with tick borne disease, all of the 56 (100%) presented ticks at the moment of the physical examination or have had a history of tick bites. From these 56 patients, 56 (100%) presented fever; 54 (96.42%) depression; 52 (92.85%) anorexia; 41 (73.21%) vomit; 36 (64.28%) diarrhea; 6 (10.70%) hematuria; 3 (5.35%) petechiae and 1 (1.78%) epistaxis. Hematological findings compatible with the disease are shown on Table (2) for red blood cell count and Table (3) for platelet count. Leukopenia was observed in some patients and reported in Table (4). Correlation between clinical signs, hematological signs and results for immunochromatographic test is shown on Table (5).

Most patients performed complete blood count were received from urban areas (n=38; 67.85%) while the other 18 (32.15%) were from rural areas. Out of these 56 patients, 22 were performed immunochromatographic commercial tests for detection of *E. canis/E. chaffeensis* and *A. platys/A. phagocytophilum* antibodies. Positive results for *E.canis/E. chaffeensis* were found in 14 (63%) of patients, while 2 (9.09%) showed positive results for *A.platys/A.phagocytophilum.* Only 3 (13.63%) showed reactivity to both antibodies and another 3 samples did not reacted to the immunochromatographic test; however, these last three patients had symptoms and hematological findings compatible with canine anaplasmosis or canine ehrlichiosis. In regard to this observation, it is possible that these three patients had other disease which present the same clinical signs, such as *Babesia* spp., *Mycoplasma* spp., *Ehrlichia* spp. or viral diseases.

It was observed that patients inhabiting rural areas were the only positive for co infection of *Ehrlichia* spp. and *Anaplasma* spp. by immunochromatography, while patients living in urban area only showed positive results for *Ehrlichia* spp. Out of the 18 canine patients living in rural areas, 8 presented positive results for *Ehrlichia* spp. and 5 for *Anaplasma* spp. including positive testing for both parasites. Relatively, there are a higher percentage of patients from rural areas that tested positive for *Anaplasma* and

Table 2: Correlation of clinical signs and hematological findings with positive results to immunochromatography in clinical ill patients.

Clinical signs	*Ehrlicia* spp. (n=14)	*Anaplasma* spp. (n=2)	Coinfection (n=3)	Non detected (n=3)
Fever	13 (92,85%)	2 (100%)	3 (100%)	2 (66,66%)
Depression	14 (100%)	2 (100%)	3 (100%)	3 (100%)
Anorexia	13 (92,85%)	1 (50%)	3 (100%)	2 (66,66%)
Vomit	12 (85,71%)	1 (50%)	2 (66,66%)	1 (33,33%)
Diarrhea	13 (92,85%)	0 (0%)	3 (100%)	1 (33,33%)
Hematuria	3 (21,42%)	0 (0%)	2 (66,66%)	0 (0%)
Petechiae	2 (14,28%)	0 (0%)	1 (33,33%)	0 (0%)
Epistaxis	0 (0%)	0 (0%)	1 (33,33%)	0 (0%)
Anemia	14 (100%)	2 (100%)	3 (100%)	3 (100%)
Thrombocytopenia	14 (100%)	2 (100%)	3 (100%)	3 (100%)
Leukopenia	8 (57,14%)	0 (0%)	3 (100%)	1 (33,33%)

Table 3: Number of animals with tick history showing hematological signs compatible with tick-borne disease.

Number of Animals	Red Blood Cell Count (M/µL)[1]	Hematocryt (V/V)[1]	Hemoglobin (g/L)[1]
2	< 2,5	< 9,0	< 3,0
12	1,6 – 2,5	9,1 – 15,0	3,1 – 5,0
16	2,6 – 3,5	15,1 – 21,0	5,1 – 7,0
5	3,6 – 4,5	21,1 – 27,0	7,1 – 9,0
10	4,6 – 5,5	27,1 – 33,0	9,1 – 12,0
4	5,6 – 6,5	33,1 – 39,0	12,1 – 15,0
6	> 6,6	> 39,1	> 15,1

[1]Anemic patients are known to possess values under 5,5M RBC, 12 Hb and 37% PCV.

