

RETROSPECTIVE STUDY OF CANINE PARVOVIROSIS IN SLOVENIA

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Summary: The aim of this retrospective study, based on the data from the archive of the Institute of Pathology, Forensic and Administrative Veterinary Medicine of Veterinary Faculty in Ljubljana, was to estimate the correlation between epidemiological parameters such as gender, age and breed of dogs that died of canine parvovirus, and the months of a year in which they died.

84 dogs, aged 1 month to 4 years, of both genders and 27 different breeds, which died of canine parvovirus from January 1987 to January 2005, were included in the study. Results of this descriptive study suggest that the age of dogs and the time of year when death occurred were correlated with canine parvovirus while there was no correlation between gender or breed and incidence of death due to canine parvovirus.

Key words: veterinary medicine; pathology; canine parvovirus; parvoviral enteritis; dogs

Introduction

Canine parvovirus is a contagious viral disease of dogs. First cases of canine parvovirus had been almost simultaneously detected for the first time in the USA, Australia and Europe in 1978 (1, 2, 3). In the following three years the disease had spread to all continents (4, 5, 6). The first case of canine parvovirus in Slovenia was diagnosed in July 1980 (7).

The disease is caused by a virus belonging to the group of parvoviruses, which also includes feline panleukopenia virus (FPV; 6). It is presumed that outbreak of parvovirus in dogs was a consequence of FPV mutation (6). Canine parvovirus is caused by two dog specific parvoviruses – canine parvovirus type 1 (CPV1) and canine parvovirus type 2 (CPV2). In contrast to low pathogenicity of CPV1, which rarely causes canine parvovirus, CPV2 is highly pathogenic (6, 8, 9). Several species of wild canids, e.g. coyotes, raccoons, foxes and wolves, are also susceptible to canine parvovirus infection (6). The most typical form of the canine parvovirus is exhibited in the form of enteritis, but the disease

can also manifest as parvoviral myocarditis or as a mixed form (6). All three forms have often fatal outcome (6). Young dogs from six weeks to one year of age are the most susceptible group for canine parvovirus, afterwards the morbidity decreases. Puppies younger than six weeks are protected by maternal immunity (10).

Materials in methods

The necropsy reports from the archive of the Institute of Pathology, Forensic and Administrative Veterinary Medicine of Veterinary Faculty in Ljubljana were reviewed in order to find out causes of death in dogs dissected in the last 18 years, from January 1987 to January 2005. Eighty four dogs in which parvovirus was diagnosed by histopathological analysis were included in this study. In 18 dogs, parvovirus was also confirmed by immunocytochemistry.

Immunohistochemical staining

Polyclonal rabbit antibody against canine parvovirus was used for immunolabelling. Serum was

raised in rabbits which were immunised with the suspension of canine parvovirus, isolat LJ-80, which was replicated on the cell culture of cell line FER (Feline Embryonic Kidney), ECACC, No 90031401. Hemagglutination titer of viral suspension was 1: 1024 (11).

Four micrometers thick, deparafinised tissue sections of jejunum, ileum, liver, kidney, spleen, thymus, lymph nodes and lung were incubated in citrate buffer (pH= 6.0) in the microwave oven for 15 minutes to expose the antigens, cooled to the room temperature and incubated in 3% hydrogen peroxide for 1 hour to inhibit the endogenous peroxidase. Polyclonal rabbit antibodies against canine parvovirus, were diluted 1: 400 in 5% bovine serum albumin in phosphate buffer saline (PBS). Tissue sections were incubated with primary antibodies at room temperature for 30 minutes, followed by washing in PBS for 5 minutes, incubated with biotinylated mixture of goat anti-rabbit and goat anti-mouse secondary antibodies (LSAB[®]2 HRP visualization system, DAKO) for 20 minutes, rewashed with PBS, incubated in streptavidin conjugated with peroxidase (DAKO) and finally incubated in 1% 3-diaminobenzidine tetrahydrochloride, containing 0.03% hydrogen peroxide, for 7 minutes. Sections were counterstained with haematoxylin, dehydrated and mounted with resin (11).

