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Detection of Canine Herpesvirus Infection on Dogs

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Authors' contributions

This work was carried out in collaboration between all authors. Author OY designed the study, authors MK and SH performed the statistical analysis, authors OA and OB wrote the protocol, authors SY and AS wrote the first draft of the manuscript. Author OA managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Canine herpesvirus (CHV) seems to be present worldwide in both domestic and wild dogs. Serologic surveys have shown a relatively high prevalence of CHV in household and colony-bred dogs. Blood samples were collected from dogs in dog shelters or dog collection centres in Konya and Antalya that showed clinical signs of the disease. In total, 141 blood samples were examined for antibodies to CHV by commercial enzyme-linked immunosorbent assay, whereas the blood leukocyte samples obtained from the dogs were assessed by immunoflouresence test (IFT) for isolation of CHV. Madin-Darby canine kidney cells were used for virus isolation. Ninety-seven samples (68.8%) were detected as seropositive; while no positive results were identified using IFT. In conclusion, the high seropositivity may indicate that CHV infection is common in dog shelters or dog collection centres in Turkey and infected animals with CHV need to be identified and quarantined.

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1. INTRODUCTION

Canine herpesvirus (CHV) is classified in the Varicellovirus genus of the *Alphaherpesvirinae* subfamily of the *Herpesviridae* family, Herpesvirales order, which has a double-stranded DNA (dsDNA) genome and has been a recognised cause of a fatal haemorrhagic syndrome of neonatal puppies [1]. CHV was first described in 1965 as a pathogen responsible for a fatal generalized haemorrhagic disease of newborn pups [2]. CHV is principally a perinatal pathogen of pregnant bitches and newborn pups and secondarily a respiratory tract pathogen of older pups and dogs [3].

CHV is related to Feline Herpesvirus (FHV) and PhocidHerpesvirus (PHV), but the infection is restricted to the members of the Canidae family [4]. The virus spreads oronasally [5], but venereal or transplacental transmission is possible [6]. Canine herpesvirus is also known to establish latent infections in dogs that can be readily reactivated by immunosuppressive drug treatment [7]. The potential of herpesvirus reactivation and shedding following periods of latency plays a crucial role in the epidemiology of these viruses [8]. Serology studies can determine whether the animal has been exposed to herpes but cannot identify if it is currently causing disease or shedding at that moment. Pathology is the most definitive way to diagnose CHV, but at that stage it is too late for the puppy [9].

The aim of this study was to determine the CHV infection status of dogs in kennels or dogs privately owned in the Konya and Antalya regions of Turkey.

2. MATERIALS AND METHODS

2.1 Animals and Samples

In this study, 141 dogs (67 kennel and 74 individual, ≤1 and ≥4 years old) were randomly sampled and tested according to their clinical symptoms (oculonasal discharge, fever, conjunctivitis, and dehydration). Corneal opacity or photophobia was not identified in any dog. No animals had been vaccinated for CHV-1. From November 2010 to February 2011 in Konya and Antalya, blood samples from 141 dogs were collected in normal serum separating tubes and also in tubes with EDTA to determine the presence of CHV antibodies and antigens. Sera and leukocyte samples were prepared from blood samples using a standard method. Sera were analysed to detect specific antibodies against CHV by commercially available indirect enzyme-linked immunosorbent assay (ELISA). The leukocyte samples were processed and inoculated on to confluent monolayer of Madin-Darby Canine Kidney (MDCK) cells using standard virological techniques. The inoculated cells were incubated at 37ºC and observed daily for the appearance of cytopathic effects (CPE). After the third passage, cells were examined by IFT for virus isolation.

2.2 Cell Cultures

The MDCK cell line was obtained from the Department of Virology, Faculty of Veterinary Medicine at the University of Selcuk. MDCK cells were grown in Dulbecco's Minimum Essential Medium (DMEM, Biological Industries, Israel) supplemented with 100 U of penicillin/mL, 100 µg of streptomycin/mL and 10% foetal calf serum (FCS, Biological Industries, Israel) for virus isolation attempts. The cells were incubated at 37ºC in a 5% carbon dioxide (CO_2) incubator.

2.3 Virus Isolation from Cell Culture

The leukocyte samples were stored in a deep freezer (-20ºC) and then rapidly thawed in a water bath at 37ºC. The MDCK cell culture was prepared in 96-well micro plates, and 2 wells were inoculated with 10 µL of the leukocyte samples. Subsequently, the micro plates were maintained at 37ºC in an incubator with 5% CO_2. The third passages of the samples grown in MDCK cell culture were applied to 24-well micro plates and stored until their use for IFT.

2.4 Indirect ELISA

In serological studies, ELISA (Institute Pourquier, Montpellier, France) was used to investigate the presence of CHV antibodies in 141 serum samples. The test was performed as per the manufacturer's instructions. The plates were then read using spectrophotometry with a 450 nm filter on an automatic ELISA reader (Rayto RT-2100, China). The results are expressed as an inhibition percentage.

