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Characterization of canine distemper clinical presentations during the epidemic outbreak 2014-2018 in Lisbon Metropolitan Area, Portugal

Caracterização de quadros clínicos de esgana durante o surto registado entre 2014 e 2018 na Área Metropolitana de Lisboa, Portugal

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Resumo

A esgana canina é uma doença infecciosa viral sistémica e altamente contagiosa que afecta essencialmente os sistemas respiratório, gastrointestinal e nervoso. É provocada por um paramixovírus do género Morbilivírus (CDV) e tem distribuição mundial. Após décadas de silêncio epidemiológico, ocorreu entre 2014 e 2018, na área metropolitana de Lisboa, um surto de esgana canina. Muitos clínicos não tinham até então visto qualquer caso da doença no exercício da sua prática diária. O presente estudo tem como objetivo caracterizar as apresentações clínicas de esgana em pacientes hospitalizados na Unidade de Isolamento e Contenção Biológica da Faculdade de Medicina Veterinária da Universidade de Lisboa durante o referido surto. Foram admitidos 41 casos suspeitos dos quais 25 foram confirmados laboratorialmente por serologia e/ou PCR. As seguintes variáveis foram investigadas: localização geográfica, género, idade, raça, sinais clínicos, método de diagnóstico laboratorial, análises clínicas, estatuto vacinal, duração da hospitalização, taxa de sobrevivência e distribuição mensal dos casos. Os sinais clínicos mais frequentemente identificados foram mioclonias (52,0%), conjuntivite (52,0%), tosse (48,0%), vómito (40,0%) e diarreia (40,0%). Em 33,3% dos casos registou-se um aumento dos leucócitos. Apenas 15,7% dos cães infectados tinha um plano vacinal completo no que respeita a proteção contra CDV. Foi calculada uma taxa de sobrevivência de 32,0%. Os resultados apresentados neste estudo reforçam a importância do cumprimento do plano vacinal na prevenção do risco de reemergência de esgana canina.

Palavras-chave: Esgana, surto, quadro clínico.

Summary

Canine distemper is a highly contagious, systemic, viral disease with a worldwide distribution. A Pramyxovirus of the genus Morbillivirus (CDV) that attacks the respiratory, gastrointestinal and nervous systems causes the disease. In Lisbon Metropolitan Area, an outbreak of CDV evolved during 2014-2018. This occurred after decades of epidemiological silence in the area. Therefore, many practitioners had never seen distemper clinical presentations. The aim of this article is to characterize the clinical presentation of canine distemper cases hospitalized at the Biological Isolation and Containment Unit of the Faculty of Veterinary Medicine, University of Lisbon during the outbreak. Forty-one suspected cases were admitted; twenty-five were laboratory confirmed through Serologic assay and/or PCR. The variables investigated were geographical location, gender, age, breed, clinical signs, laboratory diagnosis method, clinical analysis, vaccination status, hospitalization length, survival rate and monthly disease frequency. The most frequent clinical signs were myoclonus (52.0%), conjunctivitis (52.0%), cough (48.0%), vomit (40.0%) and diarrhea (40.0%). Leukocytes increased in 33.3% of the cases. Only 15.7% of the infected dogs had a complete CDV vaccination plan. The survival rate of CDV confirmed cases was 32.0%. This study reinforces the value of vaccination compliance to prevent the risk of re-emergence of canine distemper.

Keywords: Canine distemper, outbreak, clinical presentation.

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1. Introduction

Canine distemper is a multisystemic infectious disease that affects domestic dogs and a wide variety of terrestrial and aquatic carnivores, caused by a Morbillivirus of the Paramyxoviridae family (Appel *et al*., 1992; Carvalho *et al*., 2012; Sykes, 2014a). Domestic dogs are the main host for canine distemper virus (CDV) and should be considered a reservoir for other mammals (Duque-Valencia *et al*., 2019). The CDV is an enveloped, pleomorphic, single-stranded negative RNA virus, closely related with human measles and rinderpest viruses (Anis *et al*., 2018).

The CDV is highly contagious, easily inactivated by heat, dry conditions and disinfectants. Yet it is resistant to low temperature and the majority of cases in domestic dogs occur in the fall and winter. It persists within a population by inhalation of aerosolized

particles or through direct contact of susceptible animals with subclinically or clinically infected dogs. The CDV is also shed in the urine, faeces, and secretions of infected animals (Greene, 2012). Outbreaks occur with high case fatality rates (Ek-Kommonen *et al*., 1997; Kapil and Yeary, 2011; Sykes, 2014a; Pfeffermann *et al*., 2018).

The virus infects dogs of all ages but the prevalence in puppies aged 3 to 6 months is higher (Martella *et al*., 2008; Greene, 2012). Susceptible dog's exposure results mostly from inhalation of aerosolized particles from oronasal secretions of infected dogs. Usually, the incubation period is 7 to 14 days, but it ranges from 3 to 21 (Sykes, 2014a). Clinical signs vary widely, and there may be a large proportion of subclinical or slightly affected dogs (Gámiz *et al*., 2011; Greene, 2012). These variations depend on several factors including the virulence of the viral strain, the age of the animal, and the dog's immune status and presence of concomitant infections (Blixenkrone-Møller *et al*., 1993; Carvalho *et al*., 2012, Sykes, 2014a).

