SEROPREVALENCE OF CANINE *HERPES* VIRUS IN LITHUANIAN DOG POPULATION

Kristina Musayeva[1], Jakov Šengaut[2], Saulius Petkevičius[1,3], Alvydas Malakauskas[1], Gediminas Gerulis[4], Algirdas Šalomskas[1,3]
[1]*Department of Infectious Diseases, Veterinary Academy, Lithuanian University of Health Sciences*
Tilžės 18, LT-47181 Kaunas, Lithuania, E-mail: musayeva@lva.lt
[2]*Jakovo Veterinary Centre*
Gerosios Vilties 1, LT-03147, Vilnius, Lithuania
Tel. +370 5 2132982, Fax. +3705 2105049, E-mail: veterinarija@takas.lt
[3]*Department of Virology, Veterinary Institute of Veterinary Academy, Lithuanian University of Health Sciences*
Tilžės 18, LT-47181 Kaunas, Lithuania, E-mail: algirdas.salomskas@lva.lt
[4]*Department of Food Safety and Quality, Veterinary Academy, Lithuanian University of Health Sciences*
Tilžės 18, LT-47181 Kaunas, Lithuania; Tel. +370 37 36 32 08; gedulis@lva.lt

Abstract. Canine herpesvirus type 1 (CHV-1) is presumed to be enzootic in dogs all over the world, but no information was available regarding the seroprevalence to CHV-1 from European north-east countries. The aim of the present study is to determine seroprevalence to CHV-1 in the Lithuanian canine population. Twenty dogs from one breeding kennel and seventy three dogs from veterinary hospital were tested for antibodies to CHV-1 by ELISA. Seropositive animals were identified both in kennel dogs and household dogs (85% and 11%, respectively). However, the number of seropositive individuals among the kennel dogs was seven times higher compared to the pet group (RR=7.3, CI 2.2-23.2, P<0.001). The infection rate was significantly higher in oldest dogs group compared to young (P<0.001) and medium age (P<0.05) dogs. The health status and sex had no significant influence on serological status of the dogs (P>0.05).

Keywords: canine herpes virus, seroprevalence, Lithuania.

ŠUNŲ *HERPES* VIRUSŲ PAPLITIMO LIETUVOJE SEROLOGINIAI TYRIMAI

Kristina Musayeva[1], Jakov Šengaut[2], Saulius Petkevičius[1,3], Alvydas Malakauskas[1], Gediminas Gerulis[4], Algirdas Šalomskas[1,3]
[1]*Užkrečiamųjų ligų katedra, Veterinarijos akademija, Lietuvos sveikatos mokslų universitetas*
Tilžės g.18, LT-47181, Kaunas; el. paštas: musayeva@lva.lt
[2]*Jakovo veterinarijos centras, Gerosios Vilties g. 1, LT-03147, Vilnius*
tel.(8~37)5 213 2982; faks.+3705 210 5049; el. paštas: veterinarija@takas.lt
[3]*Virusologijos skyrius, VA Veterinarijos institutas, Lietuvos sveikatos mokslų universitetas*
Tilžės g. 18, LT-47181, Kaunas; el. paštas: algirdas.salomskas@lva.lt
[4]*Maisto saugos ir kokybės katedra, Veterinarijos akademija, Lietuvos sveikatos mokslų universitetas*
Tilžės g. 18, Kaunas LT-47181; tel. +370 37 36 32 08; el. paštas: gedulis@lva.lt

