

**Research Article****Investigation of the Seroprevalence of canine Herpesvirus-1 in dogs in Central Anatolian Region****Aslıhan Merve Aslan<sup>1</sup>, İbrahim Mert Polat<sup>1</sup>, İlknur Pir Yağcı<sup>1</sup>,  
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**Abstract**

In this study; seroprevalence study of Canine herpesvirus (CHV-1) , which causes infertility in breeding or pregnant dogs in various commercial dog breeding centers in the Central Anatolia region, was conducted to investigate the situation in the region.

Presented study was carried out in commercial dog breeding enterprises in various provinces and districts in the Central Anatolia Region (Ankara: Haymana, Gölbaşı, Çubuk, Etimesgut, Yenimahalle. Eskişehir: Seyit Gazi. Çankırı: Eldivan.); German Shepherd (n=13), Doberman (n=23), Rottweiller (n=31), Anatolian Shepherd Dog (Akbaş n=3, Malaklı n=9, Kangal n=29), Labrador (n=2), Mallinois (n=2), Border Collie (n=1) and Bernese Mountain Dog (n=1) , a total of 114 breeds were included. One of the samples collected from each dog in two anticoagulant (EDTA) tubes was analyzed molecularly for PCR (Polymerase Chain Reaction) process, and the other serological evaluation was made with the commercial CHV-1 ELISA kit and these results were compared. Canine Herpes Virus-1 antibody levels were found to be above 10 ng/mL in all establishments where the study was carried out. It was determined that there were many pathological findings in the reproductive history of antibody positive cases.

As a result of this study, it was thought that the dogs to be used in breeding, whether female or male, should be subjected to series of gynecological examinations and especially animals free from Canine Herpes Virus-1 should be used in production.

**Keywords:** Canine Herpesvirus, canine, infertility, Seroprevalence, PCR, ELISA

## Introduction

Canine herpes virus (CHV-1), also known as fetal hemorrhagic disease in newborns, is a viral infection that affects fertility in adult dogs. While adult dogs infected with CHV usually show no symptoms, the infection is among the leading causes of death in newborn puppies. It is known worldwide as severe viral infection of puppies (Fading Puppy Syndrome) and is characterized by mortality of up to 100% in affected puppies (Evermann J.F. ,2011). Canine Herpes Virus-1 (CHV-1) is a membranous, double-stranded DNA virus in the genus *Varicellovirus* in the *Alphaherpesviridae* subfamily of the *Herpesviridae* family (Dubovi and Maclachlan 2010). Although the virus has similar characteristics with other species, it has the ability to cause disease only in domestic dogs and the *Canidae* family thanks to the specific receptors on it. The virus is immunogenically weak and is highly resistant between pH<5 and >8 and to lipid solvents such as ether, chloroform and most disinfectants (Greene, 2012).

Seroprevalence in dog populations worldwide varies between 20-98% depending on the region (Krogenaes et al. 2012). When current studies are examined, some seroprevalence studies cannot draw adequate conclusions since infected animals can temporarily become seronegative (Greene, 2012). In a shelter study conducted in Afyon province in Turkey, blood serum samples of 94 dogs were tested for CHV-1 antibodies by indirect ELISA and 46 (48.8%) were reported to be seropositive (Gür and Acar, 2007).

Transmission is oronasal and venereal, especially during active herpes virus infection. Transplacental transmission is also possible in infected pregnant bitch. In newborn offspring, it occurs most often during emergence from the birth canal of an infected bitch or through contact with infected vaginal and nasal secretions (Greene, 2012). Newborn puppies can acquire CHV-1 infection in utero through passage through the birth canal, contact with other infected puppies, maternal oronasal secretions or fomites (although this is rare). Systemic cell-associated viremia is possible in immunocompromised or immunocompromised hosts. Less than 1 week old, experimentally infected newborn puppies are particularly susceptible to fatal generalized infections; puppies older than 2 weeks at the time of infection are relatively resistant and often develop mild or incomplete clinical disease (Greene, 2012).

CHV-1 replicates in the mucous membranes of the upper respiratory tract and external genitalia. Genital lesions can lead to transmission of the virus during mating. However, genital lesions in bitches are believed to be most important for the risk of transmission to offspring during parturition (Hill and Maré 1974; Rootwelt et al. 2009). Herpes viruses cause lifelong latent infections and can be reactivated for various reasons (Roizmann B. et al., 1992). Like many other DNA viruses, herpes viruses maintain a “persistent life strategy” (Villarreal LP. et al., 2000). Following productive infection and an initial period of antiviral response, the virus is not completely cleared from the host.

In this study, it was aimed to examine the seroprevalence of Canine Herpes Virus (CHV-1), which has thought to have a high incidence in our country and in the world, in order to evaluate the reproductive losses and economic hazards that may occur as a result of these losses in commercial dog breeding facilities in various provinces in Central Anatolia.

