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Tick-borne pathogens and disease in dogs on St. Kitts, West Indies

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ABSTRACT

Between 2009 and 2011, we conducted a case–control study of ticks and tick-associated pathogens affecting dogs on the island of St. Kitts, eastern Caribbean, including 55 cases of clinically suspected tick-borne disease (TBD) and 110 presumably healthy animals presented for elective surgeries. *Rhipicephalus sanguineus* caused year-round infestations of dogs, and 36% of the dogs in the study were infested at the time of examination. Overall, 62% of suspected TBD cases and 24% of presumably healthy dogs tested positive by PCR for infections with: *Anaplasma platys* (0% and 4%), *Babesia canis vogeli* (20% and 6%), *Babesia gibsoni* (18% and 5%), *Ehrlichia canis* (35% and 7%), and *Hepatozoon canis* (5% and 2%). Co-infections were documented in 15% of these PCR-positive dogs. Antibodies against *A. platys* or *E. canis* were noted in 36% of the dogs. Thrombocytopenia was the most common sign of infection, followed by anemia. This is the first detection of *A. platys*, *B. canis vogeli*, or *H. canis* on St. Kitts and the first detection of *B. gibsoni* in the Caribbean. We conclude that tick-borne pathogens of dogs are highly prevalent in this region and may present in dogs that appear healthy, in spite of hematologic abnormalities that may increase surgical risk.

1. Introduction

Tick-associated pathogens of dogs are diverse and can be associated with considerable morbidity, including thrombocytopenia and abnormalities of hemostasis, anemia with secondary hypoxia and organ damage, and leukopenia (Harrus and Waner, 2011; O'Dwyer, 2011; Solano-Gallego and Baneth, 2011). *Rhipicephalus sanguineus*, the brown dog tick, is widely distributed on dogs across the world; the tick is well-adapted for tropical climates and is associated with year-round infestations (Dantas-Torres, 2010). This tick has been implicated as a vector of diverse canine diseases, including: *Anaplasma platys*, *Babesia canis*, *Ehrlichia canis*, *Rickettsia* species, and *Hepatozoon canis* (via ingestion of infected ticks). Of these, *E. canis*, *B. canis*, and some *Rickettsia* may be zoonotic (Dantas-Torres, 2008; Unver et al., 2001).

Saint Kitts and Nevis is a small island federation located in the northern Lesser Antilles, in the eastern Caribbean. Previous work has provided some information about tick-borne pathogens in the region, but information regarding dogs is limited. *Rickettsia africae* was found in *Amblyomma variegatum* from several islands and is zoonotic (Kelly et al., 2003, 2010); the extent to which this tick parasitizes dogs is unknown. Other spotted-fever group rickettsiae and *Ehrlichia* spp., including *E. canis*, were reported from *R. sanguineus* from St. Kitts (Kelly et al., 2009). Similarly,

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E. canis antibodies were detected in 71% of dogs in a study from the Turks and Caicos islands (Hoff et al., 2008). Another study of 13 thrombocytopenic dogs from St. Kitts failed to detect *A. platys* or *Babesia* spp. (Kelly and Lucas, 2009); however, the study was small, and the assay used to detect *Babesia* was designed for strains found in the USA (Birkenheuer et al., 2003). In contrast, a PCR-based survey from Trinidad documented both *E. canis* and *B. canis vogeli*, and a study of dogs on Grenada detected DNA from *A. platys*, *E. canis*, *Bartonella vinsonii berkhoffii*, *B. canis vogeli*, and *H. canis*, but not *Rickettsia* (Georges et al., 2008; Yabsley et al., 2008). None of these studies reported indices of tick infestation.

We conducted this study to evaluate the presence and impact of tick-borne pathogens of dogs on St. Kitts. Dogs suspected of having tick-borne diseases (TBDs) and presumably healthy animals that were presented for elective surgery were enrolled; clinical data, tick infestation data, PCR testing (for *Anaplasma*, *Babesia*, *Ehrlichia*, *Hepatozoon*, *Rickettsia*, and *Theileria*), and testing for antibodies against *Anaplasma* and *Ehrlichia* were included.

