The Veterinary Journal 200 (2014) 257–260

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Serological and virological detection of canine herpesvirus-1 in adult dogs with and without reproductive disorders

A. Pratelli *, V. Colao, M. Losurdo

Department of Veterinary Medicine, University of Bari, Strada per Casamassima km 3, 70010 Valenzano, Bari, Italy

ARTICLE INFO

Article history:
Accepted 3 March 2014

Keywords:
Canine herpesvirus 1
Immunofluorescence
PCR
Serum neutralization
Viral shedding

ABSTRACT

Canine herpesvirus 1 (CaHV-1) is known to cause reproductive disorders in adult dogs and neonatal mortality in puppies. The seroprevalence of CaHV-1 has not been documented in Italy. Sera from 865 dogs were screened for CaHV-1 using a serum neutralization assay (SN). All CaHV-1 positive sera and 100 CaHV-1 negative sera were also tested using an in-house immunofluorescence (IF) test. Thirteen bitches with reproductive disorders and three bitches with no history of reproductive diseases were also examined clinically so that lesions associated with CaHV-1 and CaHV-1 DNA could be identified using PCR analysis of vaginal swabs.

An overall seroprevalence of 14.6% was observed using SN, and 18.6% using IF. The correlation between SN and IF was moderate. The SN assay demonstrated a greater sensitivity than IF, with a few exceptions. None of the vaginal swabs tested positive for CaHV-1 DNA. The differences in the seropositivity rates between SN and IF were not statistically significant ($P = 0.16$). Using the SN test as the reference standard, the sensitivity and specificity of IF were 29% and 95%, respectively. These results suggest that CaHV-1 is common in canine populations and could pose a threat to neonatal survival and canine fertility in breeding kennels in Italy. Vaccination of breeding bitches should be recommended if there is a history of reproductive disorders.

© 2014 Elsevier Ltd. All rights reserved.

Introduction

Canine herpesvirus 1 (CaHV-1) is classified in the genus *Varicellovirus*, subfamily *Alphaherpesvirinae*, family *Herpesviridae*. The virus was first isolated and identified almost simultaneously by three different research groups in 1965 from cases of fatal disease in puppies and fetuses and from dog kidney cell cultures (Carmichael et al., 1965; Spertzel et al., 1965; Stewart et al., 1965).

In susceptible puppies, the virus can produce a fatal systemic necrotizing and haemorrhagic disease, but the infection is usually subclinical in puppies >2 weeks old (Carmichael et al., 1965; Evermann et al., 2011). In adult dogs, CaHV-1 is associated with clinical disorders of the reproductive system, including embryonic death and/or resorption, abortion and stillbirth. Infection can cause vesicular lesions in the vestibule and vaginal mucosa and/or severe vaginitis in bitches, as well as vesicles on the penis and the preputial mucosa of dogs. CaHV-1 is sporadically associated with upper respiratory and ocular disease in older dogs (Ledbetter, 2013). Natural infection of susceptible puppies primarily occurs via the oronasal route from the birth canal during parturition (Appel, 1987), but venereal and transplacental transmission is also possible (Evermann et al., 2011).

After either acute disease or subclinical infection, dogs become latent viral carriers and CaHV-1 can be isolated from the trigeminal and lumbo-sacral ganglia, the salivary glands, tonsils and liver, even in the absence of clinical signs (Burr et al., 1996). Reactivation of latent virus might be associated with stress or, experimentally, if immunosuppressive drugs or anti-lymphocyte sera are administered (Ledbetter et al., 2012).

CaHV-1 is considered poorly immunogenic and neutralizing antibodies can fluctuate for 4–8 weeks following exposure, rarely lasting more than few months after infection (Carmichael and Greene, 1998; Nöthling et al., 2008). Numerous epidemiological factors could affect canine seroprevalence. Larger kennels and breeding kennels with histories of neonatal infection are more likely to have positive dogs, and one study reported significantly higher anti-CaHV-1 titres with increasing age, in male dogs that were mated and older bitches (Ronsse et al., 2004). It is presumed that CaHV-1 is widespread worldwide, but there is a paucity of prevalence studies reporting viral shedding and antibody titres. A long-term survey of dogs in a rehoming centre documented CaHV-1 in 9.6% of lung and 12.8% of tracheal specimens (Buonavoglia and Martella, 2007).