## Materials and methods

This study was conducted as a prospective study. The study material consisted of 114 dogs of various ages (mean=3.44), and gender (female:89, male:25), especially those with reproductive problems, which were kept in private kennels of various provinces and districts in Central Anatolia Region. Blood samples were collected from each dog in two anticoagulated (EDTA) and non-anticoagulated tubes, one of which was analyzed molecularly for PCR and the other was evaluated serologically with a commercial CHV-1 ELISA kit and the results were compared.

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## PCR Method

DNA extraction was performed by conventional method on the pre-prepared diagnostic materials to be used in PCR to detect the presence of viral nucleic acid. In the conventional PCR technique, CHV1 f: 5'-CAG GAC TAT TGG ACT TAT AGT -3' and CHV1 r: 5'- TTG CAA CAA TGC CCC TCA TAA TT-3' primer pair was used. Agarose gel electrophoresis of

PCR products was then performed and evaluated.

**ELISA Method**

A commercial ELISA test kit (DRG EIA-2481-96 CHV) and Thermo Multiskan GO plate reader were used for the determination of Canine Herpes Virus (CHV-1) antibody levels in the collected blood sera.

**Results**

**Serum Antibody Levels and Clinical Findings**

Antibody levels were above 10 ng/mL in all farms. Viral antibodies were positive (100%) in all breeders who had various problems in their reproductive history in all farms where the study was conducted. The animals sampled and average antibody levels are presented in Table 1.

**Table 1. Avarage antibody levels**

Kennel	Serum Ab (ng/mL)	n	Common Clinical Signs
A	19,70	14	Vaginal discharge
B	14,08	9	Vaginal discharge
C	16,02	7	Pup loss
D	33,85	2	Stillbirth and pup loss
E	15,80	2	Respiratory distress and neonatal death
F	12,33	3	Runny eyes and respiratory problems in puppies
G	16,29	10	Stillbirth and pup loss
H	17,40	22	Stillbirth and pup loss
I	37,81	33	Stillbirth and vaginal lesions
J	17,16	5	Respiratory distress
K	16,92	7	Pup loss

**Polymerase Chain Reaction Results**

As a result of the absence of an active disease picture during the study and the latent course of the infection, no antigenic structure was found in PCR tests.

**Distribution of Antibodies by Sex in Study Dogs**

Antibody levels of females (28 ng/mL) were higher than those of males (21.90 ng/mL) in holdings where stillbirths and pups losses were common.

**Breed Distribution**

It was determined that large breed dogs are more commonly used for commercial dog production in the Central Anatolia region where the study was conducted, and Rottweiler and Anatolian Shepherd Dogs are the dominant breeds for production purposes. In addition, when the numbers among the

breeds participating in the study were evaluated, the canine herpes virus-1 antibody level in Kangal breed dogs was higher (37 ng/mL) than all breeds. Average antibody levels according to breeds are presented in Table 2.

**Table 2. Avarage antibody levels according to breeds.**

Dog Breeds	n	Average of Serum Antibody (ng/mL)
DOBERMAN	23	18,52
GERMAN SHEPHERD DOG	13	16,10
LABRADOR RETRIVER	2	15,50
BORDER COLLIE	1	11,20
BERNESEE MOUNTAIN SHEPHERD	1	19,30
MALINOIS	2	11,15
ROTWEILLER	31	16,27
ANATOLIAN SHEPHERD DOG (KANGAL n1, MALAKLI n2, AKBAŞ n3)	n <sub>1</sub> : 31	n <sub>1</sub> : 37,00
	n <sub>2</sub> : 7	n <sub>2</sub> : 16,92
	n <sub>3</sub> : 3	n <sub>3</sub> : 32,13

**Discussion**

The mortality of the disease can reach up to 100% in newborn puppies (Davidson et al. 2003). This high mortality is due to maternal antibody levels in newborn puppies, maternal transmission of the disease during delivery, underdeveloped thermoregulation mechanism in newborn puppies and low body temperature being a favorable environment for the virus. The fact that most of the canine facilities in the study had pup losses and respiratory problems in newborns and that all siblings born in the same litter were infected demonstrates the contagiousness and mortality of the disease (Kimberlin et al. 2001).

In a study investigating canine herpes virus-1 antibodies, blood samples were evaluated by both ELISA and virus neutralization method; 26.2% seropositivity was found in the ELISA method, while a very low positivity of 5% was obtained in the virus neutralization test. In this thesis, ELISA method was used to determine the presence and seropositivity of canine herpes virus-1 antibody in kennels in Central Anatolia region. In seroprevalence determination by ELISA method, very high seroprevalence values have been detected because the infection is widespread in the world, especially in dog farms. In this study, very high seropositivity was obtained in breeding dogs in Central Anatolia region.

