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Research paper

Prevalence and risk factors for the presence of serum antibodies against canine distemper, canine parvovirus, and canine adenovirus in communities in mainland Ecuador

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ABSTRACT

The purpose of this study was to estimate the apparent prevalence and identify risk factors for antibody levels (AL) against canine distemper virus (CDV), canine parvovirus (CPV), and canine adenovirus (CAV) in three communities in the metropolitan area of Quito, Ecuador that have limited access to regular veterinary care. Whole blood samples were collected from 154 dogs presenting to three veterinary field clinics in mainland Ecuador and tested for AL against CDV, CPV, and CAV by a commercially available point-of-care ELISA. Potential risk factors for the presence of AL were analyzed. A majority of dogs had AL against CDV (66%, 95% CI = 58–73%), CPV (95%, 95% CI = 91–98%) and CAV (60%, 95% CI = 52–67%). Dogs had significantly greater odds of AL against CDV if they were > 2 years of age, from an urban community, and had previously received veterinary care. Dogs had significantly greater odds of AL against CAV if they were male, > 2 years of age, and had previously received veterinary care. Results provide baseline estimates of AL within each community and allow for the targeting of future veterinary services to communities and dogs most at risk.

1. Introduction

CDV and CPV are highly contagious, serious health threats to dogs worldwide (Frölich et al., 2000; Welborn et al., 2011; Greene and Decaro, 2012; Greene and Vandevelde, 2012). Dogs infected with CDV can shed the virus for several months; a variety of modes of transmission including through aerosolization, urine, feces, or fomites, enhance the potential for infection of at-risk animals (Sobrino et al., 2007). Clinical signs are primarily respiratory in nature; mild gastrointestinal disease is not uncommon early in the course of infection and neurological manifestations, including seizures and myoclonus, can occur in later stages. CPV is a resilient virus that can remain in the environment for long periods of time and is spread via direct contact, fomites, or by fecal contamination for up to three weeks post infection (Carmichael, 2005; Greene and Decaro, 2012). Severe gastrointestinal disease including vomiting and small bowel diarrhea are characteristic. Both CDV and CPV can present with an array of clinical signs or subclinical infection and morbidity and mortality are high in vulnerable populations (Greene and Decaro, 2012; Greene and Vandevelde, 2012). CAV-2 is also a viral respiratory disease. This virus is shed for seven days and dogs can become infected by coming into contact with contaminated urine, feces, or saliva from sick dogs (Greene, 2012). Clinical signs of CAV-2 are generally subclinical and self-limiting; morbidity is low. However, it is frequently identified as a co-pathogen with more serious respiratory infections and immunity against CAV-2 is cross-protective against infectious canine hepatitis (CAV-1), a disease with high mortality (Schulz et al., 2014; Monteiro et al., 2016). Vaccination against CDV, CPV, and CAV is a worldwide standard of preventive care with

Abbreviations: AL, antibody levels; CAV, canine adenovirus; CDV, canine distemper virus; CPV, canine parvovirus

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initial immunization recommended as early as four weeks of age. Although there has been some debate as to how often pet dogs should be vaccinated (Pollack and Carmichael, 1982; McCaw et al., 1998; Twark and Dodds, 2000; Moore and Glickman, 2004; Burr, 2006; Horzinek, 2006; Waner et al., 2006), dogs vaccinated against CDV, CPV, and CAV even once have demonstrated long-term protective immunity compared to those that are not vaccinated (Schultz, 2006; Levy et al., 2008).

