

## A Serologic Investigation of Canine Herpesvirus Type 1 Infection in Kangal Dogs

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**Abstract:** Canine Herpesvirus-1 (CHV-1) is an *Alphaherpesvirus*, the infection was detected throughout the world in dog population. In this study, 110 blood serum samples obtained from clinically healthy adult Kangal dogs, which are unvaccinated for CHV-1 in 4 private Kangal breeding kennels at Ankara province. The samples was controlled using indirect ELISA, as result of the test, out of 110 dog sera, 79 (71.8%) was found to be seropositive for CHV1. Positivity was found to be in all of the studied farms among 42.8 and 86.6% proportions. The important differences were not detected between male (21/32, 65.6%) and female (58/78, 74.3%) dogs as statistically. According to kennel records, no clinical findings related to the CHV1 infection were reported in any of the animals before, despite very high infection rates.

**Key words:** ELISA, Kangal, canine, herpesvirus, dogs, Turkey

### INTRODUCTION

Based on recent sequence data, Canine Herpesvirus-1 (CHV-1) was classified in Varicellovirus genus (Carmichael *et al.*, 1965; Spertzel *et al.*, 1965; Remond *et al.*, 1996), in an Alphaherpesviridae subfamily. The infection 1st described by Carmichael *et al.* (1965) as a fatal haemorrhagic disease. Today it is known that the infection is widespread throughout the world in dog population (Burr *et al.*, 1996; Carmichael and Grene, 1998; Carmichael *et al.*, 1965; Engels *et al.*, 1980). Although, the virus could have infects the dogs in all ages, newborn puppies are more sensitive (Apell, 1987).

CHV-1 has close antigenic relationship with Feline Herpesvirus-1 (FHV-1) (Limcumpao *et al.*, 1990). Reubel *et al.* (2002) were compared the sequences of the field CHV-1 isolates from the USA, France, Japan and Australia and they were found low sequence variability except only minor amino acid differences.

The virus usually transmitted via oronasal and genital contact with the acute infected or viremic animals newborn puppies may be infected vertically in-utero by vaginal secretions during birth from infected dam. Internal and external factors, which cause immune depression and steroid treatments are resulted with reactivation of latently existed virus (Okuda *et al.*, 1993; Burr *et al.*, 1996).

In adult dogs, the infection usually leads to upper respiratory tract infection or ocular symptoms like cornea

ulceration (Ledbetter *et al.*, 2005). Genital lesions (Karpas *et al.*, 1968; Hill and Mare, 1974; Hashimoto *et al.*, 1983), abort, stillbirth and embryonic resorbition could be seen in pregnant (Poste and King, 1971; Hashimoto *et al.*, 1982; Anvik, 1991). High mortality rate are only seeing in newborn puppies until 3 weeks old due to un-stabilised thermoregulation system, characterized by severe respiratory and gastrointestinal disorders (Carmichael *et al.*, 1965).

Kangals are one of the prominent native dogs in the Anatolia. They were classified as a bred with distinct body and mood features. They are excellent shepherd dogs, especially preferring in wild rural areas. The objective of this study was to investigate the CHV-1 infection as serologically in adult Kangal dogs.

### MATERIALS AND METHODS

**Sampled animals:** Total 110 healthy seemingly adult (1-8 years old) Kangal bred dogs (78 female-32 male) from 4 different kennels in Ankara province and around were used in the study. According to farm records, no animals were vaccinated for CHV-1 and complications associated with CHV1 infection like newborn deaths, infertility disorder or birth complications were not observed before in the studied kennels. Blood samples were taken from Vena cephalica and centrifuged at 3000×g for 10 min. within 2 h after sampling. Serums was separated to stock tubs and kept in freezer (-20°C) until testing.

**Serological test:** Canine Herpesvirus antibody ELISA test was used for detection of CHV-1 specific antibodies (Takumi *et al.*, 1990). The test was performed as producers describe (EVL firm-The Netherlands), plates were read at 450 nm and obtained OD data was calculated.

**Statistical analysis:** Statistical analysis was performed for evaluate the positivity differences between male and females. For this purpose t-test (difference in 2 proportions) was calculated (Wonnacott and Wonnacott, 1972).

**RESULTS AND DISCUSSION**

**Serological test:** Obtained blood serum samples were tested for CHV-1 specific antibodies, 79 out of 110 dogs founded positive with ELISA test. The herd-based seroprevalance values of CHV-1 were determined between 42.8 and 86.6% (Table 1). Out of 78 female Kangals, 58 was found to be positive (74.3%), seroprevalance in males was found to be 65.6% (21/32).

As a result of t-test analysis, significant differences were not found between male and females ( $p>0.05$ ).