There are studies showing that canine herpes virus

seropositivity varies depending on gender and breed. In a study conducted in Turkey, male dogs were found to be more susceptible to the agent than bitches. There are some studies with contrary findings. Nöthling et al. (2008) reported seropositivity rate as 23.8% in bitches and 17.4% in males, while Yeşilbağ et al. (2012) reported seropositivity rate as 46.8% in bitches and 44.7% in males. In this study, the number of seropositive bitches was significantly higher than the number of males. It was thought that the higher antibody levels in bitches compared to males may be due to the fact that the agent in breeding bitches reactivates and replicates with pregnancy. The fact that no clinically ill breeding animals were identified during the study period suggested that the disease was latent in the facilities in the region. In one study, the seropositivity rate in Kangal breed dogs was reported as 41.8% (23/55). (Yesilbag, et al. 2012) In this study, when both seropositivity and antibody levels were evaluated, it was determined that Kangal breed dogs were more susceptible to the disease. In addition, it was determined that the clinical signs of the disease were severe in puppies born to seropositive mother dogs and mortality was high in these puppies.

Pregnancy is an important stress factor in dogs as in all mammals. In pregnant bitches, some factors released from T-suppressor cells decrease maternal immunity. Herpes virus-positive mother dogs may develop reproductive pathologies such as early embryonic death, resorption, abortion or stillbirths, which depend on individual immunity at all stages of pregnancy. In pregnancies that end in abortion or stillbirth, pregnant bitches continue to spread the disease. As a result of vertical transmission, other breeders also become potentially infected.

All these reproductive problems were present in the kennels where the study was conducted. Individually, it was observed that some bitches who had stillbirths in the past did not get pregnant in their last matings. It was found that matings and pregnancy follow-ups were not carried out regularly and some of the males used in matings were transferred to different cities. Therefore, breeding male dogs and dog breeders were considered to be an important source of transmission and regional or inter-regional spread of the infection.

## References

1. Davidson, A. P., S. A. Grundy & J. E. Foley (2003): Successful medical management of neonatal canine herpesvirus: a

- case report. *Communication in Theriogenology*. Vol. 1, pp. 1-5 [online]: <http://ctheriogenology.lsu.edu/ct/Issues/PastIssues/2003-1/2003-1FinalWeb.pdf>
2. Dubovi, E. J. & J. N. Maclachlan (2010): *Fenner's Veterinary Virology*. 4 ed. Elsevier Inc, Boston, pp. 179 – 195.
3. Evermann, J. F., E. C. Ledbetter & R. E. Maes, (2011): Canine reproductive, respiratory, and ocular diseases due to canine herpesvirus. *Veterinary Clinics of North America: Small Animal Practice*. Vol. 41, pp. 1097-1120.
4. Greene, C. E. (2012). *Canine Herpesvirus Infection*. In: Greene C. E. *Infectious Diseases of the Dog and Cat*. 4rd ed. Saunders, pp. 48-54.
5. Hill, H. & C. J. Maré (1974): Genital disease in dogs caused by canine herpesvirus. *American Journal of Veterinary Research*. Vol. 35, pp. 669-672.
6. Kadir Yeşilbağ, Ebru Yalçın , Pelin Tuncer ,Zeki Yılmaz: *Research in Veterinary Science K. Yesilbag et al. / Research in Veterinary Science* 92 (2012) 36–39
7. Kimberlin, D., W. Lin, C. Jacobs, R. F. Dwight, A. P. Corey, L. Gruber, W. C. Rathore, M. Bradley, J. S. Diaz, P.S Kumar, M. Arvin, A. M. Gutierrez, K. Shelthon, M. Weiner, L. B. Sleasman, J. W. Mureguía de Sierra, T. Weller, S. Soon, S-J. Kiell, J. Lakeman, F. & R. J. Whitley (2001): Safety and efficacy of high-Dose intravenous acyclovir in the management of neonatal herpes simplex virus infections. *PEDIATRICS*. Vol. 108 pp. 230-238.
8. Krogenæs, A., V. Rootwelt, S. Larsen, E. K. Sjøberg, B. Akselsen, T. M. Skår, S. S. Myhre, L. H. M. Renström, B. Klingeborn & A. Lund (2012): A serological study of canine herpes virus-1 infection in the Norwegian adult dog population. *Theriogenology*. Vol. 78, pp. 153-158.
9. Nöthling J.O., Hüseyin D. , D. Steckler a, M. Ackermann ;Seroprevalence of canine herpesvirus in breeding kennels in the Gauteng Province of South Africa, *Theriogenology* 69 (2008) 276–282
10. Rootwelt, V., A. Lund & A. Krogenæs (2009): Herpes virus infection in the dog – A review. *Norsk Veterinærtidsskrift*. Vol. 121, pp. 339-347.
11. Roizmann B, Desrosiers RC, Fleckenstein B, et al: The family Herpesviridae: An update. The Herpesvirus Study Group of the International Committee on Taxonomy of Viruses. *Arch Virol* 123(3-4):425-449, 1992.
12. Sibel GÜR ,Abuzer ACAR, 2007, Fırat Üniversitesi Sağlık Bilimleri Dergisi : 21 (1): 37 – 40 <http://www.fusabil.org>
13. Villarreal LP, Defilippis VR, Gottlieb KA: Acute and persistent viral life strategies and their relationship to emerging diseases. *Virology* 272(1)2-, 2000.