The virus spreads through upper respiratory tract epithelium and tonsils, infects monocytes within the lymphoid tissue and disseminates via lymphatic and blood vessels to the reticuloendothelial system. There is direct viral destruction of a large number of lymphocytes, from the blood, tonsils, thymus, spleen, lymph nodes, bone marrow and also liver Kupffer cells, which results in lymphopenia and fever (Summers *et al*., 1984; Greene, 2012; Lempp *et al*., 2014; Sykes, 2014a). In a second phase of viremia and fever there is infection of respiratory, gastrointestinal, urinary tracts, central nervous system and skin (Greene, 2012; Lempp *et al*., 2014; Rendon-Marin *et al*., 2019). Infection can lead to abortive, clinical or subclinical disease courses (Beineke *et al*., 2009). The CDV can be excreted in all secretions, 5 days after infection, even before the start of clinical signs and continues for up to 3 to 4 months (Sykes, 2014a).

Similar to other paramyxoviruses, such as the closely related measles virus, CDV infection causes lymphoid depletion and enduring immunosuppression, which favour secondary infections (Beineke *et al*., 2009). The severe disease forms include clinical signs such as serous to mucopurulent conjunctivitis with blepharospasm and photophobia, dry cough, vomiting and diarrhoea ranging from liquid to bloody or mucous, skin rash or pustular dermatitis, hyperkeratosis of the nasal mucosa and plantar pads, neurological signs including myoclonus, seizures, tremors, opisthotonos, tetra paresis, paraparesis, proprioceptive deficits, ataxia, behavioural changes, tremors of intention, nystagmus, strabismus, blindness, walking in circles and vocalizations (Gámiz *et al*., 2011; Greene, 2012; Summers *et al*., 1984; Sykes, 2014a; Xue *et al*., 2019). Most dogs that die from distemper die from neurological complications.

On endemic areas, CDV should be included in the differential diagnosis of any febrile condition in dogs with multisystemic manifestations, reminding that the clinical picture can be distorted by viral or bacterial infections such as infectious canine hepatitis or leptospirosis, and clinical signs sometimes do not appear until late in the disease. The clinical suspicion is more problematic when only one of the presentations, respiratory, gastrointestinal or neurological, is present. Hemogram usually reveals mild anaemia and lymphopenia (Rendon-Marin *et al*., 2019). Marked monocytopenia and thrombocytopenia can be detected and sometimes left shift neutrophilia with toxic neutrophils, inclusion bodies in erythrocytes and leukocytes (Rendon-Marin *et al*., 2019; Sykes, 2014a). The cerebrospinal fluid (CSF) analysis in dogs with neurologic signs may show high protein level and high cell count, inflammatory encephalomyelitis caused by CDV with intracytoplasmic inclusions in CSF cells (Greene, 2012).

The most used diagnosis tests are RT-qPCR, serology assays for IgM/IgG detection and rapid immunochromatographic test (Greene, 2012; Sykes, 2014a). Sample collection should be adjusted according to clinical signs, in nasal, oral, conjunctival, or rectal swabs, blood or CSF aspirates (Greene, 2012). Up to 4 weeks post vaccination serologic assays can give false positive results by detecting vaccine induced antibodies, but also molecular detection can be false positive right after vaccination due to vaccine attenuate virus detection (Kapil and Yeary, 2011; Sykes, 2014a). That reinforces the need of knowing detailed vaccine history (Kapil and Yeary, 2011). A rapid immunochromatographic test can be performed during consultation to confirm the clinical suspicion but with low sensitivity but the most reliable laboratory tests are RT-qPCR or serology assays for antibody IgG/IgM detection (Greene, 2012).

Currently, there is no effective method to treat for CDV infections available in clinical practice, although favipiravir (T-705) seems to be a potential candidate for treatment as it effectively inhibited viral replication following CDV infection in vitro, still under laboratory tests (Xue *et al*., 2019). Suspect or confirmed distemper cases must be kept in isolation and clinical management consists in symptomatic and supportive treatment, aimed at limiting secondary bacterial invasion, supporting fluid balance, and controlling neurologic manifestations (Martella *et al*., 2008; Wiebe, 2015). Dogs with mild respiratory or gastrointestinal signs may recover spontaneously without treatment (Sykes, 2014a). However, in severe forms of disease, hospitalization is needed to provide fluid therapy, antiemetics, enteral feeding, antibiotics for secondary bacterial infections, oxygen therapy and nebulization. Short-term treatment of neurological signs such as seizures require the use of anticonvulsants (Greene, 2012; Sykes, 2014a). Neurological signs may appear weeks to months following recovery of respiratory or gastrointestinal forms, and become progressive, in these cases most of the times leading to death or euthanasia (Wiebe, 2015).

The vaccination for canine distemper is core in dog's vaccination schedule, recommended from 6 to 8 weeks of age, then every 2 to 4 weeks until 16 weeks

of age, a booster at 12 months of age, then revaccination every 3 years (Buczkowski *et al*., 2014; Day *et al*., 2016).