2.5 Immunofluorescence Test

The IFT was performed as described by Bulut et al. [10] for demonstrating CHV antigens. Briefly,

each well of the Lab-Tek chamber slides (Thermo Fisher Scientific, 178599, USA) was inoculated with 200 µL of MDCK cells that were diluted with DMEM + 10% calf serum, such that a cell concentration of $1x10^5$ cells/mL was obtained. After incubation, each leukocyte sample was inoculated into two wells. At the end of the third day, the medium was removed. The cell surfaces were rinsed with phosphate buffered saline (PBS) and fixed in acetone for 10 min. In a dark environment, 75 µL of the ready-to-use CHV conjugate (Catalog No: 210-13-CHV, VMRD, USA) were added to all wells. Following incubation, the conjugate was removed from the wells and allowed to dry for 10 minutes. Finally, the wells were examined under a fluorescence microscope (Olympus Bx51, Japan).

2.6 Statistical Analysis

Seropositivity ratios were evaluated by X^2 test (Minitab 12.0). A $p<0.05$ value was taken to indicate statistical significance.

3. RESULTS

Rates of antibodies of CHV according to provinces and breeding are presented in Table 1. An overall CHV seroprevalence of 68.79% (97/141) was observed by ELISA in Konya and Antalya dog shelters. No morphological change was observed in MDCK cell cultures, and a positive result was not detected by IFT. Of the 18 animals one year of age or younger, 7 dogs (38.8%) were positive for CHV antibodies. Fifteen (53.5%) of the 28 animals between one and two years of age, 21 (80.7%) of the 26 animals aged two years, 24 (82.7%) of the 29 animals aged three years, and 30 (75%) of the 40 animals four years of age or older were found to be seropositive (Table 2). Seropositivity rates of two- and three-year-old dogs were statistically higher ($p<0.05$) than the other age groups (Table 2).

Table 1. Rates of CHV antibodies according to provinces and breeding

Provinces	Type of breeding		(%)
	Kennel	individual	
Konya	56	-	72.72[a] (56/77)
Antalya	11	30	64.06[a] (41/64)
Total	67	30	68.79 (97/141)

[a]:*There was no statistical significance between provinces ($p>0.05$)*

Table 2. Age distribution of seropositive dogs

Age of dogs	Number of dogs	Number of positives (%)
≤ 1	18	7 (38.8%)[c]
1-2	28	15 (53.5%)[bc]
2	26	21 (80.7%)[a]
3	29	24 (82.7%)[a]
≥ 4	40	30 (75.0%)[ab]
Total	141	97 (68.7%)

[a, b, c] *values marked with different letters in the same line are statistically significant ($p<0.05$, χ^2 test)*

4. DISCUSSION

Canine herpesvirus, an Alphaherpesvirus, is found throughout the world in domestic and wild dogs [11]. As with other herpesviruses, CHV becomes latent and is carried by the affected individual for life, although clinical signs may not be present. Reactivation of the latent virus maybe provoked by stress (movement to new quarters, introduction of new dogs) or, experimentally, by immunosuppressive drugs (corticosteroids) or antilymphocyte serum [12]. As CHV-1 is presumed to be widespread among the dog population and as economic losses in breeding kennels after infection with CHV-1 may be disastrous, it seems necessary to determine the prevalence of CHV-1 more accurately and to evaluate its significance [11].

The specific antibodies of canine herpesviruses do not remain at a high titre in the blood for long [13]. Therefore the detection of low levels of antibodies (especially neutralization antibodies) may be difficult. Different tests have been developed for the serodiagnosis of CHV-1; the serum neutralization (SN) test and ELISA are the most frequently used. ELISA is known to be highly sensitive and specific, and it presents the advantage of being simple, automable and less labour-intensive than the SN test [11]. Indirect ELISA is preferred in European countries. An ELISA-based study in the Netherlands showed that the seroprevalence in dogs was about 40% [14]. In contrast, Takumi et al. [15] developed an ELISA method to detect antibodies against CHV-1 and observed that 26.2% was positive in Japan.

In recent years, canine herpesvirus-1 seropositivity has shown an increase in dog populations in many countries. The titres were relatively low in Europe as well as in the United States (US) until 1980. Lundgren and Clapper [16] reported a seroprevalence of 12.8%, and Fulton et al. [17] obtained a 6% positive rate in the US. Osterhaus et al. [18] reported

seropositivities of 1 to 6%, whereas Van Gucht et al. [19] found positive titres in 49.5% of tested dogs in the Netherlands. Engels et al. [20] identified 6.3% seropositivity in Switzerland, while Reading and Field [21] found a 78% seroprevalence in the English dog population. In Belgium, Ronsse et al. [11] reported a rate of 79.4%. Seropositivity for CHV-1 was found to be 48.8% (46/94) in a kennel composed of newly collected stray dogs [22] in Turkey. Acar et al. [23] identified CHV in 71.8%, whereas Yesilbag et al. [24] found 39.3% seropositivity in Turkey. The obtained value (Table 1) in this study of 68.79% (97/141) was higher than that reported by Gur and Acar [22] and Yesilbag et al. [24] but lower than Acar et al.'s [23] findings.