Table 4: Number of animals with tick history showing platelet count compatible with tick-borne disease.

Number of Animals	Platelet Count (plt)[1]
16	< 25,000
10	25,001 – 50,000
16	50,001 – 75,000
7	75,001 – 100,000
7 0	100,001 – 150,000 150,001 – 200,000

[1]Platelet count in healthy patients is over 200,000

Table 5: Number of animals with tick history showing hematological signs compatible with tick-borne disease.

Number of Animals	White Blood Cells Count (WBC)[1]
2	1,000 – 2,500
10	2,600 – 5,000
12	5,100 – 7,500
16	7,600 – 10,000
15	> 10,100

[1]Normal White blood cell count in healthy patients is over 6,000

Ehrlichia (27.77% and 38.88% respectively) than those tested for urban areas (23.18%); however, this percentage was dependent of whether the client accepted to perform the test (Table 6). Of 18 patients positive to the SNAP test, 12 were re-evaluated 15 days posterior to the antibiotic treatments. We found a 91.66% of effectively of the treatment, and only 1 patient died possibly due to bone marrow aplasia from chronic ehrlichiosis (data not show).

Panama is considered endemic for canine ehrlichiosis and canine anaplasmosis [7,8]. Although the methods to obtain the data have been different between previous records and our present finding, the amount of canine patients examined at our practice was elevated considering the time of the study and distribution of the cases, possibly due to increased observation of the owners. For example, during 2004-2009 was reported 10.12% (n=1452) of positive cases in one veterinary hospital [7]; while, since 2005-2010, 70.6% (n=201) of positive cases from 10 clinics, sampling animals with clinical illness [7,8].

Assessment of data to study *E. canis* is not always unified in different studies of the region since different authors elaborate their data and perform their diagnostic in different ways. For example, in Central America, research from Nicaragua show that randomly tested dogs showed anemia in 70% of cases (n=27), and 63% tested positive for *E. canis* by immunochromatographic test [12]. In a recent study from Costa Rica, DNA of *E. canis* was found on 3.2% (n=407) of healthy dogs and stray dogs living in parks [13]; while other studies reported 47.7% (n=301) of *E. canis* DNA on clinical ill dogs, which does not share same results due to differences in their samples [14].

Due to the behavior of *E. canis* inside its host, finding of morulae in monocytic cells occurs in only 4% of the cases [5]. While this being a strong parameter to confirm the diagnostic, it is not always the best approach for it [5]. To determine a correct prevalence among clinically ill and healthy dogs, it is necessary to perform more specific tests, such as PCR, from healthy patients as well. This could not be verified in our study since not all patients admitted at the clinic were performed specific tick borne disease tests. The only observation in regards this event is the correlation of clinical ill dogs compatible with complete blood count performed (n=56/104; 53.8%).

In the case of *A. platys*, experimentally challenged dogs with this agent showed no hematological and leukocytic alterations, only low platelet count, while those infected with *E. canis* showed severe leukopenia and thrombocytopenia, suggesting that thrombocytopenia alone a suggestive hematological finding for *A.platys* [15]. Significantly lower hematological values were found in co-infected dogs with *E.canis* and *A.platys* [15].

In this study the hematocrit from 44 dogs (78.57%) was under the normal reference value used. Immunochromatographic positive patients for *E. canis* were more likely to show not only depression and anorexia, but also gastrological signs such as vomit in 85% (n=14) of cases and diarrhea in 92,85% (n=14) of cases (table 2). More severe signs such as hematuria and petechiae were observed, but in a lower percentage of patients (21,42% and 14,28% respectively (n=14). Depression, anemia and thrombocytopenia were observed in all *Ehrlichia spp*. infected patients. On the other hand, the signs observed in *A. platys* positive patients presented clinical signs such as fever and depression in 100% of cases, and in 50% of confirmed cases anorexia and vomit (n=2). All *Anaplasma spp.* positive patients were also found to be anemic and thrombocytopenic (n=2), but not leukopenic as *Ehrlichia spp.* infected patients.