After decades of sporadic diagnosis of canine distemper, an outbreak in 2014 occurred in the Metropolitan Area of Lisbon (Machado, 2016). The event was unpredictable in time and space thus fitting the criteria of an outbreak. Until 2014, canine distemper was very rarely confirmed at VTH since its opening in 2000. The outbreak epicentre was in the municipality of Loures, then spread to the municipalities of Odivelas, Lisbon, Amadora, Sintra and Almada. Some of these infected dogs were admitted into the Veterinary Teaching Hospital of the Faculty of Veterinary Medicine (VTH) of the University of Lisbon, as first opinion cases and others were referred to VTH from veterinarians working on the affected municipalities. All cases were hospitalized at the VTH Biological Isolation and Containment Unit (BICU). The outbreak lengthened from 2014 until 2018. All clinical records of suspected and confirmed CDV cases during this period were analysed. This study aims to investigate this outbreak, through a set of parameters including georeferencing cases, potential risk factors, characterization of clinical presentations, laboratory exams, vaccination status, hospitalization course and survival rate.

2. Materials and Methods

Forty-one dogs hospitalized at BICU with clinical presentation compatible with Canine Distemper were included in the study. BICU is a multispecies facility of the VTH. It works with negative differential pressure, HEPA air filters, video surveillance system and specific standard operating procedures (SOP).

The study period was from May 2014 to June 2018.

Clinical case definition: a clinical presentation compatible with canine distemper, namely one or more of the following signs: nasal and ocular discharge, cough, vomiting, diarrhoea or neurological signs.

Laboratory criteria for diagnosis: serology assay for antibody IgG or IgM detection, or RT-qPCR.

Case classification: Suspected, a case that meets the clinical case definition, plus disease course and therapeutic response compatible with CDV infection, lacking a conclusive diagnosis test. Confirmed, a suspected case with laboratory confirmation.

Data was retrieved from the patient's medical history stored in two management software, QVet® and Guruvet® used in VTH, and then entered into a database constructed in Microsoft Office Excel 365® for Windows®, where the descriptive analysis was performed for all cases. The 41 dogs were divided into two groups: confirmed dogs (n=25; 61.0%); suspected dogs (m=16; 39.0%).

The vaccination status was established based on the Vaccination Guidelines Group of the World Small Animal Veterinary Association (WSAVA) (Day *et al*., 2016). WSAVA guidelines consider that a dog with a complete vaccination status, received the initial core vaccination at 6 to 8 weeks of age, then every 2 to 4 weeks until 16 weeks of age or older (primo-vaccination). A booster dose of vaccine given at 12-month of age and thereafter, revaccinations every 3 years. A dog with an incomplete primo-vaccination status is a puppy too young to complete primo-vaccination or any dog missing one or more doses of primo-vaccination. A vaccine delay status applies to any dog missing the 12-month booster or any core revaccination every 3 years. A not vaccinated dog corresponds to an animal that was never vaccinated. A dog with unknown vaccination status is an animal with unknown vaccination history.

Three different laboratory tests were used to confirm CDV infection, serology assay for antibody IgG or IgM detection and RT-qPCR. Biological specimens were nasal, oral, conjunctival or rectal swabs, blood samples or cerebrospinal fluid, according to the signs at the time of clinical suspicion and to the vaccination status.

The inferential statistics analysis of the confirmed cases was done in R for Windows®, version 3.5.1, with a case-control study. The case group included the 25 distemper confirmed cases and the control group consist of a random sample of 97 cases selected from BICU dog's database from dogs with a non-infectious definitive diagnosis for example neoplasia or dogs with other infectious with a clinical presentation that excluded distemper as for example cases of urinary multidrug resistant bacteria infection. The Mantel-Haenszel chi-square test (χ^2) and the Fisher's exact test were used for testing qualitative variables. ANOVA analysis was performed for quantitative variables. A confidence interval of 95% was used; a p-value ≤ 0.05 is statistically significant. The georeferencing of the cases was carried out in QGIS for Windows®, version 3.12.0.

3. Results

Both groups were analyzed for geographic location, age, gender, breed, vaccination status, diagnostic tests, clinical signs and analysis, hospitalization length, survival rate and monthly distribution of cases. For the statistical analysis, the case group included the 25 distemper confirmed cases and the control group comprised a random sample of 97 cases non distemper cases from the BICU dog's database.

The geographic location of confirmed and suspected cases was established considering the dog owner's address. Most of the cases (n=39; 95.1%) were registered in Lisbon Metropolitan Area (LMA), and only 2 (4.9%) from outside LMA, both in Alentejo. The geographic distribution of investigated dogs is plotted on Figure 1. Both cases of from Alentejo had recent history of movements between there and LMA, and most likely infected within LMA.

Figure 1 - Geographic distribution of investigated dogs. Suspected dogs (n=16) in blue and confirmed (n=25) in red, left cases in LMA (n=39) and right cases outside LMA (n=2).

The median age for both groups was 12 months (1-year-old), the mode age was 4 months and it ranged between two months and 180 months (15-years-old). All dogs were grouped in the following age classes: ≤ 1-year-old (2-11 months), ≥ 1 and < 3 years old (12-35 months), ≥ 3 and < 7 years old (36-83 months), ≥ 7 and < 10 (84-119 months) and ≥ 10 (> 120 months). The age distribution is shown on Table 1. Comparing with control, no statistically significant results were found concerning the age of confirmed cases (p=0.15).

Table 1 - Distribution of suspected (n=41) and confirmed (n=25) CDV cases by age class.