Santrauka. Šunų pirmojo tipo *herpes* virusai (ŠHV-1) išplitę daugelyje pasaulio šalių, tačiau nėra informacijos apie seroepidemiologinį paplitimą Europos Šiaurės Rytų šalyse. Mūsų darbo tikslas buvo nustatyti ŠHV-1 serologinio paplitimo mastą Lietuvos šunų populiacijoje. Dėl šių virusų imunofermentinės analizės metodu ištirti 20 šunų iš vieno veislyno ir 73 šunys iš veterinarijos gydyklos. Nustatėme, kad veislyne laikomų šunų grupėje seroteigiamų individų dalis buvo daugiau kaip 7 kartus didesnė (atitinkamai 85 proc. ir 11 proc.) nei šunų, laikomų namuose (SR=7,3; PI 2,2–23,2; p<0,001). Infekcijos dažnis vyriausių šunų grupėje buvo ženkliai didesnis nei jaunų (p<0,001) ir vidutinio amžiaus (p<0,05). Seroteigiamų šunų sveikatos būklės ir lyties rodikliai nebuvo statistiškai reikšmingi (p>0,05).

Raktažodžiai: šunų *herpes* virusas, paplitimas, Lietuva.

Introduction. Canine herpes virus type 1 (CHV-1) is a member of the family *Herpesviridae*, subfamily Alphaherpesvirinae, genus *Varicellovirus* (Fauquet et al., 2005). The infection occurs in household and kennel dogs all over the world (Rijsewijk et al., 1999; Reading and Field, 1998; Ronsse et al., 2002). The age of dogs is an important factor for the disease progress. The highest incidence of fatalities caused by CHV-1 infection occurs among newborns and puppies aged less than three weeks (Carmichael and Medic, 1978). Older dogs develop subclinical infection or local respiratory, genital or eye infections (Malone et al., 2010). Viruses are isolated from clinically healthy adult dogs, puppies delivered through c-section and from dogs with system diseases (Carmichael and Medic, 1978; Anvik, 1991). After an acute disease, the infected dogs become latent carriers (Okuda et al., 1993) and virus is found in the sacral ganglia, salivary gland, tonsils and liver in the absence of clinical symptoms (Burr et al., 1996). Antibody titres can decline quickly after primary CHV-1 infection and it is possible for the antibodies to be detected for up 15 month post infection (von König et al., 2004).

For the diagnosis of CHV-1 infection, viral isolation and serum neutralisation assays most often are used (Poste and King, 1971; Takumi el al., 1990; Okuda et al., 1993). Most recently developed and sensitive detection techniques are polymerase chain reaction (PCR) and enzyme - linked immunosorbent assay (ELISA) (Burr et al., 1996; Miyoshi et al., 1999).

In many countries, a series of seroepidemiological investigations has been carried out for epizootic incidence of herpes virus in the populations of dogs (Reading and Field, 1999; Rijsewijk et al., 1999; Ronsse et al., 2004; von König et al., 2004; Nöthling et al., 2008; Dahlbom et al., 2009). Seroprevalence of herpes virus in kennel dogs with reproductive problems was very high in Finland (100%), and lower in Belgium (47%) and Netherlands (40%) (Rijsewijk et al., 1999; Ronsse et al., 2004; Dalbom et al., 2009). In the UK it was estimated that CHV-1 is the presumable cause of infectious rhinotracheitis in kennel dogs (Erles et al., 2004). Seroepidemiological investigations in England showed that the latent form of CHV-1 infection is widespread in the household dog population (Reading and Field, 1998).

In Lithuania, the prevalence of CHV-1 in dog population has not been investigated and there is no available data about its role on the health status of dogs.

The aim of this study was to determine the prevalence of antibodies against canine herpesvirus in the serum of dogs in breeding kennel and individual dogs in Lithuania.

Material and methods. The blood samples were taken from dogs of one breeding kennel and one veterinary clinic was selected for health examination and scheduled vaccination. None of the dogs had been previously vaccinated against CHV-1.

The tested 93 dogs were of different age and breed. The blood samples for serological examination were taken aseptically from *Vena cephalica* into 5 ml vacuum test-tubes without anticoagulant. For serum preparation, samples were centrifuged at 1200 G for 10 minutes. The samples of blood serum were numbered and before the examination stored at minus 20 ºC.

