DOI: 10.1515/ap-2015-0003

Acta Parasitologica, 2015, 60(1), 21–25; ISSN 1230-2821

VERSITA

# Molecular detection of *Dirofilaria immitis*, *Hepatozoon canis*, *Babesia* spp., *Anaplasma platys*, and *Ehrlichia canis* in dogs on Costa Rica

**Lanjing Wei[1], Patrick Kelly[2], Kate Ackerson[2], Heba S. El-Mahallawy[1, 3], Bernhard Kaltenboeck[4] and Chengming Wang[1]***

[1]Jiangsu Co-Innovation Center for the Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou University College of Veterinary Medicine, Yangzhou, Jiangsu, P.R. China; [2]Ross University School of Veterinary Medicine, Basseterre, St.Kitts, West Indies; [3]Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt; [4]Auburn University School of Veterinary Medicine, Auburn, AL, USA

## Abstract

Although vector-borne diseases are important causes of morbidity and mortality in dogs in tropical areas, there is little information on these conditions in Costa Rica. In PCRs of blood from dogs in Costa Rica, we did not detect DNAs of *Rickettsia* (*R.*) *felis* and *Coxiella* (*C.*) *burnetii* but we did find evidence of infection with *Dirofilaria* (*D.*) *immitis* (9/40, 22.5%), *Hepatozoon* (*H.*) *canis* (15/40, 37.5%), *Babesia* spp. (10/40, 25%; 2 with *B. gibsoni* and 8 with *B. vogeli*), *Anaplasma* (*A.*) *platys* (3/40, 7.5%) and *Ehrlichia* (*E.*) *canis* (20/40, 50%). Nine dogs (22.5%) were free of any vector-borne pathogens while 14 (35%) were infected with a single pathogen, 11 (27.5%) with two, 4 (10%) with three, 1 (2.5%) with four, and 1 (2.5%) with five pathogens. Dogs in Costa Rica are commonly infected with vector-borne agents.

## Keywords

Costa Rica, *Dirofilaria immitis*, *Babesia gibsoni*/*vogeli*, *Anaplasma platys*, *Ehrlichia canis*, *Hepatozoon canis*

## Introduction

Canine vector-borne diseases are an important cause of morbidity and mortality in dogs worldwide and many are zoonoses. There is very limited data on vector-borne diseases in dogs from the seven Central American states with reports from only four countries, namely Costa Rica (Romero *et al*. 2011; Scorza *et al*. 2011; Rojas *et al*. 2014), Panama (Bermúdez *et al*. 2011; Pineda *et al*. 2011; Herrer and Christensen 1976) and Guatemala (Ryan *et al*. 2003) and Nicaragua (Wei *et al*. 2014). To provide further information we used PCR to investigate the prevalence of seven vector-borne agents in dogs from Costa Rica.

## Materials and Methods

A convenience sample of whole bloods was collected in EDTA from 40 dogs which were neutered in a Volunteers for Intercultural and Definitive Adventure (VIDA) Clinic in the village of Nueva Esperanaza outside Bagaces in Northwestern Costa Rica in 2012. The dogs were 6 months to 3 years of age (average 9 months) and belonged to local people from underprivileged areas (Table 1). The study was approved by the Institutional Animal Care and Use Committee of the Yangzhou University College of Veterinary Medicine of China.

The whole blood samples were stored and transported to the laboratory at 4°C where aliquots (200 µL) were frozen at -80°C until DNA was extracted using the High-Pure PCR Template Preparation Kit (Roche Molecular Biochemicals, Indianapolis, IN, USA) as described before (Zhang *et al*. 2013). The extracted DNA was eluted in 200 µl 1× $T_{10}E_{0.1}$ elution buffer and used in eight qPCRs, seven for vector-borne pathogens and one for the canine HMBS gene as an endogenous internal control. The qPCRs were performed in a 20 µl reaction system of a Roche LightCycler 480-II PCR as described previously: *Anaplasma* spp. 16S rRNA (Kelly *et al*. 2013), *Babesia* spp. 18S rRNA (Wang *et al*. 2010), *C. burnetii IS-1111* (Berri *et al*. 2009), *Dirofiliria immitis* (Wei *et al*. 2014), *E. canis* 16S rRNA (Kelly *et al*. 2013), *Hepatazoon* spp. 18S rRNA (Li *et al*. 2008), *R. felis gltA*