Co infected patients were found to have fever, depression, anorexia and diarrhea in 100% of cases, vomit and hematuria in 66,66% of cases, petechiae and epistaxis in 33,33% of cases (n=3). All of the patients that were found to be co infected presented anemia, thrombocytopenia and leukopenia, being the co infection the most pathogenic form of tick borne disease in dogs in the present study.

All animals positive to *E. canis*/*E. Chaffeensis* presented a lower hematocryt, hemoglobin and red blood cell count than those animals with only *A. platys*/*A. Phagocytophylum* positive tests, which indicates that a febrile and anemic animal is more likely to be infected with *Ehrlichia* species pathogen. On the other hand, much lower values for red blood cells parameters were found in patients coinfected with *Ehrlichia* and *Anaplasma* species (Table 6). This result is lower, however, due to the fact that pancytopenia is observed after the subclinical onset of the animal, which might depend on coexistence of another immunosuppressant disease or a high pathogenic strain of the pathogen [5,6,15].Thrombocytopenia was a finding in all of these patients, however, when positive for *Anaplasma* species on the SNAP test, the results were not as low as with the results found with the other pathogens (Table 7). Reports from another country state that the inclusions in platelets of clinical ill dogs and with platelet count compatible with canine cyclic thrombocytopenia are 84% compatible with *A. platys* [16].

Despite there is no report on Panama stating which zones poses a greater risk of ehrlichiosis and anaplasmosis, the distribution and ecology of the main vector is a first warning sign. In this country, *Rhipicephalus sanguineuss*. l. is the only known vector to both diseases and is the most common parasite

Table 6: Distribution of immunochromatography positive cases of *Ehrlichia* spp. and *Anaplasma* spp.

Humans settlements [a]	No. [b]	Positive for *Ehrlichia*spp	Positive for *Anaplasma*spp	Positive for *Ehrlichia* spp.and *Anaplasma* spp.	Negative for *Ehrlichia* spp.and *Anaplasma* spp.
		n/N (%)	n/N (%)	n/N (%)	n/N (%)
Urban city	38[c]	9/56 (16,07)	0/56 (0)	0/56 (0)	2/56 (0,03)
Rural towns	18	5/56 (8,92)	2/56 (3,57)	3/56 (5.35)	1/56 (0,01)
Total	56	14	2	3	3

[a]Urbanareas were considered as free of agriculture activities (e.g. livestock and crops). Rural areas were considered according the primary economic activity (e.g. livestock and crops), or proximity to forested areas.
[b]Corresponding to human settlements analyzed.
[c]Corresponding to neighbors of City of Panama and Colon.

Table 7: Hematological parameters from immunochromatography positive patients.