Along with canine minute virus (CnMV), CaHV-1 remains a major and severe health problem in breeding dogs and therapeutic measures to counteract the infection are not available (Pratelli and

* Corresponding author. Tel.: +39 080 4679835.
E-mail address: annamaria.pratelli@uniba.it (A. Pratelli).

http://dx.doi.org/10.1016/j.tvjl.2014.03.001
1090-0233/© 2014 Elsevier Ltd. All rights reserved.

Table 1
Sequence and position of the oligonucleotides used in PCR testing for canine herpesvirus (CaHV).

Primer	Sequence 5′ to 3′	Polarity	Target gene	Position[a]	Amplicon size
CaHV-1	TGCCGCTTTTATATAGATG	+	TK	283–301	493 bp
CaHV-2	AAGCGTTGTAAAAGTTCGT	–		758–776	

[a] Oligonucleotide positions are referred to the sequence of CaHV-1 thymidine kinase (TK) gene (GenBank accession no. X75765) for gel-based PCR amplifications.

Moschidou, 2012). CaHV-1 has been reported in many countries and the prevalence of antibodies in dogs tested worldwide varies markedly. The use of different serological methods and sampling frames within the canine population also influences test results and CaHV-1 seroprevalences in different geographical locations can vary enormously. In The Netherlands, the proportion of seropositive dogs at the end of the 1990s was particularly high (39.3%; Rijsewijk et al., 1999). The seroprevalence of CaHV-1 among dogs in the South-East of Iran was estimated to be 20.7%, specifically 22.9% and 19.1% in kennelled and owned dogs, respectively (Babaei et al., 2010). In the Gauteng Province of South Africa, among 328 canine sera tested with both serum neutralization (SN) and ELISA, 22% of the sera were positive (Nöthling et al., 2008). In a Turkish dog population, the seroprevalence by ELISA was 39.3%, but by SN was 29.4% (Yeşilbağ et al., 2012). An overall CaHV-1 seroprevalence of 45.75% was observed in the Belgian dog population (Ronsse et al., 2002), while in Norway and Finland, the seroprevalence was about 80%, suggesting that CaHV-1 is endemic in those countries (Dahlbom et al., 2009; Krogenæs et al., 2012).

Epidemiological studies have not previously been documented in Italy. In this study, sera from 865 dogs from Southern Italy were screened for CaHV-1 antibodies. Furthermore, 16 bitches with and without a history of reproductive disorders were examined clinically to identify CaHV-1 associated lesions; vaginal swabs were tested using PCR to detect viral DNA. Finally, the relationship between reproductive disorders, viral excretion and serology was evaluated to determine the potential importance of subclinical carriers of CaHV-1.

Materials and methods

Dogs and sampling

Dogs of at least 1 year of age and without a history of vaccination against herpesvirus were included in the present study. The study group consisted of 849 serum specimens collected during routine examinations at a Clinical Diagnostic Laboratory in Southern Italy. Venous blood was collected into glass tubes without heparin and serum was withdrawn and stored at –80 °C until analysis was performed. Sixteen bitches from different breeding kennels were included in the study. The vulva and vaginal vestibule were examined for CaHV-1 associated lesions. Three bitches had histories of abortion, seven bitches had histories of infertility, three bitches gave birth to puppies that were either stillborn or died in the neonatal period, and three bitches had no history of reproductive disorders. Vaginal swabs were collected in sample medium (Dulbecco Minimal Essential Medium, DMEM), and serum specimens from each bitch were stored at –80 °C until tested.

The 865 serum specimens were grouped into two categories for statistical analysis: 286 sera were collected from kennelled/breeding dogs and 579 from pet dogs.

Serum neutralization test

The CaHV-1 DK13 strain (supplied by Professor L.E. Carmichael, James A. Baker Institute for Animal Health, Cornell University) was used. The virus was propagated on A72 cell lines grown in DMEM supplemented with 10% fetal calf serum (FCS). The stock virus used in the study was at the ninth passage and had an infectivity titre of $10^{4.5}$ 50% tissue culture infective dose ($TCID_{50}$)/50 µL.