*Corresponding author: wangcm@yzu.edu.cn

**Table I**. Prevalence of tick-borne agents in dogs determined by quantitative PCRs

| Gender | Age (years) | Weight (lbs) | Tick-borne pathogens* | | | | |
|---|---|---|---|---|---|---|---|
| | | | ***Hepatazoon*** | ***Anaplasma*** | ***Babesia*** | ***Ehrlichia*** | ***Dirofilaria*** |
| M | 0.5 | 5 | – | – | *B. gibsoni* | *E. canis* | – |
| F | 1.0 | 10 | – | – | – | *E. canis* | – |
| F | 0.5 | 12 | – | – | – | – | – |
| F | 0.5 | 7 | – | *A. platys* | – | *E. canis* | – |
| M | 6.0 | 7 | – | – | *B. gibsoni* | – | – |
| F | 0.75 | 4 | – | – | – | *E. canis* | – |
| M | 1 | 7 | – | – | – | *E. canis* | – |
| M | 2.5 | 22 | – | – | – | *E. canis* | – |
| F | 3.0 | 7 | – | – | – | *E. canis* | *D. immitis* |
| M | 6 | 7 | – | – | – | *E. canis* | *D. immitis* |
| F | 0.5 | 7 | – | – | – | – | – |
| F | 0.5 | 12 | – | – | – | – | – |
| F | 0.75 | 10 | – | – | – | – | – |
| M | 1.0 | 25 | – | – | – | – | – |
| F | 0.83 | 5 | *H. canis* | *A. platys* | – | – | – |
| F | 2.0 | 10 | *H. canis* | – | *B. vogeli* | – | – |
| F | 4.0 | 12 | *H. canis* | – | *B. vogeli* | – | – |
| F | 1.0 | 14 | *H. canis* | – | *B. vogeli* | *E. canis* | *D. immitis* |
| F | 4.0 | 12 | – | – | – | *E. canis* | – |
| M | 2.0 | 18 | *H. canis* | – | – | – | *D. immitis* |
| F | 2.0 | 5 | *H. canis* | – | – | *E. canis* | *D. immitis* |
| F | 1.0 | 12 | – | – | – | – | – |
| M | 1.5 | 16 | – | – | – | – | *D. immitis* |
| F | 0.83 | 6 | – | – | – | *E. canis* | – |
| F | 1.0 | 11 | – | – | – | *E. canis* | – |
| F | 0.5 | 5 | *H. canis* | – | – | – | – |
| F | 0.5 | 18 | *H. canis* | – | – | – | – |
| M | 2.0 | 8 | *H. canis* | *A. platys* | *B. vogeli* | *E. canis* | *D. immitis* |
| F | 0.58 | 13 | *H. canis* | – | *B. vogeli* | – | – |
| F | 2.0 | 3 | *H. canis* | – | *B. vogeli* | – | – |
| M | 5.0 | 9 | *H. canis* | – | *B. vogeli* | *E. canis* | – |
| F | 2.0 | 16 | *H. canis* | – | – | *E. canis* | *D. immitis* |
| F | 0.75 | 15 | *H. canis* | – | *B. vogeli* | *E. canis* | – |
| M | 3.0 | 11 | *H. canis* | – | – | *E. canis* | – |
| M | 1.0 | 10 | – | – | – | *E. canis* | – |
| F | 0.75 | 10 | – | – | – | – | – |
| F | 1.0 | 6 | – | – | – | *E. canis* | – |
| F | 1.0 | 14 | – | – | – | – | – |
| F | 2.0 | 6 | – | – | – | – | – |
| F | 0.5 | 9 | – | – | – | – | *D. immitis* |