Patienta	Pathogen	RBC (M/µL)[1]	PCV % (V/V)[1]	Hb(g/L)[1]	Plt(x10000)[1]	WBC (x1000)[1]
1	*E. canis*/*E. chaffeensis*	2,7	18,61	6,18	8	6,0
2	*E. canis*/*E. chaffeensis*	2,2	14,87	5,01	50	3,3
3	*E. canis*/*E. chaffeensis*	2,3	15,78	5,26	62	5,7
4	*E. canis*/*E. chaffeensis*	2,0	14,07	4,54	81	3,7
5	*E. canis*/*E. chaffeensis*	1,9	12,98	4,34	94	5,7
6	*E. canis*/*E. chaffeensis*	2,2	15,24	5,03	94	9,8
7	*E. canis*/*E. chaffeensis*	2,5	17,15	5,71	140	8,3
8	*E. canis*/*E. chaffeensis*	2,7	17,86	6,16	28	4,5
9	*E. canis*/*E. chaffeensis*	2,7	15,42	5,21	44	4,5
10	*E. canis*/*E. chaffeensis*	2,8	18,91	6,40	81	6,1
11	*E. canis*/*E. chaffeensis*	3,1	9,36	7,10	96	5,4
12	*E. canis*/*E. chaffeensis*	3,1	21,49	7,07	53	6,5
13	*E. canis*/*E. chaffeensis*	4,0	27,60	9,14	57	9,8
14	*E. canis*/*E. chaffeensis*	3,0	20,21	6,85	60	7,2
15	*A. platys*/*A. phagocytophilum*	4,7	32,33	10,05	12	7,8
16	*A. platys*/*A. phagocytophilum*	4,4	30,26	10,78	54	10,9
17	Co infection	0,9	6,03	4,54	44	1,4
18	Co infection	1,2	8,00	2,74	6	2,6
19	Co infection	2,0	13,63	4,55	59	2,4

[1]Anemic patients are known to possess values under 5,5M RBC, 12 Hb and 37% PCV, platelet count in healthy patients are over 200,000 and normal White blood cell count in healthy patients is over 6,000.

in dogs in rural and urban areas, in elevations among 0-1200 m [17]. As parasite of dogs, this species seem to be the only adapted to Panamanian urban environment; however, in rural areas it shares hosts with other species of *Amblyomma* (e.g. *A. ovale, A. oblongoguttatum, A. mixtum*) and *Ixodes* (e.g. *I. affinis*) [17,18], whose role as vectors of these diseases has not been demonstrated. On the other hand, while dogs are the main host to *R. sanguineus*s. l., other *Amblyomma* or *Ixodes* ticks have alternative hosts in some stage of their life-cycle [18].

CONCLUSIONS

In spite of the short period and small number of examined animals included in this study, it has been demonstrated that *E. canis* is a common problem in the veterinary practice in the City of Panama due to the distribution of its main vector, while *A. platys* appears to be more restricted to dogs living in rural areas of the country. The possibility that the exposure of dogs to other tick species might increase the risk of cross transmission of other pathogens such as other *Rickettsia* or *Anaplasma* species [19].

Routinely the diagnostic for these diseases is made by anamnesis, clinical findings and laboratory tests compatible with the disease followed by therapeutic diagnostic. Treatment of most tick-borne pathogens is achieved by the use of tetracyclines such as doxycycline. Because of this, it is assumed most of the time that the infection that might be affecting a dog is "tick fever" or *E.canis*.

Finally, since both ehrlichiosis and anaplasmosis could represent a risk to human health [20,21], further diagnostic must include molecular tools to assess better prevalence of these diseases.

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REFERENCES

1. Dantas-Torres F, Chomel BB, Otranto D. Ticks and tick-borne diseases: a One Health perspective. Trends Parasitol. 2012; 28: 437-446.

2. Grisi L, Leite RC, Martins JR, Barros AT, Andreotti R, Cançado PH, et al.

Reassessment of the potential economic impact of cattle parasites in Brazil. Rev Bras Parasitol Vet. 2014; 23: 150-156.

3. Irwin PJ. It shouldn't happen to a dog … or a veterinarian: clinical paradigms for canine vector-borne diseases. Trends Parasitol. 2014; 30: 104-112.

4. Chomel B. Tick-borne infections in dogs-an emerging infectious threat. Vet Parasitol. 2011; 179: 294-301.

5. Harrus S, Waner T. Diagnosis of canine monocytotropic ehrlichiosis (Ehrlichia canis): an overview. Vet J. 2011; 187: 292-296.

6. Little SE. Ehrlichiosis and anaplasmosis in dogs and cats. Vet Clin North Am Small Anim Pract. 2010; 40: 1121-1140.

7. Perez A, Pile E, Torres A, Lasso J. Prevalence of canine ehrlichiosis among animals treated at the Corozal veterinary hospital of the faculty of veterinary medicine, university of Panama. ArqInstBiol (Sao Paulo). 2013; 80: 207-211.