All serum specimens were heat treated at 56 °C for 30 min to inactivate endogenous complement. Two-fold dilutions, starting from 1:2 for each specimen, were performed in duplicate and mixed with 100 $TCID_{50}$ CaHV-1 strain in 96-well tissue culture microplates (Falcon-Becton, Dickinson Labware). The plates were kept at room temperature for 90 min before 20,000 A72 cells were added to each well. Cell cultures were examined daily for 4 days. The SN titre was defined as the reciprocal of the highest serum dilution that inhibited cytopathic effect (cpe) in 50% of the cell cultures.

Immunofluorescence test

The SN-positive serum specimens and 100 SN-negative specimens were also tested for antibodies against CaHV-1 using an in-house immunofluorescence (IF) test. The 100 SN-negative sera were selected from owned dogs, from kennelled dogs and from breeding bitches.

A72 cells infected with CaHV-1 strain DK13 were placed in multi-chamber culture slides (LAB-TEK Chamber Slide System) and fixed with 80% acetone. Serial two-fold dilutions of each serum, starting from 1:50 to 1:800, were performed in phosphate buffered saline (PBS). Diluted specimens (20 µL) were placed in duplicate into wells and incubated for 30 min at 37 °C in a moist incubator. Slides were washed three times in PBS and blotted dry. Aliquots of pre-titrated goat anti-dog IgG conjugated to fluorescein isothiocyanate (FITC; Sigma Chemicals) diluted 1:50 (20 µL) were added to each well. The slides were then incubated for 30 min at 37 °C, washed again in PBS, counterstained with Evans blue (BioMérieux) and examined under a fluorescent microscope. Approximately 75% of the cells in each well were infected, providing an internal negative control for the non-specific binding of antibodies to the cell monolayer. The titre was read as the highest serum dilution that produced detectable levels of fluorescence in foci of virus-infected cells. Antibody titres <1:50 were scored as negative.

DNA extraction and PCR analysis

DNA was extracted from 200 µL of viral suspension from vaginal swabs using the DNeasy Tissue Kit (Qiagen), following manufacturer's instructions. DNA from each specimen was eluted in 200 µL of elution buffer and diluted 1:10 in distilled water prior to molecular analysis to decrease residual inhibitors of DNA polymerase activity to an ineffective concentration. A conventional PCR assay, targeting the TK gene of CaHV-1, was performed as described previously by Schulze and Baumgärtner (1998) and modified by Decaro et al. (2010). PCR amplification was conducted using LA PCR Kit Ver. 2.1 (TaKaRa Bio) in a 50 µL reaction containing 1 µmol/L of primers CaHV-1 and CaHV-2 (Table 1), LA PCR Buffer (Mg^{2+} plus) 1 x, 8 µL of dNTP mixture (corresponding to 400 µmol/L of each dNTP), 2.5 units of TaKaRa LA Taq and 10 µL of 1:10 diluted template DNA. The cycling protocol consisted of preheating at 94 °C for 3 min, followed by 40 cycles of 94 °C for 30 s, 53 °C for 30 s and 72 °C for 1 min, with a final extension at 72 °C for 10 min. The PCR products were detected by electrophoresis in 1.5% agarose gel and visualization under UV light after bromide ethidium staining.

Statistical analysis

Data were analyzed using freeware (R Software, version 2.8.1)[1]. The differences between the proportions of positive sera detected by SN and IF, respectively, were compared using chi-squared tests, applying the Yates correction (for continuity) for dichotomous variables. To determine if dog housing was a risk factor, the chi-square test was also used to assess the statistical association between disease status and housing type. Statistical significance was set at $P < 0.05$.

Results

Of the 849 serum specimens collected during routine examinations and tested by SN, 124 were positive and 725 were negative (titre <2). Positive sera had titres of 2–4 ($n = 65$) to up to 64 ($n = 59$). Among the 16 sera collected from breeding bitches with and without reproductive disorders, only two specimens (12.5%) had positive results, with titres of 2–16 (Table 2). The two positive specimens belonged to the group of seven breeding bitches with a history of infertility. No significant differences in mean antibody titres were observed between bitches with and without histories of reproductive disorders ($P = 0.16$). The percentage of positive specimens did not differ between the kennel/breeding population (17.1%) and the owned dogs (13.2%).

[1] See: http://www.r-project.org/ (accessed 2 March 2014).

Download English Version:

https://daneshyari.com/en/article/5798027

Download Persian Version:

https://daneshyari.com/article/5798027

Daneshyari.com