AL are commonly used to estimate levels of immunity in a given population and can assist in the development of rational vaccination campaigns. Measurement of antibody titers through hemagglutination inhibition (CPV) and virus neutralization (CDV and CAV) is typically considered the gold standard for such assessments; however, results from a commercially available point-of-care test kit (VacciCheck, Biogal Galed Labs, Kibbutz Gal'ed, Israel) have been correlated with those from such diagnostic laboratory tests (Mazar et al., 2009; Butler and Crawford, 2013). These test kits offer immediate results feasible for conducting assessments in the field. Studies of dog populations in Ecuador's Galapagos Islands (utilizing both testing methodologies) indicate prevalence rates of 22–38% for CDV, 89–100% for CPV, 67% for CAV-1, and 40–66% for CAV-2 (Levy et al., 2008; Diaz et al., 2012). Seroprevalence of CDV and CPV in the neighboring South American countries of Chile and Brazil has also been described (Courtenay et al., 2001; Acosta-Jamett et al., 2011). Currently, the prevalence of AL against CDV, CPV, and CAV throughout mainland Ecuador is unknown. The objectives of this study were to obtain preliminary estimates of the prevalence of AL against CDV, CPV, and CAV in three communities within mainland Ecuador that lack access to regular veterinary care and to identify historical and clinical factors associated with the presence of AL against CDV, CPV, and CAV in these communities.

2. Materials and methods

2.1. Study locations

Three University of Florida-Universidad San Francisco de Quito cooperative veterinary field clinics in mainland Ecuador were selected as study sites. Two urban clinic locations in north (Carcelén, 0.08 °S, 78.47 °W) and south (La Ecuatoriana, 0.30 °S, 78.54 °W) Quito were each selected by the Metropolitan Agency of Control and the Quitumbe Zonal Administration because of the perceived overpopulation of stray dogs in those communities. Socioeconomic status and access to veterinary care is variable in Carcelén; the poverty rate is 17%. Citizens of La Ecuatoriana generally have a low socioeconomic status, limited access to veterinary care, and a poverty rate of 26.8%. The rural Itulcachi community (0.02 °S, 78.20 °W) is located on the boundary between owned and non-owned free roaming dog populations due to geographic and socioeconomic characteristics. In addition, the field clinic program offers free veterinary services, including vaccination against CDV, CPV, and CAV, to this community on an annual basis. This community has a poverty rate of 64% and veterinary services are limited to those provided by the field clinic program once per year (Institute of the City of Quito, 2010).

2.2. Animals

All dogs estimated to be at least 12 weeks of age by dental chronometry that presented to the field clinics in June 2013 were included in the study. All dogs had a designated caretaker, however, common ownership practice in Ecuador includes allowing such dogs to roam free during the daytime (Diaz et al., 2012; Grijalva Rosero, 2014). Standardized medical health forms were completed with information on each dog. Patient signalment, physical examination findings, previous veterinary history and community of origin were collected and analyzed as potential risk factors for AL.

2.3. Sample collection

Each sample was collected prior to administration of modified-live vaccination against CDV, CPV, and CAV. Whole blood (3 mL) was collected by jugular or cephalic venipuncture from each dog and stored in a sterile EDTA blood collection tube until analysis. Samples were stored in a cooler and analyzed within 12 h.

2.4. Serologic testing

Whole blood samples were tested for AL in the form of IgG against CDV, CPV, and CAV using a point-of-care ELISA according to the manufacturer's instructions (VacciCheck, Biogal Galed Labs, Kibbutz Gal'ed, Israel). Respectively, sensitivity and specificity of the assay is 97–100% and 79–83% for CDV, 88–99% and 75–100% for CPV, and 96% and 82% for CAV (Mazar et al., 2009; Butler and Crawford, 2013). In lieu of the manufacturer-supplied capillary tube and piston, a calibrated pipettor was used to conduct testing. Test results were interpreted visually using the provided CombScale.

2.5. Statistical analyses

Descriptive statistics of the sample population are reported. Apparent prevalence was calculated by dividing the number of dogs with AL by the number of dogs tested for CDV, CPV, and CAV, respectively. The confidence intervals for the apparent prevalences were calculated using the Wald asymptotic method for binomial data. Simple chi-square analyses were used to calculate relative risk of differences in seroprevalence with potential historical and clinical risk factors. Stepwise regression was used to develop a statistical model to assess interaction of the variables (SAS 9.3, SAS Institute Inc., Cary, NC). P-values less than or equal to 0.05 were considered significant.

The study protocol was approved by the University of Florida Institutional Animal Care and Use Committee.