**R. felis* and *C. burnetii* were not detected in the whole blood samples of any those dogs. "-" denotes the absence of bacterial DNA in the whole blood of the designated dog

(Hii *et al*. 2013) and the canine HMBS gene (Wang *et al*. 2012). The melting curve analysis for probes annealing to the PCR products was determined by monitoring the fluorescence from 45°C to 80°C following the completion of PCRs, and the first derivatives of F4/F1 were evaluated to determine the probe melting temperature ($T_m$). The PCR

products were verified by gel electrophoresis and nucleotide sequencing using forward and antisense primers (GenScript, Nanjing, China) was used to confirm the identity of the organism detected.

## Results and Discussion

The PCR for the HMBS gene was positive on all samples indicating successful extraction of amplifiable DNA. Our PCRs for pathogens were positive for 5 of the 7 organisms tested, namely *E. canis* (20/40, 50%), *H. canis* (15/40, 37.5%), *Babesia* spp. (10/40, 25%; including 2 dogs with *B. gibsoni* and 8 with *B. vogeli*), *D. immitis* (9/40, 22.5%) and *A. platys* (3/40, 7.5%). *R. felis* and *C. burnetii* were not detected. Altogether, 77.5% (31/40) of the dogs were positive with 35% having evidence of infection with a single agent, 27.5% with two, 10% with three, 2.5% with four, and 2.5% with five agents (Table I).

In a recent PCR study of dogs from the central, northwestern and eastern areas of Costa Rica, a high percentage of dogs (47%; 69/146) was also found positive for vector-borne agents, mainly *E. canis* (34%), *A. platys* (10%), *B. vogeli* (8%) and *H. canis* (8%). Infections with *E. canis* have further been shown to be very prevalent in another study in Costa Rica (47%; 148/310; Romero *et al*. 2011). The vector of *E. canis* is *Rhipicephalus* (*R.*) *sanguineus* which occurs worldwide (Dantas-Torres, 2008) and it is likely, then, that infections with *E. canis* are widespread in Central America. Veterinarians in the region should have a high index of suspicion of infections in their patients (Kelly *et al*. 2013; Shaw *et al*. 2001) and, as human cases of infection with *E. canis* are known (Perez *et al*. 2006), human health workers should be alerted to the possibility of infections in patients with contact with dogs and their ticks.

*R. sanguineus* is also the vector of *B. vogeli* (Solano-Gallego and Baneth 2011), *H. canis* (Baneth *et al*. 2007) and *A. platys* (Alleman *et al*. 2008) and, in the only other report on these organisms in Central America, they were found to be prevalent in Costa Rica with 8%, 7.5% and 10% of dogs positive, respectively (Rojas *et al*. 2014). As with *E. canis* the widespread distribution of their vector makes it likely that infections with these organisms are also common in dogs throughout Central America. Although these organisms generally cause few clinical signs (Alleman *et al*. 2008; Baneth 2011; Solano-Gallego and Baneth 2011) and do not appear to be a major threat to the health of dogs in the region, it should be noted that we and Rojas *et al*. (2014) both found mixed infections were common and it is possible the effects of one agent may exacerbate (Brown *et al*. 2006; Kelly *et al*. 2013; Rojas *et al*. 2014) or ameliorate (Matthewman *et al*. 1993) the effects of another. The organisms also appear not to be important zoonotic agents with no reports of *B. vogeli* or *H. canis* infections in people (Esch and Petersen 2013) and only one report of *A. platys* in a person (Maggi *et al*. 2013).

