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Infectious diseases in dogs rescued during dogfighting investigations

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ABSTRACT

Dogs used for dogfighting often receive minimal preventive health care, and the potential for spread of infectious diseases is high. The purpose of this study was to describe the prevalence of infectious diseases in dogs rescued from fighting operations to guide medical protocols for their immediate and long-term care. A total of 269 pit bull-type dogs were seized in a multi-state investigation. Fleas were present on most dogs, but few ticks were observed. Testing performed at intake included packed cell volume (PCV), serology and PCR for vector-borne pathogens, and fecal analysis.

The most common infections were *Babesia gibsoni* (39%), '*Candidatus* Mycoplasma haematoparvum' (32%), *Mycoplasma haemocanis* (30%), *Dirofilaria immitis* (12%), and *Ancylostoma* (23%). Anemia was associated with *B. gibsoni* infection (63% of infected dogs, odds ratio = 2.5, $P < 0.001$), but not with hemotropic mycoplasmas or *Ancylostoma*.

Pit bull heritage and dogfighting are known risk factors for *B. gibsoni* infection, possibly via blood transmission from bites and vertical transmission. Hemotropic mycoplasmas have a similar risk pattern. Empirical care for dogs from dogfighting cases should include broad-spectrum internal and external parasiticides and monitoring for anemia. Dogfighting case responders should be prepared for mass screening and treatment of *B. gibsoni* and heartworm infections and should implement protocols to prevent transmission of infectious and zoonotic diseases in the shelter and following adoption. Former fighting dogs and dogs with possible dog bite scars should not be used as blood donors due to the risk of vector-borne pathogens that can escape detection and for which curative treatment is difficult to document.

Introduction

Although dogfighting is a felony offense in all 50 states and under federal law, this cruel bloodsport is still entrenched in communities across the United States. Growing public awareness of these crimes has led to more frequent seizures of dogs by law enforcement agencies (Lockwood, 2011, 2013). These agencies often partner with humane organizations to provide care for seized dogs, which are considered legal evidence. Dogs may be housed for months in traditional or temporary animal shelters while the legal cases proceed (Lockwood, 2013).

Fighting dogs typically receive minimal preventive care and are kept chained outside in poor conditions. Multiple dogs are housed in each 'dog yard' and are exposed to other fighting dogs during breeding, training, and fights. The potential for spread of infectious diseases is high. Since dogfighting is an underground illegal activity, little is known about the infectious diseases carried by dogs from organized fighting rings.

Historically, dogs seized in animal fighting investigations were routinely euthanized due to the belief that their heritage and training made them unsafe. Recently, however, rescue organizations have begun assessing their health, behavior, and suitability for adoption in response to widespread rehoming interest. Rescue operations often transport dogs around the country for adoption. While this lifesaving trend is laudable, there is potential risk for sending dogs harboring infectious diseases to unsuspecting owners or to regions where the infections are not currently endemic. The purpose of the study reported here was to describe the prevalence of infectious diseases in dogs rescued from fighting operations. This information will support an evidence-based foundation of medical protocols for biosecurity, disease screening, treatment, and long-term follow-up care for this unique population.

Materials and methods

Animals

The study included 269 dogs seized from eight scenes in four states (Alabama, Georgia, Mississippi, and Texas) during a federal dogfighting investigation in August and September, 2013. The dogs were pit bull-type phenotypes, only one of which

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had cropped ears. Ages estimated based on dentition included neonates (eight dogs, <6 weeks), juveniles (65 dogs, 6 weeks to 5.9 months), and adults (196 dogs, ≥6 months). A total of 52% of the dogs were female and 48% were male. The dogs were triaged at the scenes, vaccinated with a SC commercial rabies vaccine, and transported to Florida. They were housed in a climate-controlled warehouse that served as a temporary animal shelter until the legal cases were completed. Fleas were present on most dogs, but few ticks were observed. Ticks were not collected for species identification. Upon intake to the temporary shelter, dogs received intranasal vaccines containing modified-live *Bordetella bronchiseptica*, parainfluenza, and adenovirus-2, subcutaneous vaccines containing modified-live distemper virus, adenovirus-2, parainfluenza, and parvovirus, and topical moxidectin/imidacloprid for internal and external parasitism. Adult dogs were housed individually in chain-link portable kennels. Puppies were housed with littermates and nursing dams were housed with litters. Adoptable dogs diagnosed with *B. gibsoni* were treated with atovaquone (13.4 mg/kg orally q 8 h with a fatty meal) compounded into capsules (Wedgewood Pharmacy) and azithromycin (10 mg/kg orally q 24 h) for 10 days as previously described (Kirk, 2014). Dogs diagnosed with dirofilariasis were treated with a macrocyclic lactone monthly, an oral doxycycline regimen consisting of 1 month on and 2 months off throughout their custody, and with melarsomine following release from legal custody (Kirk, 2014).