Age class (years)	**Suspected cases Group**		**Confirmed cases Group**	
	n	%	n	%
< 1	19	46.3	10	40.0
≥ 1 and < 3	10	24.4	6	24.0
≥ 3 and < 7	5	12.2	5	20.0
≥ 7 and < 10	3	7.3	2	8.0
≥ 10	4	9.8	2	8.0
Total	41	100	25	100

Considering gender, males were overrepresented in both groups: suspected (n=32; 78.0%); confirmed (n=18; 72.0%). Females represented only 22.0% (n=9) on the suspected group and 28.0% (n=7) on the confirmed group. Nevertheless, comparing to control no statistically significant results were found considering the gender of confirmed distemper cases (p=0.1).

Regarding breed, in the suspected group there were 51.2% mixed breed dogs (n=21) and 48.8% pure breeds (n=20). Similar results were obtained for the confirmed group: 52.0% of mixed breeds (n=13), 48.0% of pure breeds (n=12). Comparing to control a statistically significant result was found suggesting that breed may be a protection factor for canine distemper (p=0.036; OR=0.4336; 0.1775<OR< 1.0592).

According to WSAVA guidelines (Day *et al*., 2016), the study population was divided in vaccinated dogs and unvaccinated dogs. The vaccinated dogs group included all cases that fulfilled WSAVA criteria; the unvaccinated dogs group added dogs with vaccination plans incomplete or out of date, and all dogs that were never vaccinated. Ten dogs (24.4%) were excluded due to unknown vaccination status; six of them belonged to the confirmed group. In the suspected group, 83.9% (n=26) were unvaccinated and only 16.1% (n=5) were vaccinated. Out of the nineteen confirmed CDV cases with a vaccination status known, sixteen (84.2%) were not vaccinated and only three (15.7%) were vaccinated. Comparing to control, no statistically significant result was found regarding the vaccination status of confirmed cases (p=0.1).

The clinical presentation included neurological (n=30; 73.2%) gastrointestinal (n=20; 48.8%) and respiratory (n=21; 51.2%) signs, for suspected cases. The confirmed cases presented mainly neurologic (n=19, 76.0%), respiratory (n=15; 60.0%) and gastrointestinal (n=12; 48.0%) signs. A wide variety of canine distemper clinical signs was found in suspected and confirmed cases as shown in Figure 2. The most frequent clinical signs among the suspected group were conjunctivitis (n=17, 41.5%), myoclonus (n=17, 41.5%), cough (n=15, 36.6%) and vomiting (n=15, 41.5%). The same Top 4 clinical signs were found in the confirmed group: conjunctivitis (n=13,

52.0%), myoclonus (n=13, 52.0%), cough (n=12, 48.0%), vomiting (n=10, 40.0%), plus diarrhoea (n=10, 40.0%). Hyperkeratosis was only observed in confirmed cases.

The hemogram of investigated patients are compiled on Table 2. In general, the results are within range values. Focusing on the confirmed group the hematocrit was normal in 13 dogs (56.5%); it was decreased in 9 dogs (39.1%) and increased in only 1 dog (4.4%). Concerning platelet counts, 14 dogs (66.7%) were normal, 6 dogs (28.6%) had a decrease, and only 1 dog (4.8%) showed an increase. White blood cells' counts revealed that, total leucocytes were normal in 10 (47.6%), increased in 7 (33.3%) and decreased in 4 (19.0%). The lymphocytes were normal in 12 (60.0%) dogs, decreased in 7 (35.0%) and increased in only 1 dog (5.0%). The segmented neutrophils were normal in 9 dogs (45.0%), in-creased in 8 dogs (40.0%) and decreased in 3 dogs (15.0%). Banded neutrophils were normal in 18 dogs (90.0%), increased in 2 dogs (10.0%) and did not decrease in any dog (0%).