Statistical analyses

Data about gender, age and breed of dogs and month of their death was determined from the records about anamnesis.

For determination of effects of chosen epidemiological parameters on canine parvovirus, dogs were divided into two groups by gender: 70 males and 14 females; in three groups by age: 50 puppies up to 6 months of age, 19 dogs from 6 months to 1 year and 5 dogs older than 1 year; into four groups by breed: 14 German Shepherds, 9 Rottweilers, 6 Labrador Retrievers and 20 mixed breeds.

Regarding the month of the death, dogs were divided into 12 groups, and by the year of death into 18 groups.

Relative ratio of deaths caused by parvovirus from the total number of dissected dogs (2486) was calculated individually for each of the above groups. The differences between groups for each epidemiological parameter were determined using standard statistical methods: correlation and regression analysis, t-test and chi test, with $p < 0.05$, which were considered as statistically significant.

Results

Anamnesis and diagnosis of canine parvovirus

In the last 18 years, 2486 dog carcasses underwent pathological examination at the Institute of Pathology, Forensic and Administrative Veterinary Medicine of Veterinary Faculty in Ljubljana in the last 18 years. Typical macroscopic lesions in the small intestine (mostly hemorrhagic enteritis), which were diagnosed as parvoviral enteritis using histopathological examination, were found in 84 dogs (3.4 % of all examined dogs).

In 55 dogs that died due to parvovirus infection, sudden onset with vomiting, bloody diarrhea, depression and loss of appetite were noticed by their owners or veterinarians. All these dogs died in one or at most two days after the onset, or were euthanized due to severe illness. Five dogs died suddenly, without previous clinical signs. For 24 dogs there was no anamnesis concerning the clinical status prior to death.

Ten dogs were vaccinated against canine parvovirus, three dogs were not vaccinated while for 71 dogs there were no data concerning the vaccination.

Epidemiological analysis

Sex of dogs

Seventy dogs (83.3 %) that died due to canine parvovirus were males and 14 (16.7 %) were females (Diagram 1). Statistical analysis revealed significant difference between sexes in dogs that died due to parvovirus ($P < 0.01$).

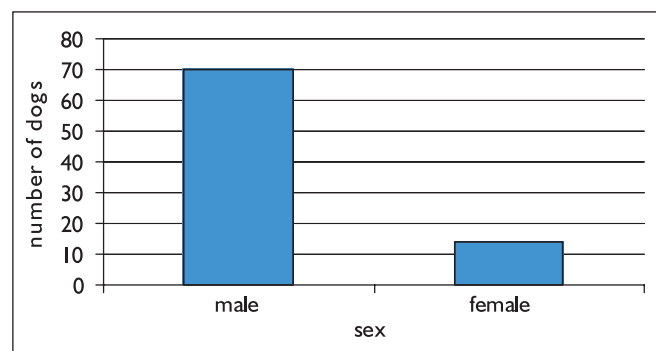


Diagram 1: Sex ratio of dogs that died due to canine parvovirus

The age of dogs

Fifty dogs (67.6 %) that died of canine parvovirus were younger than six months, 19 dogs (25.7 %)

were aged from six months to one year, and only five dogs (6.8 %) were older than one year. For 10 dogs included in our study there were no data about their age (Diagram 2). Comparison between age groups and the death due to canine parvovirus revealed statistical significance between groups ($P < 0.01$).