Sample collection

Blood for routine health screening was collected by jugular or cephalic venipuncture into two EDTA tubes, one serum separator tube, and two heparinized microhematocrit tubes during examination the first week in custody. Serum was harvested by centrifugation. Fecal samples were collected after defecation within 4 days of intake. Use of surplus blood and feces following routine health screening was approved by the University of Florida Institutional Animal Care and Use Committee (Protocol 201308177, November 11, 2013).

Sample analysis

One set of EDTA blood samples was tested onsite for *Dirofilaria immitis* (heartworm) antigen and for antibodies against *Anaplasma phagocytophilum*, *Anaplasma platys*, *Borrelia burgdorferi*, *Ehrlichia canis*, and *Ehrlichia ewingii* (SNAP 4Dx Plus Test, IDEXX Laboratories).

Serum was tested for *B. gibsoni* antibodies by ELISA as described (Goo et al., 2008) with slight modifications. In brief, 96-well plates were coated with full-length rBgTRAP with 6× His-tag. The plates were incubated with serum samples diluted to 1:200, followed by color development with horseradish peroxidase-conjugated anti-dog IgG and optical density measured at 650 nm. Samples were considered positive if the optical density was greater than the mean plus 3 standard deviations of the values from control samples collected from an Alaskan population of dogs determined to be free of *B. gibsoni* by PCR.

The packed cell volume (PCV) was determined by centrifugation of the microhematocrit tubes. For the purposes of this study, anemia was defined as PCV < 26% in neonates, PCV < 31% in juveniles, and PCV < 37% in adults (Hoskins, 2001).

The second set of EDTA blood samples was tested at a commercial reference laboratory for vector-borne pathogens by real-time PCR (Tick/Vector Comprehensive RealPCR Panel Canine, IDEXX Laboratories), including *Anaplasma phagocytophilum* (msp2 [p44], DQ519570), *A. platys* (groEL, heat shock protein, AY848753), *Babesia* spp. (ssrRNA, AF271082), *Bartonella* spp. (citrate synthase gene, AJ439406), *Mycoplasma haemocanis* (ribosomal RNA ssrRNA, AF197337), '*Candidatus* Mycoplasma haematoparvum' (ribosomal RNA ssrRNA, AY383241), *Ehrlichia canis* (disulfide oxidoreductase [dsb] gene, AF403710), *E. ewingii* (disulfide oxidoreductase [dsb] gene, AY428950), *E. chaffeensis* (disulfide oxidoreductase [dsb] gene, AF403711), *Hepatozoon canis* (ssrRNA, AF176835), *H. americanum* (ssrRNA, AF176836), *Leishmania* spp. (glycoprotein gp63, YO8156), *Neorickettsia risticii* (ribosomal RNA 16S RNA, AF184082), and *Rickettsia rickettsia* (GroEL heat shock protein, AJ293326). Real-time PCR was performed with six quality controls, including quantitative PCR-positive controls, PCR-negative controls, negative extraction controls, quantitative DNA internal sample quality control targeting the host 18S rRNA gene complex, an internal positive control spiked into the lysis solution, and an environmental contamination monitoring control. Samples positive by PCR for *Babesia* spp. were submitted for species-specific real-time PCR testing, including *B. canis* (heat shock protein 70, AB248735), *B. canis vogeli* (heat shock protein 70, EF527401), *B. canis rossi* (heat shock protein 70, AB248738), *B. felis* (ITS-2, AY965742), *B. gibsoni* (heat shock protein 70, AB248731), and *B. conradae* (ITS-2, AY965742. All assays were designed and validated according to industry standards.[1]

Fecal samples were processed by zinc sulfate centrifugation to screen for ova and parasites at a commercial laboratory (IDEXX Laboratories) and tested for *Giardia* spp. antigen by ELISA (SNAP Giardia Test, IDEXX Laboratories).