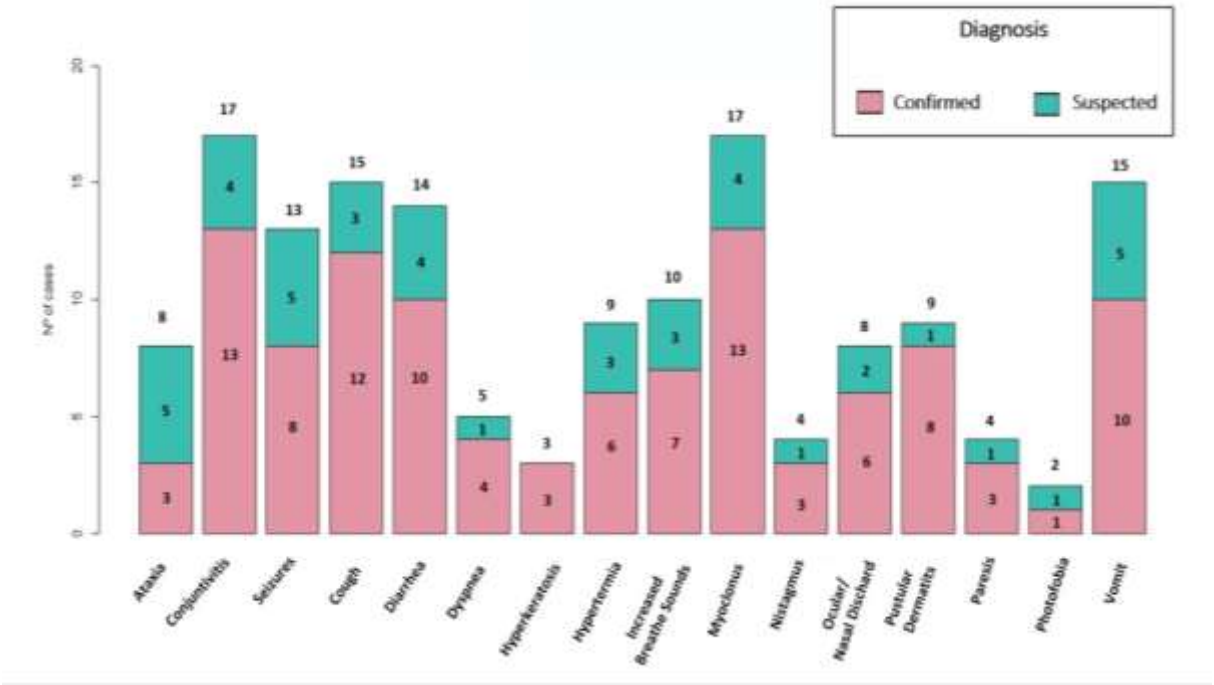

Figure 2 - Distribution of clinical signs in the suspected group (n=16) and in the confirmed group (n=25).

Table 2 - Hemogram results of suspected (n=41) and confirmed (n=25) CDV cases

Results	Suspected group							Confirmed group						
	Increase		Normal		Decrease		Total	Increase		Normal		Decrease		Total
	n	%	n	%	n	%		n	%	n	%	n	%	
Hematocrit	2	5.3	19	50.0	17	44.7	38	1	4.4	13	56.5	9	39.1	23
Platelets	1	2.8	22	61.1	13	36.1	36	1	4.8	14	66.7	6	28.6	21
White blood cells	15	41.7	16	44.4	5	13.9	36	7	33.3	10	47.6	4	19.0	21
Lymphocytes	4	12.5	20	62.5	8	25.0	32	1	5.0	12	60.0	7	35.0	20
Segmented neutrophils	16	50.0	13	40.6	3	9.5	32	8	40.0	9	45.0	3	15.0	20
Banded neutrophils	6	18.8	26	81.3	0	0.0	32	2	10.0	18	90.0	0	0.0	20

The findings regarding the diagnostic test used in the confirmed group (n=25) were the following: 18 dogs (72.0%) had a positive IgM serology test (unvaccinated animals), 5 dogs (20.0%) had a positive RT-qPCR, and 1 dog (4.0%) had an IgG positive serology on CSF. RT-qPCR positive tests were obtained in a variety of biological samples: blood (n=1); CSF (n=1), conjunctival/oronasal swabs (n=2); blood and feces (n=1). Concerning the suspected group (n=16): 10 dogs (62.5%) remained suspected

due to inconclusive serology results, 4 dogs (25.0%) lacked laboratory testing, 2 dogs (12.5%) because of inconclusive RT-qPCR, due to insufficient sampling.

The median length of hospitalization was 3.0 days, ranging from 1 to 20 days (n=41). The median length of hospitalization of the confirmed group (n=16) was 3.0 days, ranging from 1 to 20 days. The median length of hospitalization of the suspected group (n=25) was 2.0, ranging from 1 to 7 days. No statistically significant differences were found (p=0.38). The survival rate at discharge was 48.0% for the confirmed group and 63.4% for the suspected group.

The temporal distribution of cases is observed in Figures 3 and 4. The monthly distribution of suspected and confirmed distemper cases plotted on Figure 3 shows more admissions at BICU in the winter months of December and January (IPMA, 2020). Distribution during the study period (Figure 4) shows the emergence of cases in 2014 with peek of frequency in 2016 and 2017, and a decline in 2018.

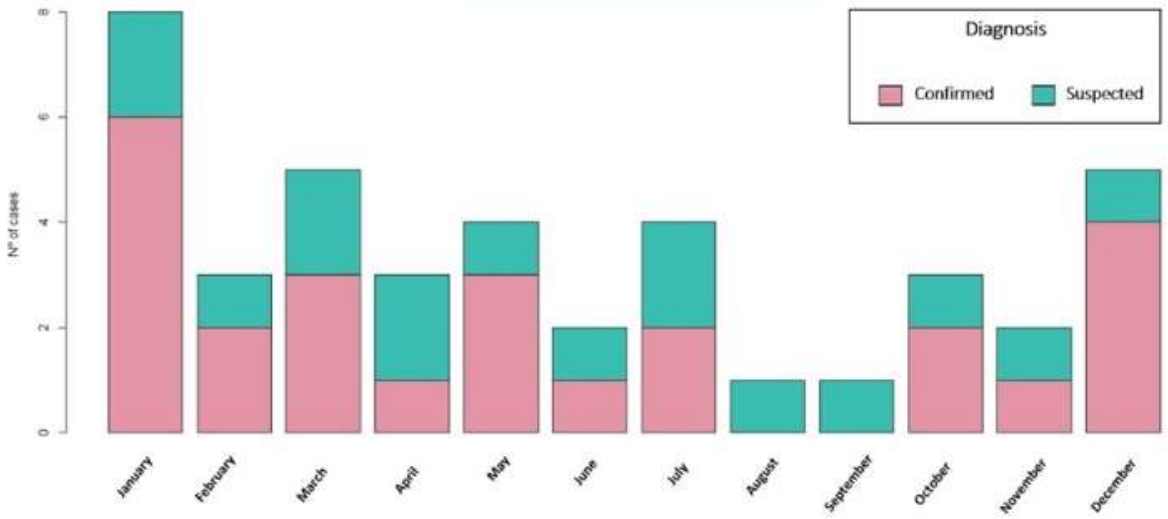

Figure 3 - Monthly distribution of suspected and confirmed CDV cases.