3. Results

Samples were collected from a total of 154 dogs including 81 [52.6%] females and 73 [47.4%] males. A total of nine dogs [5.8%] were previously neutered (five females and four males). Most dogs were known or estimated to be 6–12 months of age (54 [35.1%]), followed by those > 24 months of age (49 [31.8%]), those 13–24 months of age (40 [26%]), and those < 6 months of age (8 [5.2%]). Age was not recorded for three dogs; all other data points were recorded and these dogs were included in all other analyses. Physical examination findings were normal for most dogs (105 [68.2%]). The most common abnormal physical examination findings included ophthalmologic (12 [24.5%]), dermatologic (9 [18.4%]), and orthopedic disease (6 [12.2%]). Dogs with external parasites in the absence of other abnormal physical examination findings (n = 6) were considered normal. Most samples were from dogs living in urban communities (88 [57.1%]) and just over half of the caregivers indicated that their dog had previously received some sort of veterinary care during its lifetime (79 [51.3%]); specific type of care was not described.

3.1. Antibody levels

A total of 101 (65.6%, 95% CI = 58–73%) dogs had AL against CDV, 146 (94.8%, 95% CI = 91–98%) against CPV, and 92 (59.7%, 95% CI = 52–67%) against CAV. Among those ≤2 years of age, 54 (52.9%), 94 (92.2%), and 48 (47.1%) dogs had AL against CDV, CPV, and CAV, respectively. Among those > 2 years of age, 46 (93.9%), 49 (100%), and 43 (87.8%) dogs had AL against CDV, CPV, and CAV, respectively. Among sexually intact dogs that were evaluated, 94 (64.8%), 137 (94.5%), and 85 (58.6%) had AL against CDV, CPV, and CAV, respectively. A total of 82 (53.2%) dogs had AL against all 3

Table 1
Risk factors associated with antibody levels (AL) against canine distemper virus (CDV), canine parvovirus (CPV), and canine adenovirus (CAV) in 154 dogs from three communities in mainland Ecuador.

Risk factor		CDV				CPV				CAV			
		No. antibody +	OR	95% CI	p-value	No. antibody +	OR	95% CI	p-value	No. antibody +	OR	95% CI	p-value
Sex	Female	48	REF	–	–	76	REF	–	–	42	REF	–	–
	Male	53	1.82	0.92-3.59	0.0916	70	1.54	0.35-6.66	0.7223	50	2.02	1.04-3.90	0.0481
Age	≤2 years	54	REF	–	–	93	REF	–	–	48	REF	–	–
	> 2 years	46	0.10	0.03-0.30	< 0.0001	50	0.11	0.0062-1.93	0.0528	43	0.15	0.06-0.36	< 0.0001
Physical examination	Abnormal	30	REF	–	–	48	REF	–	–	28	REF	–	–
	Normal	71	0.756	0.37-1.53	0.470	98	3.43	0.41-28.67	0.4370	64	0.85	0.43-1.70	0.7251
Com-munity	Rural	36	REF	–	–	65	REF	–	–	37	REF	–	–
	Urban	65	2.36	1.19-4.64	0.0164	81	0.18	0.02-1.48	0.1388	55	1.31	0.68-2.50	0.5069
Previous veterinary care	No	33	REF	–	–	63	REF	–	–	27	REF	–	–
	Yes	65	4.60	2.20-9.59	< 0.0001	79	6.27	0.71-55.05	0.0944	62	5.23	2.56-10.69	< 0.0001

pathogens including 20 (50%) in La Ecuatoriana, 30 (63%) in Carcelén, and 32 (94.1%) in Itulcachi.

3.2. Risk factors for AL

Dogs > 2 years of age, those from an urban community, and those that had previously received veterinary care had greater odds of AL against CDV. No risk factor was a significant predictor of AL against CPV. Dogs > 2 years of age, males, and those that had previously received veterinary care had greater odds of AL against CAV [Table 1]. Each of the identified risk factors maintained significance after stepwise regression and logistical modeling [Table 2].

4. Discussion

The majority of dogs presenting to three field clinics in Ecuador had AL against CDV (66%) and CAV (60%), with virtually all dogs having AL against CPV (95%). A greater proportion of dogs > 2 years of age had AL against CDV and CAV compared to dogs ≤2 years of age. In addition, dogs from regions with greater human population density and those with previous veterinary care were more likely to demonstrate detectable AL.