During the experiment, the data about the age, breed and sex of tested dogs as well as health status of seropositive dogs (eye diseases, respiratory or reproductive disorders) were collected. For determining the influence of age on the incidence of CHV-1 infection, the experimental dogs were divided into three groups: group of young dogs (n=61, newborns and up to 4 years old), group of medium age dogs (n=23, from 4 to 7 years old), and a group of dogs over 7 years old (n=9). The all three groups included 40 males and 53 females.

The serum tests were performed using commercial diagnostic *DRG*® Canine Herpes Virus Ab (EIA-2481) ELISA kit (DRG International, Inc., USA) and following the manufacturer's recommendations. A microtitre plate with 96 wells was covered with CHV-1 antigen. The control and experimental samples were diluted 20-fold and poured into wells. The incubation at 37 ºC lasted for 1 hour. After the incubation, the content of the microtitre plate was shaken out and the wells rinsed two times. The rinsed wells were filled for incubation with peroxidase conjugated antibodies. After repeated rinsing, the wells were filled with substrate solution. The concentration of antibodies in a sample was indicated by the blue colour appearing during the reaction and turning yellow after suppression of reaction with acid. The test results were estimated by measuring the optical density (OD) of samples at wave length λ = 450 nm using spectrophotometer Thermo Scientific Multiskan EX (Thermo electron corporation, China, 2005).

The serological test by ELISA method was performed in 2010 at the Department of Infectious diseases of Veterinary Academy, Lithuanian University of Health Sciences.

The statistical analysis of obtained data was carried out using software Graph Prism 3.0^{TM}. The confidence interval (CI) and relative risk (RR) at probability of 95% were calculated. The Student's significance level and the obtained data were regarded significant when values were $P< 0.05$.

The tests were performed observing the Law of the Republic of Lithuania on the Care Keeping and Use of Animals No 8-500 ("Valstybės žinios" 28 11 1997, No 108) and related documentation.

Results. The total of 93 dogs was examined for CHV-1 infection. Not a single dog had been vaccinated against CHV-1.

The data in Table 1 shows that the average of 26.8% of blood samples (CI 18.9–36.7%) contained canine herpes virus antibodies. Seroprevalence in kennel dogs was significantly higher (RR=7.3, CI 2.2–23.2) than in household dogs ($P<0.001$).

Table 1. **Seroprevalence of CHV-1 in different groups of dogs**

Groups of dogs	Number of examined dogs	Number of seropositive dogs		CI, %
		Number	%	
Breeding kennel	20	17	85	64.0–94.8
Household dogs	73	8	10.9	5.7-20.2
Total	93	25	26.8	18.9–36.7

Analysis of age effects showed that CHV-1 infection occurs in all age groups of dogs but the number of seropositive individuals in the group of the oldest dogs (7–10 years old) was considerably higher than in the groups of young ($P<0.001$) and medium age ($P<0.05$) dogs (Table 2). It would be useful to compare the age grouping of kennel and household dogs. There is the possibility that they will be different.

Table 2. **The age effect on seroprevalence of CHV-1**

Groups of dogs (age in years)	Number of examined dogs	Number of seropositive dogs (%)	CI, %
Young dogs (<4)	61	9 (14.8)	7.96–25.72
Medium age dogs (4–7)	23	8 (34.8)	18.81–55.11
Older dogs (7–10)	9	8 (88.9)	56.5–98.0

Analysis of the health status of seropositive dogs showed that 13 (52%) samples were taken from infected dogs and 12 (48%) from the clinically healthy dogs yet this difference was not statistically significant (P>0.05).

Also it was determined that 14 (35%) of examined males and 11 (20.7%) of bitches were seropositive but the difference was not statistically significant (P>0.05).