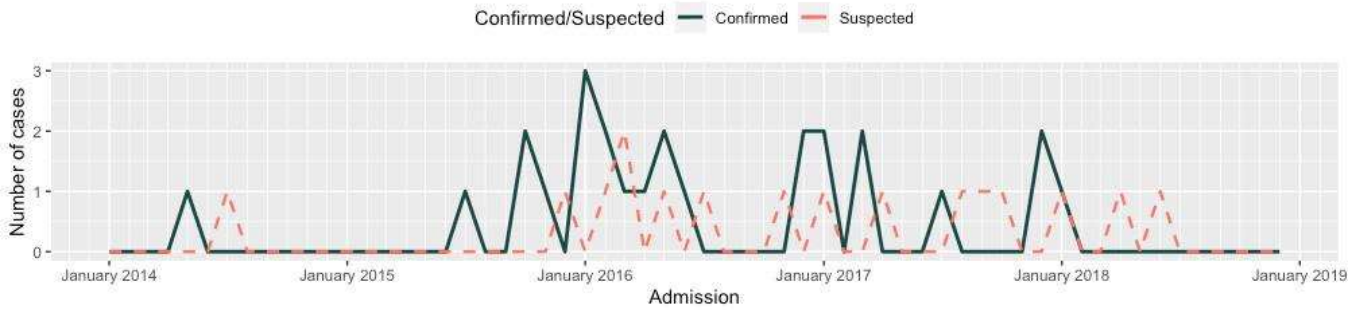

Figure 4 - Distribution of suspected and confirmed CDV cases during the study period.

3. Discussion

The 2014-2018 outbreak of canine distemper in Lisbon Metropolitan Area was investigated. Forty-one dogs were studied, of which twenty-five had a conclusive distemper diagnosis and sixteen were suspected. As observed in Figure 4, it was considered an outbreak event of canine distemper because we could recognize the beginning and the upward phase of the outbreak following its descending phase. The available data did not allow confirmation nor rejection of possible consecutive resurgence events after the initial clinical cases, or to distinguish different waves of the same epidemic as the complete viral genome virus was not sequenced to characterize the field strain. The outbreak was extinct due to the gradual development of immunity in the population of dogs because of vaccination and natural exposition to CDV virus. As our results pointing a reduced rate of vaccination compliance may explain the occurrence of cases during a 4-year period.

The geographical area encompasses 18 municipalities divided by the two banks of the Tagus River. It is the most densely inhabited metropolitan area in Portugal with almost three million residents, covering an area of 3015 square kilometres (AML, 2021). The geographical dispersion of cases shown in Figure 1 illustrates the easy spread of CDV during the

outbreak. Transmission was probably due to the contact of susceptible dogs with infected dogs improperly vaccinated or unvaccinated (Anis *et al*., 2018), stray dogs (Kapil *et al*., 2008) or wildlife animals, namely red foxes (*Vulpes vulpes*) (Alexandre *et al*., 2020; Beineke *et al*., 2015; Ek-Kommonen *et al*., 1997; Trebbien *et al*., 2014) in the infectious phase or with viable virulent products dispersed in the ecosystem (Greene, 2012). The lack of data about dog movements, the role of hunting dog packs, plus scarce data about wild CDV reservoirs in Portugal (Santos *et al*., 2009) did not allow in depth investigation of location as a potential risk factor. Even though two locations drew our attention. Some hospitalized, suspected and confirmed cases (n=6) were referred to BICU from public and private shelters. This were clusters of disease, probably due to the hosting of infected dogs. Several veterinarians working on Floodplain and Coastal Park of Loures (Parque da Várzea e Costeiras de Loures) area described various cases, some fatal, with clinical presentations compatible with canine distemper, but unfortunately without a definitive diagnosis, also a few hospitalized infected dogs in our study wandered in this ecosystem of rivers, streams and marshes that may have prolonged the survival of CDV on the environment. Some of these dogs were hunting dogs.

The months with more confirmed cases were December and January, the wettest and coldest months in Portugal during the outbreak period (IPMA, 2020). A strong reduction of cases was observed in summer months and no cases were verified at the BICU in August and September. This is consistent with CDV being a fragile virus, enveloped, relatively unstable outside the host, sensitive to high temperatures and low relative humidity, tending to vanish from the ecosystems during summer (Ek-Kommonen *et al*., 1997; Greene, 2012; IPMA, 2020; Sykes, 2014a). This seasonal pattern characterized by very dry summers persisted from 2014 to 2018 (IPMA, 2020) and must have contributed to reduce the magnitude of the outbreak.