[1] See: Applied Biosystems, User Bulletin #3 http://tools.thermofisher.com/content/sfs/manuals/cms_041001.pdf (Accessed 26 February, 2016).

Statistical analysis

Statistical analysis was conducted in two phases. In phase 1, two authors (SC, JL) calculated the prevalence of vector-borne and intestinal pathogens. For the purposes of statistical analysis, dogs positive for *Babesia gibsoni* by either PCR or serology were considered infected. Descriptive statistics for PCV and anemia were calculated, and asymptotic χ^2 tests were used to test for unadjusted bivariate associations between the presence of anemia and positive results for *Babesia gibsoni*, hemotropic mycoplasmas, or *Ancylostoma*. A value of $P < 0.05$ was considered significant. All calculations were made with statistical software (SigmaStat for Windows 3.5, Systat Software).

Because the prevalence of vector-borne pathogens and intestinal parasitism varied among the eight investigation scenes, further analysis of PCV was conducted to control for the cluster effect of scene by a third author (JS). The Mantel–Haenszel method (Mantel and Haenszel, 1959) was used to calculate the odds ratio (OR) and 95% confidence interval (95% CI) for anemia, defined as the ratio of the odds of anemia when the vector-borne pathogen was present to that if it was absent. This also provided a *P*-value adjusted for clusters. Multiple linear regression was used to compare PCV results by calculating the average difference (PCV when pathogen was present minus PCV when pathogen was absent), adjusted for scene and for co-infections with any of three other major pathogens. A value of $P < 0.05$ was considered significant. All calculations in this phase were conducted with statistical software (Statistical Analysis System [SAS] software version 9.3, SAS Institute).

Results

Pathogens

Vector-borne pathogens were present in most dogs (164/269, 61%; Table 1). The most common vector-borne pathogens were *B. gibsoni* and hemotropic mycoplasmas, the prevalence of which varied widely across the eight investigation scenes (Fig. 1). No dogs had evidence of infection with *Anaplasma* spp., *Bartonella* spp., *Ehrlichia* spp., *Hepatozoon* spp., *Leishmania* spp., *Neorickettsia risticii*, *Rickettsia rickettsii*, or *Babesia* spp. other than *B. gibsoni*.

Results of PCR and serologic testing for *B. gibsoni* were in agreement for 93% of dogs, including 84 that were positive by both PCR and serology and 165 that were negative by both PCR and serology. Two dogs were PCR-positive and serology-negative, and 18 were PCR-negative and serology-positive. For the purposes of statistical analysis, dogs that were positive by either test (104 dogs; 39%) were considered infected. One dog that was PCR-negative and serology-positive later gave birth to *B. gibsoni*-infected puppies. This dam was retested via PCR after diagnosis in her puppies and she tested positive for *B. gibsoni*.

Co-infections were more common than isolated infections. A total of 76/269 (28%) dogs were co-infected with both *B. gibsoni* and one or both canine hemotropic mycoplasmas (Table 2). There was a

Table 1
Prevalence of vector-borne pathogens in 269 dogs seized from eight scenes in a federal dogfighting investigation.

	Test method	Dogs tested (*n*)	Dogs positive (*n*)	Percent positive
Babesia gibsoni	Serology	261	102	38
Babesia gibsoni	PCR	269	86	32
'*Candidatus* Mycoplasma haematoparvum'	PCR	269	86	32
Mycoplasma haemocanis	PCR	269	80	30
Dirofilaria immitis[a]	Serology	196	23	12
Anaplasma spp.	PCR	269	0	0
Anaplasma spp.	Serology	269	0	0
Bartonella spp.	PCR	269	0	0
Ehrlichia spp.	PCR	269	0	0
Ehrlichia spp.	Serology	269	0	0
Hepatozoon spp.	PCR	269	0	0
Leishmania spp.	PCR	269	0	0
Neorickettsia risticii	PCR	269	0	0
Rickettsia spp.	PCR	269	0	0
Borrelia burgdorferi	Serology	269	0	0

[a] Testing for *D. immitis* was performed only on adult dogs 6 months and older.