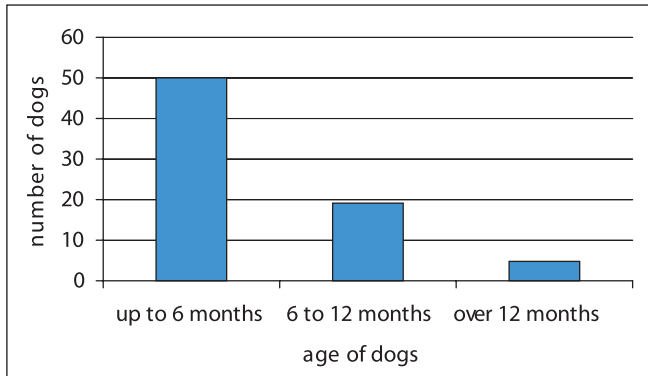


Diagram 2: Age distribution of dogs that died due to canine parvovirus

Breeds of dogs

The highest percentage of deaths, 23.8 % (20 dogs) was noticed in mixed breeds, followed by German Shepherds with 16.7 % (14 dogs), Rottweilers with 10.5 % (nine dogs), Labrador Retrievers with 7.1 % (six dogs) and Poodles with 5.9 % (five dogs). Single cases of canine parvovirus were diagnosed in other 21 pure breeds. The differences between different breeds and the number of deaths due canine parvovirus was not statistically significant ($P > 0.05$).

The year of the death

Twenty dogs died due to canine parvovirus in 1991 (10.3 % of all dogs). In 1987 and from 2001 to 2003 there were no deaths due to canine parvovirus. The percentage of deaths in other years ranged from 1.29 to 8.4 %.

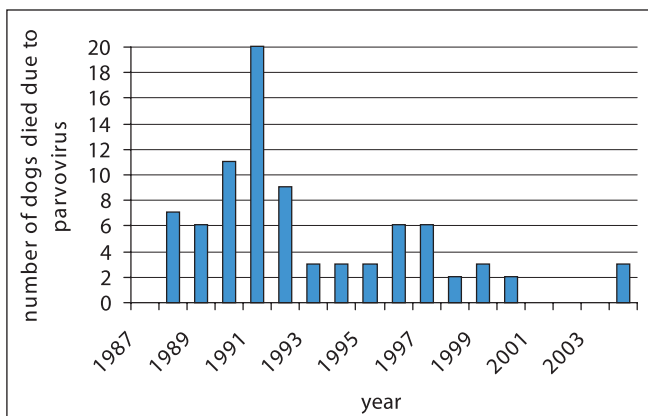


Diagram 3: Number of dogs that died due to canine parvovirus in the last 18 years

The month of the death

The highest percentage of deaths due to canine parvovirus was noticed in August (16.7 %), followed by September (11.9 %), October, December and January (10.7 % each), May (9.5 %) and November (7.1%). The percentage of deaths ranged from 2.3 to 5.9 % in other months of the year (Diagram 4). The difference between months and deaths due to canine parvovirus was statistically significant ($P < 0.001$).

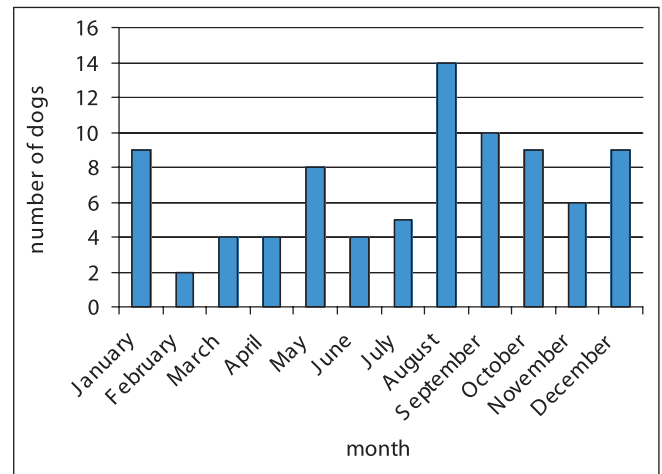


Diagram 4: Number of dogs that died due to canine parvovirus by the month in which they died.

Discussion

In the last 18 years, 2486 dogs were dissected at the Institute of Pathology, Forensic and Administrative Veterinary Medicine of Veterinary Faculty in Ljubljana and among them, 84 died or were euthanized due to canine parvovirus. In all cases, canine parvovirus was suspected due to clinical signs and/or characteristic intestinal lesions, and was confirmed by histopathological examination of the intestine and in 18 dogs also with immunohistochemical reaction using polyclonal antibodies against canine parvovirus (11). Several authors described microscopic changes in small intestine, i.e. necrosis of crypt's epithelia with crypt's dilatation, atrophy of intestinal villi, necrotic enterocytes with enlarged nucleus and focal mucosal collapse, as typical for parvovirus (6, 7, 18). Similar lesions were found also in the small intestines of dogs included in our retrospective study. Ruiz Romero et al. (19) also found necrosis of crypts and atrophy of villi in small intestine of dogs and confirmed parvovirus by immunohistochemical staining (19).