Both study groups, suspected and confirmed distemper cases, had a median age of 1-year-old ranging from 4 months to 15 years. Narrowing the analysis to the confirmed group, the most represented age group was less than 1 year (40.0%). Y*et al*l age classes were affected. This is in accordance with the known distribution of canine distemper across all ages, but with increased susceptibility in young animals (Cleaveland *et al*., 2000; Sykes, 2014a). Even though no statistically significance was found considering the age of the confirmed cases (p=0.15).

Despite a higher frequency of males, on both suspected and confirmed groups, in comparison with females, no statistically significant results were found concerning the gender of confirmed cases (p=0.1). This result is consistent with many canine distemper epidemiological studies reporting the absence of significant differences in males and females' frequency of CDV infections (Arjo *et al*., 2003; Headley and Graça, 2000).

According to our data analysis, breed may be a protection factor for distemper (p=0.036; OR=0.4336; 0.1775<OR<1.0592). This may be a biased result reflecting more health investment given to pure breed dogs, like vaccination, as well as a more contained dog lifestyle.

To prevent CDV infection, puppies must receive a primary vaccination from 6 to 8 weeks of age until 16 weeks or later and rappel until 1-year age, and then every 3 years for adult dogs (Day *et al*. 2016). The proportion of immune dogs is essential for individual protection but also for the development of herd immunity that prevents the occurrence of outbreaks (Day *et al*., 2016; Greene, 2012; Waner *et al*., 2006). Data obtained from clinical reports show the dogs investigated in this study were poorly immunized against core infectious diseases, as the proportion of vaccinated animals was less than 20% in both groups: 16.1% in the suspected group, 15.7% in the confirmed group. This similarity explains that no statistically significant result was found regarding the vaccination status of dogs diagnosed with distemper (p=0.1). The global economic recession of early 2010s affected severely Portugal (Day *et al*., 2016; Krugman, 2017). This financial crisis and recession may have reduced compliance of dog owners in preventive health care, leading to a progressive decline in the dog population's immunity against CDV, and consequently this outbreak emergence in the Lisbon Metropolitan Area (Day *et al*., 2016; Wensley, 2013;). Older animals tend to present with delayed vaccination schedule and younger animals not vaccination or lacking vaccine boosts. In both cases exposing animals to risk of contract CDV. A similar scenario happened in Finland where canine distemper reappeared in dogs in 1990 after a 16-year absence, culminating in an outbreak in 1994-1995. It was concluded that a critical decrease in the dog population's immunity during 1990 to 1994 was the main explanation for the outbreak (Xue, X. *et al*., 2019). The existence of cases among vaccinated dogs (n=3, 16.1%) in our study might be related with vaccination failures in puppies due to interference of maternal antibodies, failure to comply with revaccinations, but also to possible antigenic differences between the vaccine strains and the currently circulating wild-type strains. This problem was already found in the US (Norris *et al*., 2006), suggesting the need to develop an updated CDV vaccine (Anis *et al*., 2018) and should be investigated urgently in Europe.

As presented previously, both groups displayed similar clinical signs patterns (Figure 2), with all major signs of CDV infection present in both groups, except hyperkeratosis that was only observed in the confirmed group. The most frequently recorded clinical signs were also similar: conjunctivitis, myoclonus, cough and vomiting in the suspected group. Conjunctivitis, myoclonus, cough, vomiting and diarrhoea in the confirmed group. This reinforces the rational that some dogs that remained on the suspected group would have been confirmed if their owners had the willingness and economic capacity to

pay for conclusive diagnostic tests. The clinical presentation in the confirmed group is consistent with the wide variety of clinical presentations that in severe forms lead to hospitalization and the disease course (Sykes, 2014a; Wiebe, 2015). Infected dogs usually presented hyperthermia, anorexia, followed by conjunctivitis (serous to purulent), dry cough, vomit, diarrhoea (Greene, 2012; Sykes, 2014a), and neurologic signs that tend to develop 1 to 6 weeks after acute disease (Greene, 2012). Neurologic signs were mainly myoclonus, hyperesthesia, seizures and paresis (Carvalho *et al*., 2012; Mahajan *et al*., 2018). Probably due to the late appearance of this sign during a chronic course of the disease (Rendon-Marin *et al*., 2019; Sykes, 2014a), hyperkeratosis was only observed in the confirmed group.

The results of blood analysis, hematocrit and platelets were not helpful to differentiate the two groups (Table 2). Even though some researchers report mild anemia and thrombocytopenia in canine distemper patients (Barger, 2003; Greene, 2012; Sykes, 2014a), most of the dogs investigated showed normal hematocrit and platelet count. The same trend was found for white blood cell counts in both groups, but there was a high proportion of leukocytosis in the suspected group and a high proportion of leukopenia in the confirmed group. Lymphopenia was present in a higher frequency in the confirmed group than on the suspected group. The opposite was observed concerning neutrophilia (segmented and banded). These results suggest the presence of bacterial infections (respiratory or abdominal) usually associated with leukocytosis due to neutrophilia (Sykes, 2014b; Sykes, 2014c). On some distemper suspected dogs, bacterial infections were the final diagnosis, but they could be concomitant infections masking cases of distemper (Hiebl *et al*., 2019). In the confirmed group lymphopenia was a more frequent finding, often cited on the literature (Greene, 2012; Martella *et al*., 2008; Rudd *et al*., 2010).