2. Materials and methods

2.1. Animal enrollment

All work was performed in accordance with an approved Institutional Animal Care and Use Committee protocol. Dogs were seen between December 2009 and November 2011 and were enrolled upon presentation for veterinary care. Cases of suspected TBD were identified by clinicians at the Ross University School of Veterinary Medicine. Cases were defined as dogs that presented with unexplained weight loss, lethargy, fever, or anemia, and with a history of tick exposure. Diagnostic procedures typically included physical examination, complete blood count (CBC; VetScan HM5, Abaxis, Union City, CA), and serum biochemistry panel (VetScan VS2, Abaxis). Controls were dogs presented for routine surgery that were believed to be healthy. Pre-surgical screening included a physical examination, CBC, and pre-anesthetic biochemistry panel. We enrolled every dog in this category from which remnant whole blood from pre-surgical screening was available.

Clinical records for each dog were reviewed, and the following data were recorded: sex, age, reproductive status, rectal temperature, presence of lymphadenopathy, presence of ticks, history of tick control, CBC constituents (hematocrit and absolute counts of red blood cells, platelets, and leukocytes), serum biochemistry results (alkaline phosphatase, ALP; alanine aminotransferase, ALT; total protein; and globulin), and serologic test results.

2.2. Tick collection and control

Ticks were collected into vials containing 70% ethanol and identified using published keys (Elbl and Anastos, 1966; Pegram et al., 1987). Medical records were reviewed to identify the methods used for tick control, including the type of product and consistency of use, as well as purchase history of tick control products. Clients reporting rare or intermittent use of product were categorized as having no tick control program.

2.3. Blood samples

Following clinical testing, remnants of EDTA whole blood were obtained for each dog. A 200 μL aliquot was reserved for DNA extraction, and remaining whole blood was centrifuged at 1500 rpm for 8 min. Plasma was harvested and transferred to a clean tube. Whole blood and plasma were stored at −20 °C, pending testing.

DNA was extracted from each 200 μL aliquot of frozen whole blood using a DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA), according to the manufacturer's directions, with the following modification: DNA was eluted in 100 μL of buffer using a 3-min incubation on the column. DNA samples were incubated at 65 °C for 15 min to ensure inactivation of nucleases and stored at −20 °C until tested.

2.4. PCR screening

The 18S rRNA gene of *Babesia* and *Theileria* was detected using nested PCR (Jefferies et al., 2007). Positive samples were subjected to restriction fragment length polymorphism analysis using *Hinf*I (New England Biolabs, Ipswich, MA), confirmed by repeat amplification, and sequenced. The 18S rRNA gene of *Hepatozoon* was detected using a conventional PCR assay (Otranto et al., 2011); positive samples were sequenced. Real-time PCR assays were used to screen for Anaplasmataceae and *Rickettsia* (Loftis et al., 2006). Samples testing positive for Anaplasmataceae were further screened using a probe-based assay for *E. canis* (Baneth et al., 2009), and all samples testing negative for *E. canis* were selected for re-amplification, using nested PCR primers for the 16S rRNA gene, and sequencing.

PCR products selected for sequencing were purified using a QIAquick PCR Purification kit (Qiagen) and submitted for DNA sequencing at a commercial laboratory (Davis Sequencing, Davis, CA). Sequences were aligned using BioEdit software and species were identified by comparison, using BLAST, with published sequences. GenBank accession numbers for sequences from this study are: JX112780–JX112785.

2.5. Serologic testing

Sera or plasma samples were screened using rapid benchtop lateral flow assays (SNAP® 3Dx®/4Dx® test kits, IDEXX, Westbrook, ME). Two samples were screened using the SNAP® 3Dx® test kit (1 sample from a suspect TBD case and 1 from a healthy dog), and all other samples were screened with the SNAP® 4Dx® kit. The 4Dx® test screens for *Dirofilaria immitis* antigen, antibodies against *Borrelia burgdorferi*, antibodies against *E. canis*/*E. ewingii*, and antibodies against *A. phagocytophilum*/*A. platys* (IDEXX, package insert). The 3Dx® kit omits the assay for *Anaplasma* antibodies.

Table 1
Comparison of dogs suspected to have tick-borne disease with healthy dogs presented for elective surgery.