Previous reports indicate that almost all dogs will have AL against both CPV and CDV after vaccination, even in dogs with unknown vaccination histories (Litster et al., 2012), and that such immunity will last for years (Schultz et al., 2010). For these reasons, a more uniform distribution of AL against these pathogens would be expected in the study population if it was indicative of previous vaccination. Such a pattern was found in the rural Itulcachi community which, despite being of the lowest socioeconomic status and having the least access to local veterinary care, had the highest proportion of dogs with AL against all three pathogens (94%). This is thought to be attributed largely to the annual vaccination campaigns conducted within this community.

Table 2
Adjusted logistic model for assessment of risk factors associated with antibody levels (AL) against canine distemper virus (CDV) and canine adenovirus (CAV) in 3 communities in mainland Ecuador.

	Risk Factor	OR	95% CI
CDV	Previous veterinary care	4.17	1.79-9.74
	Urban community	3.51	1.51-8.14
	> 2 years	8.25	2.62-26.04
CAV	Previous veterinary care	5.34	2.39-11.94
	> 2 years	6.33	2.38-16.87
	Male	2.67	1.18-6.05

Given the high morbidity and mortality of CPV, the near complete evidence of protection against CPV (95%) likely indicates the population of dogs surveyed survived natural infection with CPV. Conversely, dogs without AL likely succumbed to the disease and therefore were not available for assessment. The use of monovalent CPV vaccines is also a possibility, though is considered less likely as these are not typically used in the region of interest. Positive test results due to maternal antibody detection is also possible, however, only six samples were from dogs between 12 and 20 weeks of age, the period during which maternal antibodies are most likely to be detected (Welborn et al., 2011), and detection of CPV AL was not consistent even among this cohort. The lack of gastrointestinal signs in the study population make clinical infection unlikely in the majority of cases although direct testing for parvoviral antigen was not performed; instances of subclinical infection cannot be ruled out.

As primarily respiratory infections with high morbidity and lower mortality than CPV, previous exposure to vaccinates or natural exposure are likely sources of observed AL against CDV and CAV. As discussed with CPV, AL detection could represent current CDV infection, though this is considered unlikely given the lack of clinical signs suggestive of active disease (i.e., respiratory, gastrointestinal, neurologic). Given the typical lifestyle of dogs in the sample communities, both direct and indirect disease transmission between infected and susceptible dogs at some point in their life is common. As a non-enveloped virus resistant to environmental inactivation, opportunities for natural exposure to CAV are particularly abundant (Greene, 2012). The observed risk factors for AL against CDV and CAV support this idea: AL were found against both pathogens in older dogs and those that had previously received veterinary care. The age of these dogs makes maternal antibody detection unlikely and allows ample time for natural exposure to take place; similar age associations with AL were identified in Santa Cruz, Galapagos (Diaz et al., 2012). In that report, dogs ≥1 year of age had greater odds of seropositivity for CAV. Having a history of veterinary care could indicate previous vaccination against CDV and CAV but, particularly in the case of CDV, AL against a greater proportion of dogs would be expected if this were the case. Veterinary care in these communities is typically delivered as was described in this report – intermittent mass vaccination campaigns in a field clinic setting. Although clinic staff take precautions to minimize disease transmission, such scenarios create opportunities for increased pathogen exposure. Similarly, the enhanced risk for AL CDV in dogs from an urban community suggests a higher rate of pathogen transmission in dogs living in areas with a higher population density. In Santa Cruz, Galapagos, dogs from rural communities were more likely to demonstrate AL against CAV; however, in that report, previous vaccination prior to taking up residence in the rural regions was suspected (Diaz et al., 2012). The finding of increased AL against CAV in (intact) male dogs is not

surprising and is likely due to enhanced roaming and opportunities for direct contact with other exposed dogs. Male dogs were also identified as having higher prevalence of seropositivity against CAV on Isabela Island, Galapagos (Levy et al., 2008).