The definitive diagnosis of canine distemper was challenging because the disease presented a wide variety of clinical presentations; a proportion around 16% of the studied dogs were vaccinated with live-attenuated vaccines; many practitioners had never seen canine distemper following decades of rare sporadic cases in the area. In the present study, a high proportion of suspected dogs (62.5%) remained in this category due to inconclusive serology, unknown vaccination history or the record of a recent vaccination boost that could be responsible for a false-positive result. In fact, 31.3% (n=5) of suspected cases had a recent vaccination boost that invalidate a definitive diagnosis. These cases occurred isolated, well distributed across study period. Inconclusive RT-qPCR was obtained in 12.5% (n=2) of the suspected dogs, due to insufficient sampling as a single site sample in a multisystemic disorder is not enough to exclude disease. In both cases dog presented neurologic signs, but PCR was performed either in fecal or blood samples. These results support the need to collect multiple specimens according to clinical signs (Kapil and Yeary, 2011; Sykes, 2014a).

Our results reinforce the relevance to perform an accurate anamnesis with a complete vaccination history, and to make a good physical exam allowing for the detection of a wide range of clinical signs (Amude *et al*., 2006). The most used test to confirm distemper was IgM serology in unvaccinated dogs (72.0%), as it requires a simple blood sample and it is affordable (Greene, 2012; Kubo *et al*., 2008). RT-qPCR was performed in 20.0% of the samples. It detects antigen, it is a very sensitive and specific test (Amude *et al*., 2007), but much more expensive than IgM serology. In patients presenting neurologic signs, RT-qPCR in CSF is the gold standard diagnostic test. However, because it requires an invasive and expensive sample collection technic, which is rather dangerous in dogs presenting seizures and therefore unstable for anesthesia (Amude *et al*., 2007), it was not possible to use it in this study.

Dogs of the confirmed group (n=25) stayed 1 more day at BICU than dogs of the suspected group (n=16), median lengths of hospitalization were respectively 3 days, ranging from 1 to 20 days, and 2 days, ranging from 1 to 7 days. Two reasons may explain this difference; on the one hand, some suspected dogs suffered from other health conditions with better outcomes and shorter hospitalization periods, and on the other hand, dog owners that could not pay laboratory tests to confirm distemper could not afford long hospitalization stays. This last condition was very impactful on the average length of stay.

The outcome of distemper varies depending on the clinical presentation, dogs with mild symptoms may recover almost without veterinary supervision, but severe cases benefit from hospitalization with supportive treatment (Wiebe, 2015). The development of different clinical signs throughout the course of the disease is possible, especially with regard to neurologic signs (Greene, 2012). The survival rate is also influenced by the clinical presentation as about 30% of dogs develop neurological signs (Sykes, 2014a), in which survival is reduced (Greene, 2012; Sykes, 2014a). This same pattern was observed in our study. At discharge from first hospitalization, the survival rate of confirmed CDV cases was 48.0%, but as some dogs were later readmitted, the final survival rate dropped to 32.0%. Considering all admissions, the survival rate for dogs hospitalized for 3 or less days (median hospitalization length) was 30.8% in comparison with a survival rate of 33.3% for animals hospitalized more than 3 days. These results are consistent with other studies (Machado, 2016; Xue, X., 2019).

4. Conclusions

Canine distemper was successfully controlled in many countries since the development of distemper modified live vaccines in the 1950s, followed by routine vaccinations of dog populations with polyvalent

vaccines against core canine infectious diseases (distemper, parvovirosis and infectious hepatitis) with these vaccines. In Portugal, scheduled canine distemper vaccinations began in late 1970s. This immunization strategy led to disease control and then to epidemiological silence in Lisbon Municipality, here and there interrupted, by rare sporadic cases. Until the CDV outbreak of 2014-2018 that spread into six of the eighteen municipalities of the Lisbon Metropolitan Area and caught unaware a generation of young veterinarians who had never seen this multisystemic infectious disease with a wide variety of clinical presentations. To make the scenario more complicated only 15.7% of the infected dogs hospitalized at BICU had been subjected to a complete CDV vaccination plan, mainly due to the 2010s global economic recession that affected Portugal badly. That is why it is relevant to characterize and share information about canine distemper outbreaks among the veterinary community because there have been reports of canine distemper reemergence in several countries. In another perspective, this study reinforces the need to improve the communication with dog owners regarding the importance of strict compliance to vaccination schedules. Relaxation in vaccination, progressively leads to loss of herd immunity predisposing to the occurrence of epidemic outbreaks. The CDV can be quickly disseminated by packs of stray dogs, hunting dogs and red foxes, increasingly seen in urban environments of the Lisbon Metropolitan Area.

In addition, this study demonstrates the improved value of the systematic collection, analysis and sharing of clinical data for outbreak identification, but it is crucial to improve surveillance of infectious diseases, in order to prevent future events. Particularly in distemper surveillance to monitor which CDV strains circulate in the ecosystems, to ensure the early detection of new CDV variants might be essential, as various episodes of distemper in vaccinated dogs have been reported worldwide.

Finally, an upsurge of canine distemper may impair the success of the Iberian wolf (*Canis lupus signatus*) conservation program and the action plan for the reintroduction of the Iberian lynx (*Lynx pardinus*) in force in Portugal.

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