	Suspected tick-borne disease	Healthy	Probability[a]
Number of dogs	**55**	**110**	
Age: mean ± SD	2.54 ± 2.53 yrs	1.93 ± 2.17 yrs	0.114
Female (%)	28 (50.1%)	94 (85.5%)	$\mathbf{1.9 \times 10^{-6}}$
Intact (%)	44 (80.0%)	96 (87.3%)	0.219
Temperature: mean ± SD	101.9 ± 2.1 °F	101.7 ± 0.9 °F	0.324
Tick infested (%)	24 (43.6%)	36 (32.7%)	0.170
Lymphadenopathy (%)	23 (41.8%)	30 (27.3%)	0.059
Number of dogs with CBC data	**53**	**110**	
Anemia only (%)	5 (9.4%)	11 (10.0%)	0.909
Thrombocytopenia only (%)	10 (18.9%)	26 (23.6%)	0.492
Thrombocytopenia + anemia (%)	30 (56.6%)	9 (8.2%)	$\mathbf{1.1 \times 10^{-11}}$
Leukocytosis (%)	10 (18.9%)	15 (13.6%)	0.385
Leukopenia (%)	6 (11.3%)	3 (2.7%)	**0.024**
Number of dogs with PCR testing	**55**	**110**	
Single infections			
Anaplasma platys	0 (0%)	4 (3.6%)	0.152
Babesia canis vogeli	6 (10.9%)	6 (5.5%)	0.203
Babesia gibsoni	8 (14.5%)	5 (4.5%)	**0.025**
Ehrlichia canis	11 (23.6%)	8 (7.2%)	**0.003**
Hepatozoon canis	1 (1.8%)	2 (1.8%)	1.00
Co-infections			
B. canis vogeli + *B. gibsoni*	0 (0%)	1 (0.9%)	0.478
E. canis + *Babesia* spp.	6 (10.9%)	0 (0%)	$\mathbf{4.2 \times 10^{-4}}$
E. canis + *H canis*	2 (3.6%)	0 (0%)	**0.044**
None detected	21 (38.2%)	84 (76.4%)	$\mathbf{1.5 \times 10^{-6}}$
Number of dogs with 4DX SNAP testing	**54**	**109**	
Anaplasma only	1 (1.8%)	9 (8.3%)	0.109
Ehrlichia canis only	13 (24.1%)	10 (9.2%)	**0.010**
Anaplasma + *Ehrlichia canis*	13 (24.1%)	12 (11.0%)	**0.029**
Neither	27 (50.0%)	78 (71.6%)	**0.007**

[a] Probabilities calculated using chi-squared testing, with the exception of age (*T*-test; two-sample with equal variance). Significant values are shown in bold type.

2.6. Statistical analysis

Pearson's chi-squared tests were used to compare categorical data between groups. Student's *T* tests were used to compare age, blood cell concentrations, or biochemical data between groups.

3. Results

3.1. Dogs

Fifty-five suspected cases of TBD, and 110 dogs presented for routine surgery were included in this study; in the latter case, the dogs were believed to be healthy at the time of admission. The distribution of age was similar for suspect TBD cases and for healthy animals. A higher proportion of the healthy dogs were female, due to the number of routine ovariohysterectomies (Table 1).

On physical examination, the majority of the dogs were afebrile, with similar rectal temperatures in suspect TBD cases and dogs presented for elective surgeries (Table 1). Lymphadenomegaly of one or more lymph nodes was observed in 38% of the dogs in this study, with no difference between the two groups of dogs. Lymphadenomegaly affecting at least three pairs of peripheral lymph nodes was seen in 27% of TBD suspects and 13% of healthy dogs ($p = 0.022$) but was not associated with any particular pathogen. Thrombocytopenia and anemia were more frequent in clinically ill dogs. Leukopenia was also more frequent in TBD suspects (Table 1), typically due to a stress leukogram with lymphopenia and low to low-normal neutrophil concentrations.

3.2. Tick-associated pathogens

Using sensitive PCR assays and sequence confirmation, *A. platys*, *B. canis vogeli*, *B. gibsoni*, *E. canis*, and *H. canis* were detected in dogs. *Rickettsia* was not detected in any dogs in this study. Overall, pathogen DNA was detected in 36% of the dogs in this study; 62% of the suspected TBD cases and 24% of controls had PCR evidence of one or more pathogens ($p < 0.001$; Table 1). Co-infections with more than one agent were documented in 9 dogs; this was within the range of what would be expected by chance. When antibody tests for *Anaplasma* and *Ehrlichia* were included, an additional 7 TBD suspect cases (13%) and 23 healthy dogs (20%) had evidence of exposure to one or both agents. No association between dog age or gender and pathogen status was found. There was no association between ALP or ALT activity between TBD suspects and healthy dogs, or between dogs with and without evidence of tick-associated pathogens (data not shown). Due to the high prevalence of pathogens in dogs believed to be healthy, both groups of dogs were combined when analyzing clinical data to identify signs associated with each pathogen (Tables 2 and 3).

Table 2
Hematologic findings in all dogs with PCR evidence of tick-associated pathogens, compared to dogs with no detectable tick-borne agents.