Discussion and conclusions. According to the obtained data, the present study was the first attempt for determining CHV-1 infection in the Lithuanian population of dogs. The study revealed that the prevalence of CHV-1 in the Lithuanian dog population is rather high (average of 26.8%) yet the incidence of this infection largely depends on the environmental risk factors. Investigations performed in other countries also showed that seroprevalence of CHV-1 may vary from 3 to 90% in different geographical localities. For example, the number of seropositive kennel dogs in Finland and England exceeded 80% (81.5 and 94% respectively) (Reading and Field, 1999; Dahlbom et al., 2009). The reported number of seropositive dogs in kennels of other countries is slightly lower: Belgium 45.8%, Netherlands 39.3% (Rijsewijk et al., 1999; Ronsse et al., 2002). A number of investigations have proved that CHV-1 infection is more widespread among the dogs kept in groups. By serological tests CHV-1 antibodies have been identified in 27.9% of kennel dogs in Italy, 62.1% of kennel dogs in Turkey and in 3.1% (Italy) and 39% (Turkey) of household dogs (Sagazio et al., 1998; Yesilbag et al., 2010). The study carried out in Turkey showed high prevalence of CHV-1 (71.8%) among the kennel clinically healthy dogs (Acar et al., 2009).

According to our data, the concentration of dogs is directly responsible for the rates of CHV-1 infection. In kennel dogs, the prevalence of CHV-1 was significantly higher (85%; PI 63.1–94.8; SR=7.3, PI 2.2–23.2; P<0.001). Comparable results have been reported by Dahlbom who identified 81.5% of seropositive blood samples in the Finnish breeding kennels (Dahlbom et al., 2009). This pattern of CHV-1 prevalence among dogs kept in groups presumably is predetermined by its transmission via contact, sexual intercourse or aerogenically. Thus when dogs are kept in close proximity and are intensively bred, there occur favourable conditions for transmission of viruses (Ronsse et al., 2002).

According to our data, seroprevalence of CHV-1 among the household dogs accounted for 11%. Similar results have been reported by researchers from other countries: Switzerland 6.3%, Iran 19.1% (Engels et al., 1980; Babaei et al., 2010). It is obviously that individually kept dogs have not so many contacts with other pets and are less intensively bred. Yet there are controversial reports about higher seroprevalence of CHV-1 among household dogs (Reading and Field, 1999).

The age effect on the prevalence of CHV-1 infection was analysed in several scientific studies performed by Raeding (1999), Yesilbag (2010). It was concluded, that there is no relation between the age of dogs and the number of seropositive dogs (Reading and Field, 1999; Yesilbag et al., 2010). However, we have determined that the number of seropositive individuals was significantly lower in the group of younger dogs (14.8%) compared to medium age and older dog groups (34.8% and 88.9% respectively).

These data similar to the data obtained by Ronsse (2004), Babaei (2010) who have reported that the probability of herpes virus infection is higher in older dogs (Ronsse et al., 2004; Babaei et al., 2010). This can be explained by the fact that older dogs have higher possibility to be exposed of different risk factors which affect their immune system (such as mating, change of keeping conditions, different diseases, whelping, etc.). These factors create favourable conditions for the spread of herpes virus in dogs population (Rijsewijk et al., 1999; Ronsse et al., 2004).

Our study did not prove the influence of sex on CHV-1 prevalence. This was in concert with results obtained by Ronsse (2004) and Yesilbag (2010) (Ronsse et al., 2004; Yesilbag et al., 2010).

It has been determined that during the acute canine herpes virus infection the clinical manifestation may be variable. For this reason, the disease can be hardly diagnosed without laboratory tests. Erles have pointed out that the clinical signs of CHV-1 infection occur later than signs of other viral infections. In the majority of cases, the CHV-1 infection causes serious respiratory diseases (Erles et al., 2004; Kawakami et al., 2010). Dahlbom et al. determined that 100% of dogs with reproduction disorders and 65% of clinically healthy dogs were CHV-1 seropositive (Dahlbom et al., 2009). Ronsse examined 27 breeding bitches regarding the CHV influence on breeding disorders determined that there was no dependence between the number of bitches with and without reproduction disorders and the increased titre of antibodies (Ronsse et al., 2005). Ledbetter reported that dog eye diseases, such as conjunctivitis and ulcerative keratitis, may be induced by CHV-1 (Ledbetter et al., 2006; 2009). Yet according to our data, the health status of dogs does not affect the spread of CHV-1. During the present study, the numbers of seropositive individuals in the groups of sick and healthy dogs were comparable. This can be accounted by the fact that antibodies in the

infected and recovered dogs persist for a long time in the ganglia of nervous system and can be isolated from the relatively healthy dogs showing no clinical signs of infection (Acar et al., 2009; Babaei et al., 2010).