The PCR we designed for *D. immitis* was found to be reliable, consistently giving positive results for the positive control, and also sensitive, detecting down to one copy of the standard in a PCR reaction. When used in the study the PCR showed nine dogs (9/40, 22.5%) had evidence of DNA of *D. immitis*, the agent of mosquito transmitted canine heartworm disease which is an important disease of dogs worldwide (McCall *et al*. 2008; Cuervo *et al*. 2013). In the only other report on *D. immitis* in Central America, 2% of dogs studied in the center of the western region of Costa Rica were seropositive (Scorza *et al*. 2011). The far higher prevalence in our PCR study might be because our dogs were from underprivileged areas where heartworm preventatives are unaffordable. The current data indicates dogs in Costa Rica, and likely the region, are at risk of infection with *D. immitis* and animal health workers should be recommending the use of heartworm preventatives where possible. Human health workers should also be aware that *D. immitis* can infect people (Simon *et al*. 2005).

Melting point analysis of our *Babesia* positive PCR reactions and genomic sequencing of the PCR products showed that two of the dogs had DNA of *B. gibsoni* ($T_m$ ~67 °C) and 8 dogs had *B. vogeli* ($T_m$ ~60 °C) (Wang *et al*. 2010). This organism is found in northern Africa, southern Asia, Australia, Europe, the USA and the Caribbean (Kelly *et al*. 2013). *Babesia gibsoni* is thought to be transmitted by dogs fighting or by ticks, in particular *R. sanguineus* (Taboada and Lobetti 2006). There are no reported human infections (Esch and Petersen 2013) but in dogs infections usually result in acute signs including fever, pallor, splenomegaly and anorexia (Ayoob *et al*. 2010). Dogs that recover generally become chronic subclinical carriers with significantly reduced platelet counts (Matsuu *et al*. 2004). Unfortunately diagnosis is not easy, generally requiring serology and PCR (Ayoob *et al*. 2010), and treatment of *B. gibsoni* sometimes fails (Iguchi *et al*. 2013).

Of the agents that were not detected in our study, *C. burnetii* is the agent of Q fever in people which occurs worldwide, apart from New Zealand (Cutler *et al*. 2007). Our negative findings for *C. burnetii* are consistent with there being no reports of *C. burnetii* in people or animals in Central America. We would note, however, that only few seropositive dogs are also PCR positive (1:25) and dogs have only been implicated in one outbreak of Q fever (Buhariwalla *et al*. 1996). Determining the true importance of *C. burnetii* in Central America requires studies of people and livestock which are the major reservoirs and sources of infection.

We also found no evidence of *R. felis*, an emerging pathogen principally associated with cat fleas (*Ctenocephalides* felis) and causing flea-borne spotted fever in people (Abdad *et al*. 2011). It occurs on all continents except Antarctica and recent evidence suggests dogs might be reservoir hosts with only subclinical infections (Hii *et al*. 2011). It is unexpected that we did not find the organism as

it occurs in cat fleas in Costa Rica (Troyo *et al*. 2012), and also in Guatemala and Panama (Bermudez *et al*. 2011; Hun *et al*. 2011). Further studies are indicated to characterize infections in dogs and their possible role as sentinels of human infections.

In conclusion, our study adds to the scant data on vector-borne diseases of dogs and vector-borne zoonoses in Central America. Further we provide the first evidence of *B. gibsoni* in Central America while confirming the presence of important canine vector-borne pathogens in the region. Finally, many of the pathogens can infect people and health workers need to be aware of the possibility of infections in their patients, particularly those that have contact with dogs and their parasites.

**Acknowledgments.** This project was supported by grants from the National Natural Science Foundation of China (NO. 31272575), the Ross University School of Veterinary Medicine, and by the Priority Academic Program Development of Jiangsu Higher Education Institutions. The authors sincerely appreciate Xiaojing Zhu, Yongpeng Zhang and Lei Jiang from Yangzhou University College of Veterinary Medicine for their technical help.