	# of Dogs	Hematocrit (%)	Platelets ($\times 10^3/\mu L$)	WBC ($\times 10^3/\mu L$)
No pathogen DNA	104*	41.1 ± 7.8 a	269 ± 157 a	13.9 ± 6.2 a
Anaplasma platys DNA	4	41.6 ± 8.2 a,b	110 ± 65 b,c	12.3 ± 2.9 a
Babesia canis vogeli DNA	12	35.0 ± 6.8 b	287 ± 290 a,b	14.0 ± 11.1 a
Babesia gibsoni DNA	13	25.9 ± 13.2 c,d	135 ± 154 b,c	12.7 ± 4.8 a
Ehrlichia canis DNA	18*	33.3 ± 9.6 b,c	95 ± 106 c	11.4 ± 5.2 a
Hepatozoon canis DNA	3	46.0 ± 4.6 a	209 ± 103 a,b	11.9 ± 2.9 a
B. canis vogeli + *B. gibsoni*	1	48.9**	240**	11.7**
E. canis + *Babesia* DNA	6	19.6 ± 6.8 d	69 ± 79 c	9.4 ± 5.0 a
E. canis + *H. canis* DNA	2	24.4 ± 2.0 c,d	58 ± 1 b,c	12.1 ± 2.3 a

Letters (a, b, c, d) indicate statistical significance; values with the same letter are not statistically different ($p > 0.05$).
* CBC data were not available for one dog with no detectable pathogens and one with DNA from *E. canis*.
** Sample size insufficient for statistical analysis.

E. canis DNA was detected in the blood of 27 dogs, including 19 dogs with *E. canis* alone and 8 that were co-infected with either *H. canis* ($n = 2$) or *Babesia* (4 with *B. canis vogeli*, 1 with *B. gibsoni*, and 1 with both *Babesia* spp.). *Ehrlichia* organisms were seen in three (11%) blood films, two from dogs with *E. canis* and one from a dog co-infected with *E. canis* and *B. canis vogeli*. All three of these dogs were clinically ill, suspect TBD cases. Dogs with either PCR or serologic evidence of *E. canis* were more likely to be identified as possible TBD cases than as healthy (Table 1), although several infected dogs were believed to be healthy upon presentation. In contrast, all eight of the co-infected dogs were identified as suspect TBD cases. PCR positivity for *E. canis* was associated with anemia, thrombocytopenia, and slightly reduced total leukocyte counts, with no discernable pattern among specific leukocytes (Table 2).

In addition to the six co-infected dogs with *Ehrlichia* and *Babesia*, dogs infected solely with *B. canis vogeli* ($n = 12$) or *B. gibsoni* ($n = 13$) were identified. Piroplasms were seen on the blood films of one dog with *B. canis vogeli* (8%), two dogs with *B. gibsoni* (15%), and one dog co-infected with *E. canis* and *B. gibsoni*; all four of these dogs were clinically ill, suspect TBD cases. Overall, dogs with *B. canis vogeli* were equally likely to be identified as suspect TBD cases or healthy, whereas *B. gibsoni* was more frequent in the TBD suspects (Table 1). Both species of *Babesia* were associated with anemia, and *B. gibsoni* was also associated with thrombocytopenia (Table 2). Among all dog with PCR confirmed infections, hematocrits were lowest in dogs co-infected with *E. canis* and *Babesia* spp. and in dogs with *B. gibsoni* (Table 2). Infection with *B. gibsoni* was also associated with an increase in total protein or globulins (36% of PCR-positive dogs, $p = 0.016$).

Four dogs in the study (2%) had PCR evidence of *A. platys* infection. All four dogs were believed to be healthy on presentation, were normothermic, and had thrombocytopenia; three also had antibodies against *Anaplasma*. Organisms were not visible on these blood films.

Hepatozoon canis was detected in 5 dogs (3%), three dogs infected only with *H. canis* and the two dogs co-infected with *E. canis* (Table 1). No CBC or biochemical abnormalities were associated with this pathogen (Table 2). Organisms were seen in the blood films from both co-infected dogs but not on blood films from dogs with only *H. canis*.

3.3. Serologic testing

A rapid lateral flow assay was used to screen dogs for heartworm antigen and antibodies against *Anaplasma*, *Ehrlichia*, and *B. burgdorferi*.