The results of this study showed that CHV-1 seroprevalence is moderately high in the Lithuanian dog population. Thus better knowledge of the specific features of CHV-1 infections requires further comprehensive studies determining the influence of different risk factors on the CHV-1 etiology. Further experimental, studies are required to explore presence of genetic resistance. Extended studies are necessary to determine the prevalence of the CHV-1 infection. Routine vaccination is proposed as a measure of infection control, especially in kennels.

References

1. Acar A., Gur S., Dogan I., Akca Y. A. Serologic Investigation of Canine Herpesvirus Type 1 Infection in Kangal Dogs. Journal of Animal and Veterinary Advances, 2009. V. 8(7). P.1377–1380.

2. Anvik J.O. Clinical considerations of canine herpesvirus infection. Veterinary Medicine, 1991. V. 4. P.394–403.

3. Babaei H., Akhtardanesh B., Ghanbarpour R., Namjoo A. Serological Evidence of Canine Herpesvirus-1 in Dogs of Kerman City, South-East of Iran. Transboundary and Emerging Diseases. 2010. V.57 (5). P. 348–351.

4. Burr P.D., Campbell M.E.M., Nicolson L. and Onions D.E. Detection of canine herpesvirus 1 in a wide range of tissues using the polymerase chain reaction. Veterinary Microbiology. 1996. V.53. P. 227–237.

5. Carmichael L.E., Medic B. L. S. Small-plaque variant of canine herpesvirus with reduced pathogenicity for newborn pups. Infection and immunity. 1978. V.20 (1). P. 108–114.

6. Dahlbom M., Johnsson M., Myllys V., Taponen J., Andersson M. Seroprevalence of canine herpesvirus-1 and *Brucella canis* in Finnish breeding kennels with and without reproductive problems. Reproduction in Domestic Animals, 2009. V. 44. P. 128–131.

7. Engels M., Mayr-Bibrack B., Ruckstuhl B., Metzler A., Wyleret R. The sero-epizootiology of canine herpesvirus infections in Switzerland and preliminary studies with a vaccine. Zentralbl Veterinarmed B. 1980. V. 27. P. 257–267.

8. Erles K., Dubovi E. J., Brooks H., Brownlie J.W. Longitudinal study of viruses associated with canine infectious respiratory disease. Journal of Clinical Microbiology, 2004. V. 42. P. 4524–4529.

9. Fauquet C.M., Mayo M.A., Maniloff J., Desselberger U., Ball L.A. Virus Taxonomy: Classification and Nomenclature of Viruses, Eighth Report of the International Committee on Taxonony of Viruses, Elsevier Academic Press, London, 2005. http://www.sciencedirect.com/science/article/pii/S0378113510000908#bbib36

10. Yesilbag K., Yalcin E., Tuncer P., Yilmaz Z. Seroprevalence of canine herpesvirus-1 in Turkish dog population. Research in Veterinay Science, 2010. P.1–4.

11. Kawakami K., Ogawa H., Maeda K., Imai A., Ohashi E., Matsunaga S., Tohya Y., Ohshima T., Mochizuki M. Nosocomial Outbreak of serious canine infectious tracheobronchitis (Kennel Cough) caused by canine herpesvirus infection. Journal of Clinical Microbiology, 2010. V. 48. P. 1176–1181.

12. Ledbetter E.C., Riis R.C., Kern T.J., Haley N.J., Schatzberg S.J. Corneal ulceration associated with naturally occurring canine herpesvirus- 1 infection in two adult dogs. Journal of the American Veterinary Medical Association, 2006. V. 229. P. 376–384.