## References

Abdad M., Stenos J., Graves S. 2011. *Rickettsia felis*, an emerging flea-transmitted human pathogen. *Emerging Health Threats Journal*, 4, 7168. DOI: 10.3402/ehtj.v4i0.7168

Alleman A.R., Wamsley H.L. 2008. An update on anaplasmosis in dogs. *Veterinary Medicine*, 103, 212–220

Ayoob A.L., Hackner S.G., Prittie J. 2010. Clinical management of canine babesiosis. *Journal of Veterinary Emerging Critical Care* (San Antonio), 20, 77–89. DOI: 10.1111/j.1476-4431.2009.00489.x

Baneth G., Samish M., Shkap V. 2007. Life cycle of *Hepatozoon canis* (Apicomplexa: Adeleorina: Hepatozoidae) in the tick *Rhipicephalus sanguineus* and domestic dog (*Canis familiaris*). *Journal of Parasitology*, 93, 283–299. DOI: 10.1645/GE-494R.1

Baneth G. 2011. Perspectives on canine and feline hepatozoonosis. Veterinary Parasitology. 181, 3–11. DOI: 10.1016/j.vetpar.2011.04.015

Bermudez C.S., Zaldívar A.Y., Spolidorio M.G., Moraes-Filho J., Miranda R.J., Caballero C.M., Mendoza Y., Labruna M.B. 2011. Rickettsial infection in domestic mammals and their ectoparasites in El Valle de Antón, Coclé, Panamá. *Veterinary Parasitology*, 177, 134–138. DOI: 10.1016/j.vetpar.2010.11.020

Berri M., Rekiki A., Boumedine K.S., Rodolakis A. 2009. Simultaneous differential detection of *Chlamydophila abortus*, *Chlamydophila pecorum* and *Coxiella burnetii* from aborted ruminant's clinical samples using multiplex PCR. *BMC Microbiology*, 9, 130. DOI: 10.1186/1471-2180-9-130

Brown G.K., Canfield P.J., Dunstan R.H., Roberts T.K., Martin A.R., Brown C.S., Irving R. 2006. Detection of *Anaplasma platys* and *Babesia vogeli* and their impact on platelet numbers in free-roaming dogs associated with remote Aboriginal communities in Australia. *Australian Veterinary Journal*, 84, 321–325. DOI: 10.1111/j.1751-0813.2006.00029.x

Buhariwalla F., Cann B., Marrie T.J. 1996. A dog-related outbreak of Q fever. *Clinical Infectious Diseases*, 23, 753–755. DOI: 10.1093/clinids/23.4.753

Cuervo P.F., Mera Y., Sierra R., Waisman V., Gerbeno L., Sidoti L., Albonico F., Mariconti M., Mortarino M., Pepe P., Cringoli G., Genchi C., Rinaldi L. 2013. Detection of *Dirofilaria immitis* in mid-western arid Argentina.*Acta Parasitologica*, 58, 612–614. DOI: 10.2478/s11686-013-0177-z

Cutler S.J., Bouzid M., Cutler R.R. 2007. Q fever. *Journal of Infection*, 54, 313–318. DOI: 10.1016/j.jinf.2006.10.048

Dantas-Torres F. 2008. The brown dog tick, *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae): from taxonomy to control. *Veterinary Parasitology*, 152, 173–185. DOI: 10.1016/j.vetpar.2007.12.030

Esch K.J., Petersen C.A. 2013. Transmission and epidemiology of zoonotic protozoal diseases of companion animals. *Clinical Microbiology Reviews*, 26, 58–85. DOI: 10.1128/CMR.00067-12

Herrer A., Christensen H.A. 1976. Natural cutaneous leishmaniasis among dogs in Panama. *American Journal of Tropical Medicine and Hygiene*, 25, 59–63. DOI: 10.1590/S0074-02762011000800021

Hii S.F., Abdad M.Y., Kopp S.R., Stenos J., Rees R.L., Traub R.J. 2013. Seroprevalence and risk factors for *Rickettsia felis* exposure in dogs from Southeast Queensland and the Northern Territory, Australia. *Parasites & Vectors*, 6, 159. DOI: 10.1186/1756-3305-6-159