Antibodies against *Ehrlichia* were detected in 49 dogs, 29% of which were PCR-positive for *E. canis* alone, 8% were co-infected with *E. canis* and *Babesia* spp., 4% were co-infected with *E. canis* and *H. canis*, and 12% were PCR-positive only for *Babesia* spp. or *H. canis*. Of the 106 *Ehrlichia* antibody-negative dogs, 5% were PCR-positive for *E. canis* alone, 2% were co-infected with *E. canis* and *B. canis vogeli*, and 23% were infected with other pathogens. Overall, the positive predictive value of *E. canis* antibodies for ehrlichemia (PCR positivity for *E. canis*) was 0.41 (95% CI 0.27–0.55), and the negative predictive value was 0.93 (95% CI 0.89–0.98). Antibodies against *Ehrlichia* were not statistically associated with hematologic abnormalities (Table 3) or with clinical signs of illness. However, an increase in total protein or globulins was observed in 36% of dogs with antibodies against *Ehrlichia* ($p < 0.001$).

Antibodies against *Anaplasma* were equally prevalent in suspect TBD cases and controls (Table 1). Antibodies were detected in 35 dogs; *A. platys* was found, using PCR, in only 3 of these (9%). Antibodies against *Anaplasma* were associated with reduced platelet counts, with 28 (80%)

Table 3
Hematologic findings among all PCR-negative dogs, according to serologic evidence of exposure to *Anaplasma* or *Ehrlichia*.

	# of dogs	Hematocrit (%)	Platelets ($\times 10^3/\mu L$)	WBC ($\times 10^3/\mu L$)
No antibodies	75	41.0 ± 8.1 a	299 ± 162 a	14.2 ± 6.4 a
Anaplasma antibodies	7	44.7 ± 5.4 a	158 ± 93 b	13.7 ± 4.1 a
Ehrlichia antibodies*	13	41.7 ± 7.0 a	228 ± 130 a	12.6 ± 7.1 a
Anaplasma + *Ehrlichia*	9	38.1 ± 7.5 a	163 ± 77 b	12.3 ± 3.6 a

Letters (a, b) indicate statistical significance; values with the same letter are not statistically different.
* CBC data were not available for one dog with *E. canis* antibodies.

Table 4
Presence of tick infestation at the time of examination for dogs treated with different tick control products.

Tick control product	# of dogs with product	Number (%) infested
None	76	36 (47.4%) a
OTC shampoos and powders	8	4 (50.0%) a
Flumethrin topical	7	4 (57.1%) a
Fipronil spray	8	4 (50.0%) a
Fipronil top-spot	20	2 (10.0%) b
Selamectin top-spot	9	1 (11.1%) b
Amitraz collar	12	1 (8.3%) b
Fipronil top-spot + Amitraz collar	10	1 (10.0%) b

Letters (a, b) indicate statistical significance; values with the same letter are not statistically different.

of antibody-positive animals exhibiting thrombocytopenia ($p = 0.006$). When analysis was restricted to PCR-negative dogs, this association remained significant (Table 3).

Local transmission of canine heartworm does occur on St. Kitts, but only two dogs in this study tested positive for heartworm antigen. Both dogs were also positive using a modified Knott's test, but neither had clinical signs specific to heartworm infestation. One dog was infected with *Babesia canis vogeli*, with mild thrombocytopenia, and the other was PCR-negative for all pathogens, had antibodies against both *Anaplasma* and *E. canis*, and was both thrombocytopenic and anemic.

3.4. Tick infestation

Ticks were observed on 36% (60/165) of the dogs. A total of 268 ticks were submitted for identification from 43 of these dogs; identified ticks were exclusively *R. sanguineus* and consisted of 82 females, 94 males, 88 nymphs, and 4 larvae. The majority of these ticks were visibly engorged with blood. Ticks were submitted throughout the year, and adult ticks were seen in every month. Tick infestation prevalence at presentation was similar between dogs suspected of having TBD and healthy dogs (Table 1) and was not associated with pathogen status by PCR (data not shown). The tick control products are summarized in Table 4; of these products, top-spot preparations of fipronil or selamectin or amitraz collars were associated with reduced tick infestation.

4. Discussion

Prior to this work, *E. canis* was the only known canine tick-borne pathogen on St. Kitts; however, thrombocytopenia in dogs was a problem of unknown etiology (Kelly and Lucas, 2009). This work expands on prior work and documents *A. platys*, *B. canis vogeli*, *B. gibsoni*, and *H. canis* from St. Kitts. *Rickettsia* species were not detected using PCR. With the exception of *B. gibsoni*, these same agents were recently reported from dogs on Grenada, also in the eastern Caribbean (Yabsley et al., 2008). *Babesia gibsoni* has never been reported from the Caribbean but is enzootic in parts of the United States; this pathogen may have been introduced to St. Kitts by the importation of infected dogs. However, several of the dogs testing positive for *B. gibsoni* were born on St. Kitts and had never traveled, indicating that, regardless of origin, *B. gibsoni* is now established on the island.