CHV-1 is occurs in canine population throughout the world (Engels *et al.*, 1980; Rijsewijk *et al.*, 1999; Ronsse *et al.*, 2002). Serologic surveys show that infection has been detected in high levels in the European countries. The rate of the infection are high, especially in kennels, it could be reach to 90% at some of them (Apell, 1987).

In this study, the presence and the seroprevalance of CHV-1 infection were investigated serologically in adult Kangal dogs. Kangal is native bred of Sivas province in Central Anatolia. Their reproduction has been keeping under control for bred purity in a state and some private barns. In this study, blood serum samples collected from 4 private Kangal breeding Kennels in Ankara province and around. All sampled dogs were unvaccinated for CHV-1 and adult. As result of the ELISA test, out of 110 serum samples, 79 (71.8%) were found to be positive for CHV-1 specific antibodies. Seroprevalance proportions varied between 42.8 and 86.6% in kennel basis.

Table 1: The sampled dogs, number and proportion of CHV-1 seropositives

Kennel	No. animals	CHV-1 Ab (+)	CHV-1 (%)
1	21	9	42.8
2	26	19	73.0
3	33	25	75.7
4	30	26	86.6
Total	110	79	71.8

Following acute CHV-1 infection, uncoated viral genome persist as circular double-stranded DNA within the cell nucleus in the wide range of neuronal and lymphoid tissue especially in trigeminal ganglia, by the way the virus not fully cleared from the host (Okuda *et al.*, 1993; Burr *et al.*, 1996), but specific antibodies may be found in blood sera. Like most of the other Herpesviruses, laboratory diagnosis of the infection has mainly based on serological techniques.

Canine Herpesvirus is weak immunogenic, specific antibodies are not stay for a long time in blood in a high titer (Carmichael and Grene, 1998), therefore Virus Neutralisation test may be failed to detect low level antibodies. Takumi *et al.* (1990) were developed indirect ELISA and detected 26.2% positivity with this test while, 5% with neutralisation in 557 dogs.

The previous serologic studies using virus neutralisation test showed that prevalence were <10% (6, 7) but the studies using indirect ELISA in European countries showed that infections rate was nearly 40% in kennels. Rijsewijk *et al.* (1999) were collected 224 dog sera samples in kennels in Netherlands and founded 39.3% positivity, Ronsse *et al.* (2004) was detected 40% positivity in Belgium. Seropositivity for CHV-1 was reported as 48.8% (46/94) in a kennel composed of newly collected stray dogs (Gur and Acar, 2007). Obtained value in this study (71.8%) was higher than previous report. This difference may be explained by management conditions. In previous report (Gur and Acar, 2007), the dogs has been keeping in adjacent but separate cages, there was no direct contact between them. In this study, close age groups of Kangals has been living freely together in a restricted area. Close contact seems as most important factor increasing the incidence.

As a bred characteristic, Kangal dogs are generally mating with same partner during their whole life, this type of mating creates risk particularly for the newborns.

According to a cross-sectional study (Ronsse *et al.*, 2004), lots of factors effects the incidence of CHV1 infection like unhygienic conditions, using of external dogs for reproduction and especially large kennel size. The animals used in this study have been breeding in small barns with good and clean care. According to the farm records, no dog was added to the kennels at least for 2 years except kennel 2, positivity was found to be 73% in kennel 2, but proportions in other 2 kennels (3-4) were higher, thus infection may be originated from stud animals in this kennels.

We know that incidence was higher in kennels than individual dogs. Because of latent structure of the infection like other alphaherpesviruses, specific antibody positive individuals carry the virus during whole life. Even though prognoses are generally mild or sub-clinical in adult dogs, virus could easily reactivate in birth season

due to birth trauma and hormonal factors (Johnston *et al.*, 2001), by the way newborn puppies at CHV-1 positive kennels are under risk (Ronsse *et al.*, 2004).

### CONCLUSION

CHV-1 infection's presence and proportion was detected for the 1st time in Kangal dogs with this study. Obtained proportion in this study showed that, CHV-1 infection are very high in Kangal kennels in Ankara province. Although, Kangal dogs are not a bred under the risk of extinction, number of pure bred dogs is not many and need to propagate in control.

We know that immune suppressive factors are facilitating the viral reactivation. According to farm records, no clinical problem were observed in both puppies and bitches related CHV1 in studied groups before probably due to good feeding and routine vaccination for other prominent infections. High proportion may be result of inappropriate management condition like intensive breeding and mating style. Further experimental, studies are required to explore presence of genetic resistance. Routine vaccination for CHV1 infection generally has not been applying in field in Turkey. Extended studies are necessary to determine the prevalence of the CHV1 infection, routine vaccination was proposed to take control the infection, especially in kennels.

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