Hun L., Troyo A., Taylor L., Barbieri A.M., Labruna M.B. 2011. First report of the isolation and molecular characterization of *Rickettsia amblyommii* and *Rickettsia felis* in Central America. *Vector-Borne Zoonotic Diseases*, 11, 1395–1397. DOI: 10.1089/vbz.2011.0641

Iguchi A., Matsuu A., Fujii Y., Ikadai H., Hikasa Y. 2013. The in vitro interactions and in vivo efficacy of atovaquone and proguanil against *Babesia gibsoni* infection in dogs. *Veterinary Parasitology*. 197, 527–533. DOI: 10.1016/j.vetpar.2013.06.006

Kelly P.J., Xu C., Lucas H., Loftis A., Abete J., Zeoli F., Stevens A., Jaegersen K., Ackerson K., Gessner A., Kaltenboeck B., Wang C. 2013. Ehrlichiosis, babesiosis, anaplasmosis and hepatozoonosis in dogs from St. Kitts, West Indies. *PLoS One*, 8, e53450. DOI: 10.1371/journal.pone.0053450

Li Y., Wang C., Allen K.E., Little S.E., Ahluwalia S.K., Gao D., Macintire D.K., Blagburn B.L., Kaltenboeck B. 2008. Diagnosis of canine *Hepatozoon* spp. infection by quantitative PCR. *Veterinary Parasitology*, 157, 50–58. DOI: 10.1016/j.vetpar.2008.06.027

Maggi R.G., Mascarelli P.E., Havenga L.N., Naidoo V., Breitschwerdt E.B. 2013. Co-infection with *Anaplasma platys*, *Bartonella henselae* and *Candidatus Mycoplasma* haematoparvum in a veterinarian. *Parasites & Vectors*, 6, 103. DOI: 10.1186/1756-3305-6-103

Matsuu A., Kawabe A., Koshida Y., Ikadai H., Okano S., Higuchi S. 2004. Incidence of canine *Babesia gibsoni* infection and subclinical infection among Tosa dogs in Aomori Prefecture, Japan. *Journal of Veterinary Medical Science*, 66, 893–897. DOI: 10.1292/jvms.66.893

Matthewman L.A., Kelly P.J., Bobade P.A., Tagwira M., Mason P.R., Majok A., Brouqui P., Raoult D. 1993. Infections with *Babesia canis* and *Ehrlichia canis* in dogs in Zimbabwe. *Veterinary Record*, 133, 344–346. DOI: 10.1136/vr.133.14.344

McCall J.W., Genchi C., Kramer L.H., Guerrero J., Venco L. 2008 Heartworm disease in animals and humans. *Advances in Parasitology*, 66, 193–285. DOI: 10.1016/S0065-308X(08)00204-2

Perez M., Bodor M., Zhang C., Xiong Q., Rikihisa Y. 2006. Human infection with *Ehrlichia canis* accompanied by clinical signs in Venezuela. *Annals of the New York Academy of Sciences*, 1078, 110–117. DOI: 10.1196/annals.1374.016

Pineda V., Saldaña A., Monfante I., Santamaría A., Gottdenker N.L., Yabsley M.J., Rapoport G., Calzada J.E. 2011. Prevalence of

trypanosome infections in dogs from Chagas disease endemic regions in Panama, Central America. *Veterinary Parasitology*, 178, 360–363. DOI: 10.1016/j.vetpar.2010.12.043

Rojas A., Rojas D., Montenegro V., Gutiérrez R., Yasur-Landau D., Banet,h G. 2014. Vector-borne pathogens in dogs from Costa Rica: First molecular description of *Babesia vogeli* and *Hepatozoon canis* infections with a high prevalence of monocytic ehrlichiosis and the manifestations of co-infection. *Veterinary Parasitology*, 199, 121–128. DOI: 10.1016/j.vetpar.2013.10.027