In general, our clinical findings in PCR-positive animals corresponded with expected effects of each pathogen: *A. platys* was associated with thrombocytopenia; *E. canis* with reduced leukocytes, thrombocytopenia, and anemia; and *B. gibsoni* with thrombocytopenia and anemia (Otranto et al., 2011; Unver et al., 2001). The most severe infections, associated with significant thrombocytopenia, anemia, and consistent identification of animals as suspect TBD cases, were found in dogs co-infected with *E. canis* and *Babesia* species. In spite of thrombocytopenia, overt illness was not seen in any cases of *A. platys*, and several dogs infected with *E. canis*, *B. canis vogeli*, and *B. gibsoni* were also believed to be healthy prior to testing. Among dogs with only *E. canis*, thrombocytopenia and anemia were often marked and severe, but changes in leukograms were mild, without consistent changes in any specific leukocyte population, and increases in liver enzymes (ALP or ALT) were uncommon. However, increases in either total protein or globulins were observed in a third of dogs with *E. canis* antibodies, suggesting chronic immune stimulation; this may explain the low prevalence of these indicators of acute disease in PCR-positive dogs. Our inability to detect consistent clinical characteristics of *B. canis vogeli* and *H. canis* infections may similarly reflect the presence of chronic, subclinical or mild infections (O'Dwyer, 2011; Otranto et al., 2011). The high prevalence of chronic infections and the similarity in presentation between these infections creates a challenge for the clinical diagnosis of dogs. Disease agents were rarely visible on blood films, noted only in 9/34 of the acutely ill animals with PCR evidence of infection and in none of the healthy-appearing animals. PCR was required for sensitive detection of the organisms.

Rapid antibody tests were also evaluated for their use as a clinical indicator of infection. Although the test can detect antibodies against *E. canis/E. ewingii* or *A. phagocytophilum/A. platys*, neither *A. phagocytophilum* or its vector (*Ixodes* spp.), nor *E. ewingii* or its vector (*Amblyomma americanum*), have been found on St. Kitts. This allows us to use the test specifically for antibodies against *A. platys* or *E. canis*. However, antibodies against *E. canis* were poorly predictive of PCR positivity, illness, or hematologic abnormalities beyond the hyperglobulinemia discussed above. This suggests that antibody positive dogs included a mixture of chronically infected animals and dogs who had recovered from *E. canis* infection. Among dogs with antibodies against *A. platys*, 80% were thrombocytopenic, whereas only 9% were PCR-positive. PCR detection in chronically infected dogs with thrombocytopenia might lack sensitivity, since the host cells for the pathogen are found at low concentrations. This suggests that rapid antibody testing can assist with diagnosis of chronic *A. platys* infections but has poor predictive value for *E. canis*; in either case, testing for *Babesia* and *Hepatozoon* species is also needed. This limits the utility of rapid antibody testing to guide clinical treatment.

Consistent with the detection of several pathogens transmitted by *R. sanguineus*, we documented extensive, year-round, infestation of dogs with *R. sanguineus*. Because most of these ticks also contained canine blood from the

host animal, the ticks were not tested for pathogens by PCR. Significant differences were seen between the efficacy of different tick-control products. Of particular interest is the poor efficacy of topical flumethrin and the spray formulation of fipronil; two possible explanations are improper use by dog owners, although medical records were reviewed in an attempt to identify this, or acaricide resistance. Further work needs to be done to test this possibility.

Overall, we documented the presence of several previously unreported tick-borne pathogens on St. Kitts. With the exception of *B. gibsoni*, these pathogens were also detected in Grenada and probably infect dogs throughout the entire Lesser Antilles. Testing using PCR was shown to be the most accurate means of diagnosis, but, at present, this testing is not readily available in the Caribbean. Veterinary clinicians in this region are faced with both diagnostic and treatment challenges, including: the lack of effective tick control, poor predictive value of antibody-based tests, limited access to PCR-based diagnostic testing, and the lack of effective treatments for piroplasms in dogs. These factors all contribute to the high prevalence of TBD in this region.

Disclosure

No competing financial interests exist.

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