Poor hygienic conditions, the size of colony and its management may affect the reactivation of latent infections and subsequent viral spread in large colonies [7]. Babaei et al. [25] suggested that kennel dogs had a higher infection rate (22.9%) compared with privately owned dogs (19.1%). Babaei et al. [25] and Ronsse et al. [11] reported that a significant difference was not observed between privately owned and kennel dogs. These researchers suggested that the CHV-1 prevalence is not necessarily higher in dog colonies and that overcrowding or management factors may play roles that are more important. On the other hand, Dalhbom et al. [26] found that dogs in large kennels had higher titres than dogs from smaller units. Ronsee et al. [5] also reported that dogs living in kennels that contained more than six dogs had higher antibody titres. After both symptomatic and asymptomatic infections, dogs remain latently infected, and the virus may be excreted at unpredictable intervals over periods of several months or years. However, due to the aforementioned reasons, the virus may be reactivated sometimes after infection. Shelters, especially crowded environments, can pose a risk for new-born pups.

Oronasal and venereal transmission are considered the main routes of CHV infection, but foetuses can also be infected in utero [6,27]. The most important route of transmission of CHV is oronasal. No differences in titres were found between mated and unmated dogs, and nasal transmission was suggested to have been the route of infection by Ronsse et al. [9]. Venereal transmission has also been reported by these authors but is far less common [5]. Bitches in the third trimester of pregnancy may transmit the infection transplacentally to the foetuses [6]. Experimentally infected dogs have been shown to shed the virus in nasal secretions irrespective of the routes of inoculation [28]. In this study, the prevalence found in dogs living in kennels was higher than that of individual dogs (Table 1). This high rate might be a result of oronasal contaminations, which can occur due to overcrowded conditions and the shape of the shelter management. In the present study, we failed to detect CHV-1 within blood samples using IF. Our inability to isolate CHV-1 may be particularly relevant to the types of samples used [29].

In this study, increased seropositivity was identified ($p<0.05$) in dogs two and three years of age (Table 2). The high seropositivity detected in animals hosted in crowded kennels not suitable in terms of hygiene might be a result of the management and the frequency of exposure to the virus. The higher antibody titres found in older dogs may indicate a booster effect following repeated exposure to CHV1 [30]. Babaei et al. [25] suggested that kennel dogs had a higher infection rate (22.9%) compared with privately owned dogs (19.1%), and the prevalence was higher for animals three years of age and older. Ronsee et al. [5] and Babaei et al. [25] both reported that the route of transmission of infection seropositivity increased with age. In our study, the high seropositivity detected in older animals hosted in crowded environments and improper shelters indicated that venereal transmission should also be considered.

Seo et al. [31] reported that seroprevalance increases with age. Lacheretz and Cognard [32] found the seroprevalance in dogs less than two years of age (0.7%) was significantly lower than in dogs upto the age of six. Ronsee et al. [5] observed that especially in bitches, progressive seroconversion with aging occurred, and they suggested that antibody titres increase around puberty and during the first two years of life. Researchers [5] notified that no dog younger than six months appeared seropositive. This finding can be explained by the shorter duration of exposure and reduced contact with the virus, but it could also be related to hormonal effects that enhance herpesvirus infection or reactivation and antibody production during puberty [5]. Dahlbom et al. [26] reported relationship between CHV-1 infection and reproduction problems as dogs from kennels with such problems had significantly higher CHV-1 titres than dogs from kennels without reproduction problems.

CHV-1 antibody titres may be affected by many factors. As the main reason for screening CHV-1 antibody titres in clinical conditions is to assess the disease's role in fertility disorders, other parameters, such as kennel history, clinical evaluation and complementary laboratory results, should be considered for conclusive etiologic diagnosis. Oronasal and venereal transmission appear to play a role in the spread of infection. Prevention is currently largely based around management, such as keeping puppies warm to reduce the likelihood of systemic infection. Routine vaccination for CHV-1 infection generally has not been applied in the field in Turkey. Extended studies will be necessary to determine the prevalence of CHV-1 infection; routine vaccination has also been proposed to control the infection. Close contact seems to be the most effective factor that increases the incidence of infection. Latency and factors triggering reactivation of CHV-1 are considerably important. Therefore, further epidemiological studies should be carried out on CHV-1.

5. CONCLUSION

In conclusion, CHV is commonly seen in dog shelters in the Konya and Antalya regions and poses a threat to other dogs.

ETHICAL APPROVAL

As per international standard or university standard written ethical permission has been collected and preserved by the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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