8. Santamaria A, Calzada JE, Saldana A, Yabsley MJ, Gottdenker NL. Molecular diagnosis and species identification of Ehrlichia and Anaplasma infections in dogs from Panama, Central America. Vector Borne Zoonotic Dis. 2014; 14: 368-370.

9. Gray J, Dantas-Torres F, Estrada-Peña A, Levin M. Systematics and ecology of the brown dog tick, Rhipicephalus sanguineus. Ticks Tick Borne Dis. 2013; 4: 171-180.

10. Arraga-Alvarado CM, Qurollo BA, Parra OC, Berrueta MA, Hegarty BC, Breitschwerdt EB. Case report: Molecular evidence of Anaplasma platys infection in two women from Venezuela. Am J Trop Med Hyg. 2014; 91: 1161-1165.

11. Weiss DJ, Wardrop KJ, editors. Schalm's veterinary hematology. John Wiley & Sons; 2011.

12. Lara VR, Arancibia DM, Saenz M, Bonilla JL. Hallazgo de Ehrlichiosis canina causada por E. canis en una Comunidad del Municipio de León, Nicaragua. REDVET. Revista electrónica de Veterinaria. 2010; 1695: 7504.

13. Barrantes-González AV, Jiménez-Rocha AE, Romero-Zuñiga JJ, Dolz G. Serology, molecular detection and risk factors of Ehrlichia canis infection in dogs in Costa Rica. Ticks Tick Borne Dis. 2016; 7: 1245-1251.

14. Romero LE, Meneses AI, Salazar L, Jiménez M, Romero JJ, Aguiar DM, et al. First isolation and molecular characterization of Ehrlichia canis in Costa Rica, Central America. Res Vet Sci. 2011; 91: 95-97.

15. Gaunt SD, Beall MJ, Stillman BA, Lorentzen L, Diniz PP, Chandrashekar R, et al. Experimental infection and co-infection of dogs with Anaplasmaplatys and Ehrlichiacanis: hematologic, serologic and molecular findings. Parasit & Vectors. 2010; 3: 1.

16. Ferreira RF, de Mello FigueiredoCerqueira A, Pereira AM, Guimarães CM, de Sa AG, da Silva Abreu F, et al. *Anaplasma platys* diagnosis in dogs: comparison between morphological and molecular tests. Int J Appl Res Vet M. 2007; 5: 113.

17. Bermúdez SE, Castro AM, Trejos D, García GG, Gabster A, Miranda RJ, et al. Distribution of Spotted Fever Group Rickettsiae in Hard Ticks (Ixodida: Ixodidae) from Panamanian Urban and Rural Environments (2007-2013). Ecohealth. 2016; 13: 274-284.

18. Bermúdez C, Miranda C. Distribution of ectoparasites of Canis lupus familiaris L. (Carnivora: Canidae) from Panama. Revista MVZ Córdoba. 2011; 16: 2274-2282.

19. Szabó MP, Pinter A, Labruna MB. Ecology, biology and distribution of spotted-fever tick vectors in Brazil. Front Cell Infect Microbiol. 2013; 3: 27.

20. Bouza-Mora L, Dolz G, Solórzano-Morales A, Romero-Zuñiga JJ, Salazar-Sánchez L, Labruna MB, et al. Novel genotype of Ehrlichia canis detected in samples of human blood bank donors in Costa Rica. Ticks Tick Borne Dis. 2017; 8: 36-40.

21. Dumler JS, Choi KS, Garcia-Garcia JC, Barat NS, Scorpio DG, Garyu JW, et al. Human granulocytic anaplasmosis and Anaplasma phagocytophilum. Emerg Infect Dis. 2005; 11: 1828-1834.

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