The estimates of AL against CPV and CAV in the communities evaluated are similar to those reported on Isabela Island, Galapagos (Levy et al., 2008), though the prevalence of AL against CDV was substantially higher (65.6% vs. 22%). In an effort to prevent the introduction of disease into the Galapagos, vaccination of dogs was prohibited until 2017 (Ministerio del Ambiente, 2019). Given this limitation, the seropositivity discovered there likely represents that acquired primarily through natural exposure (or illegal vaccination), while that found in mainland Ecuador could also include that acquired from intermittent vaccination campaigns.

Seroprevalence of CDV and CPV in other South American countries has also been reported. Studies of domestic dogs in Chile also found higher rates of AL against CDV and CPV in dogs from urban regions as compared to those from rural regions (Courtenay et al., 2001; Acosta-Jamett et al., 2015). Overall seroprevalence in these reports ranged from 34 to 80% for CDV and 65–92% for CPV which is consistent with the 74% for CDV and 92% for CPV reported in the current study. In the Chilean reports, dogs > 12 months of age also had significantly greater odds of seropositivity for both pathogens than those < 12 months of age. Seroprevalence in a rural population of Brazilian domestic dogs was substantially different than those reported in Ecuador and Chile with rates of 9% and 13% reported for CDV and CPV respectively (Courtenay et al., 2001).

While the majority of dogs had evidence of AL against CDV, 34% of dogs sampled and 47% of dogs ≤2 years of age had no such protection. Most importantly, the overall proportion was lower than that desired to create herd-level immunity and prevent widespread disease outbreaks as well as that thought to limit the efficacy of vaccination campaigns (Coleman and Dye, 1996; Plans-Rubió, 2012; Belsare and Gompper, 2015). On the neighboring Galapagos island of Santa Cruz, AL were reported to be 36% for CDV, 89% for CPV, and 40% for CAV (Diaz et al., 2012). Outbreaks of CDV have been reported in Santa Cruz, resulting in both deaths due to disease and culling of hundreds of dogs (Levy et al., 2008). Given the relatively low rate of AL, these data suggest prioritizing efforts to minimize the spread of CDV in particular, especially in younger dogs, could help minimize morbidity and mortality. Widespread vaccination against CDV along with assessment of wildlife vectors, minimization of opportunities for canine transmission and education of community dog caretakers should assist in the reduction of disease. Veterinarians may also prioritize the use of vaccine products known to induce rapid immunity and quickly overcome maternal antibody interference in these populations (e.g., recombinant and modified-live virus products) to ensure adequate protection.

There are several limitations to the current study, common to many seroprevalence surveys. First, although the accuracy of the antibody test utilized has been evaluated, the potential for a low number of false positive and false negative test results exists (Mazar et al., 2009; Butler and Crawford, 2013). By default, the true prevalence of AL in the canine population in mainland Ecuador is unknown and may have an impact on the accuracy of test kit results, particularly in the direction of underestimating negative samples (i.e., reduced negative predictive value) (Moore and Glickman, 2004). Second, quantitative titer analysis was not attempted, which may provide more objective insight into whether or not the seropositivity detected was the result of previous infection or vaccination. Third, the use of antibodies as a measure of "protection" ignores the role of the innate immune system in disease protection and will not identify dogs that may mount a protective immune response to actual disease challenge. Fourth, sample collection was conducted over a finite period of time and may not reflect alterations in disease exposure or population characteristics that may occur with seasonal variations (e.g., ambient temperature, rainfall patterns, etc.) or with regional movement of both domestic and wild animal populations. Finally, the population of dogs that presented to the field clinics for evaluation may not be representative of the entire canine population in mainland Ecuador. Sample randomization or systematic sampling techniques could have provided seroprevalence estimates more reliably extrapolated to the regional population. Despite the fact that only samples from owned dogs presented for veterinary care were analyzed, it is normal practice in each of the study communities for such dogs to roam free during the daytime. Therefore, it is thought that the risk of exposure to infectious diseases in the sample population is likely similar to both owned and unowned dogs in the communities of interest that were not evaluated directly.

The current report provides baseline data on a population of animals for which AL prevalence has not been previously reported. This information is relevant to those developing practical immunization protocols in the region as well as provides a benchmark for future epidemiologic studies and the operation of future vaccination campaigns in the communities evaluated.

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