13. Ledbetter E. C., Sung G. K., Edward J. D. Outbreak of ocular disease associated with naturally-acquired canine herpesvirus-1 infection in a closed domestic dog colony. Veterinary Ophthalmology, 2009. V. 12(4). P.242–247.

14. Malone E.K., Ledbetter E.C., Rassnick K.M., Kim S.G., and Russell D. Disseminated Canine Herpesvirus-1 Infection in an Immunocompromised Adult Dog. Journal of Veterinary Internal Medicine, 2010. V. 24. P. 965–968.

15. Miyoshi M., Takiguchi M., Yasuda J., Hashimoto A., Takada A., Okazaki K., Kida H. Structure of the immediate early gene of canine herpesvirus. Archives of Virology, 1999. V. 144. P. 407–420.

16. Nöthling J.O., Hüssy D., Steckler D., Ackermann M. Seroprevalence of canine herpesvirus in breeding kennels in the Gauteng province of South Africa. Theriogenology, 2008. V. 69. P. 276–282.

17. Okuda Y., Hashimoto A., Yamaguchi T., Fukushi H., Moris S., Tani M., Hirai K., Carmichael L. Repeated Canine Herpesvirus (CHV) reactivation in dogs by an immunosuppressive drug. The Cornell Veterinarian, 1993. V.83. P. 291–302.

18. Poste G., King N. Isolation of a herpesvirus from the canine genital tract: association with infertility abortion and stillbirths. Veterinary Records, 1971. V. 88. P. 229–233.

19. Reading M. J., Field H. J. A serological study on canine herpesvirus- 1 infection in the English dog population. Archives of Virology, 1998. V. 143. P. 1477–1488.

20. Reading M. J., Field H. J. Detection of high levels of canine herpes virus-1 neutralising antibody in kennel dogs using a novel serum neutralisation test. Research in Veterinary Science, 1999. V. 66. P. 273–275.

21. Rijsewijk F. A., Luiten E. J., Daus F. J., van der Heijden R. W., van Oirschot J. T. Prevalence of antibodies against canine herpesvirus 1 in dogs in The Netherlands in 1997–1998. Veterinary Microbiology, 1999. V. 65. P. 1–7.

22. Ronsse V., Verstegen J., Onclin K., Guiot A. L., Aeberle´ C., Nauwynck H. J., Poulet H. Seroprevalence of canine herpesvirus-1 in the Belgian dog population in 2000. Reproduction in Domestic Animals, 2002. V.37. P. 299–304.

23. Ronsse V., Verstegen J., Onclin K., Farnir F., Poulet H. Risk factors and reproductive disorders associated with canine herpesvirus-1 (CHV1). Theriogenology, 2004. V. 61 (4). P. 619–636.

24. Ronsse V., Verstegen J., Thiry E., Onclin K., Aeberlč C., Brunet S., Poulet H. Canine herpesvirus-1(CHV-1): clinical, serological and virological patterns in breeding colonies.Theriogenology, 2005. V. 64. P. 61–74.

25. Sagazio P., Cirone F., Pratelli A., Tempesta M., Buonavoglia D., Sasanelli M., Rubino G. Infezione da herpesvirus del cane: diffusione sierologica in Puglia. Obiettivi e Documenti Veterinari, 1998. V.5. P. 63–67.

26. Takumi A., Kusanagi K., Tuchiya K., Xuan X., Azetaka M., Takahashi E. Serodiagnosis of Canine Herpes Infection – Development of an Enzyme-Linked Immunosorbent Assay and Its Comparison with Two Improved Methods of Serum Neutralization Test. Japanese Journal of Veterinary Science, 1990. V. 52 (2). P. 241–250.

27. Von König M., Neiseke J., Thiel H. J. Prevalence of canine herpesvirus 1 (CHV-1) in German kennels. Tierärztliche Umschau, 2004. V. 59. P. 559–565.

Received 8 March 2012
Accepted 11 January 2013