Romero L.E., Meneses A.I., Salazar L., Jiménez M., Romero J.J., Aguiar D.M., Labruna M.B., Dolz G. 2011. First isolation and molecular char-acterization of *Ehrlichia canis* in Costa Rica, Central America. *Research in Veterinary Science*, 91, 95–97. DOI: 10.1016/j.rvsc.2010.07.021

Ryan P.R., Arana B.A., Ryan J.R., Wirtz R.A., Wortmann G.W., Rizzo N.R. 2003. The domestic dog, a potential reservoir for *Leishmania* in the Peten region of Guatemala. *Veterinary Parasitology*, 115, 1–7. DOI: 10.1016/S0304-4017(03)00158-4

Scorza A.V., Duncan C., Miles L., Lappin M.R. 2011. Prevalence of selected zoonotic and vector-borne agents in dogs and cats in Costa Rica. *Veterinary Parasitology*, 183, 178–183. DOI: 10.1016/j.vetpar.2011.06.025

Shaw S.E., Day M.J., Birtles R.J., Breitschwerdt E.B. 2001. Tick-borne infectious diseases of dogs. *Trends in Parasitology*, 17, 74–80. DOI: 10.1016/S1471-4922(00)01856-0

Simón F., López-Belmonte J., Marcos-Atxutegi C., Morchón R., Martín-Pacho J.R., What is happening outside North America regarding human dirofilariasis?*Veterinary Parasitology*, 133, 181–189. DOI: 10.1016/j.vetpar.2005.03.033

Solano-Gallego L., Baneth G. 2011. Babesiosis in dogs and cats-expanding parasitological and clinical spectra. *Veterinary Parasitology*, 181, 48–60. DOI: 10.1016/j.vetpar.2011.04.023

Taboada J., Lobetti R. 2006. Babesiosis. In: Greene CE, editor. Infectious Diseases of the Dog and Cat. Saunders Elsevier. Missouri. 722–736

Troyo A., Álvarez D., Taylor L., Abdalla G., Calderón-Arguedas Ó., Zambrano M.L., Dasch G.A., Lindblade K., Hun L., Eremeeva M.E., Estévez A. 2012. *Rickettsia felis* in *Ctenocephalides felis* from Guatemala and Costa Rica. *American Journal of Tropical Medicine and Hygiene*, 86, 1054–1056. DOI: 10.4269/ajtmh.2012.11-0742

Wang C., Ahluwalia S.K., Li Y., Gao D., Poudel A., Chowdhury E., Boudreaux M.K., Kaltenboeck B. 2010. Frequency and therapy monitoring of canine *Babesia* spp. infection by high-resolution melting curve quantitative FRET-PCR. *Veterinary Parasitology*, 168, 11–18. DOI: 10.1016/j.vetpar.2009.10.015

Wang C., Mount J., Butler J., Gao D., Jung E., Blagburn B.L., Kaltenboeck B. 2012. Real-time PCR of the mammalian hydroxymethylbilane synthase (HMBS) gene for analysis of flea (*Ctenocephalides felis*) feeding patterns on dogs. *Parasites & Vectors*, 5, 4. DOI: 10.1186/1756-3305-5-4

Wei L., Kelly P., Ackerson K., Zhang J., El-Mahallawy HS., Kaltenboeck B., Wang C. 2014. First report of *Babesia gibsoni* in Central America and survey for vector-borne infections in dogs from Nicaragua. *Parasites & Vectors*, 7, 126. DOI: 10.1186/1756-3305-7-126

Zhang J., Wei L., Kelly P., Freeman M., Jaegerson K., Gong J., Xu B., Pan Z., Xu C., Wang C. 2013. Detection of *Salmonella* spp. using a generic and differential FRET-PCR. *PLoS One*, 8, e76053. DOI: 10.1371/journal.pone.0076053

**Received**: April 7, 2014
**Revised**: July 14, 2014
**Accepted for publication**: July 25, 2014