Since 1980, when the first case of canine parvovirus has been diagnosed and described in Slov-

enia (7), deaths due to canine parvovirus were diagnosed almost every year. The number of deaths increased in 1990, 1991 and in 1992, while a slow fall was noticed afterwards until 2001, when no case of death due to canine parvovirus was diagnosed. The first new outbreak occurred in November 2004. There is no data in the literature concerning the disease frequency in the world during the last decades.

The most likely reason for decreased number of deaths due to canine parvovirus is greater owner's awareness and regular preventive vaccination against canine parvovirus. The new outbreak of canine parvovirus could be related to the new canine parvovirus mutant that has been just found in Italy (12). This hypothesis is supported by the facts that this was the first outbreak of disease after three years and, that all three puppies were vaccinated with standard vaccine against canine parvovirus. The second possible explanation for the new outbreak is inappropriate time of vaccination. It is known that there is a critical period from two to four weeks, when vaccination is fairly unsuccessful. In this period, the maternal antibody titer is so high, that a puppy is unsusceptible to preventive vaccination, but at the same time too low to protect the animal against infection. For that reason the vaccination in the above cited period is ineffective. Therefore it is suggested that the assessment of the titer of antibodies against canine parvovirus should be determined before preventive vaccination (13).

83.3 % of dogs, which died due to canine parvovirus, were males, and 16.7 % were females. The difference between genders in the death ratio due to canine parvovirus is statistically significant. In spite of this result we cannot conclude that males are more susceptible for canine parvovirus infection. The major susceptibility of male dogs will be confirmed, if there will be an equal number of male and female dogs in the Slovenian dogs' population. Unfortunately, we don't have the data about the exact number of male and female dogs in Slovenia. Other authors (4) reported that the canine parvovirus infection was more frequent in male dogs, but statistical analyses could not confirm significant differences between sexes and morbidity (4, 5).

The highest percentage of deaths, 67.7 %, was noticed in dogs younger than six months, and the lowest, 6.7 %, in dogs older than one year. From six months to one year 25.7 % of dogs died due to canine parvovirus. Comparison between the age groups and death due to canine parvovirus showed statis-

tical significance. We have demonstrated that age affects the death due to canine parvovirus. Very high percentage of deaths, 80 %, was found in puppies younger than six months by Pospischill and Yamaho (14) and 74.5 % morbidity was reported by Ernst et al. (4) at the same age. The most likely reason that dogs younger than six months are at the greatest risk is very high affinity of canine parvovirus for tissues with high mitotic activity (10). Puppy's immune system, which depends on the maternal antibodies, also has an important role in the fight against canine parvovirus (15). Maternal immunity which lasts two to three months makes morbidity of puppies at this age very low. Singular cases of canine parvovirus in puppies are probably a consequence of subclinical form of canine parvovirus during the mother's pregnancy (4). The mitotic activity in the cells decreases with age, therefore the percentage of older dogs that die due to canine parvovirus is very low. Deaths in older dogs are mostly related to the high invasions with intestinal parasites, bacterial and viral infections that all cause immunosuppression, damage of intestinal mucosa and consequently provoke increased mitotic activity of intestinal cells (4). The statistically significant difference between the age of dogs and morbidity due to canine parvovirus was reported in the study of Ernst et al. (4).

23.8 % of Slovenian dogs, which died due to canine parvovirus, were mixed breeds, followed by German Shepherds, Rottweilers, Labrador Retrievers and Poodles. Using statistical analysis we found that the difference between groups of different breeds and the number of deaths due to canine parvovirus was not statistically significant. We have demonstrated that breed does not have influence on death due to canine parvovirus. Several authors wrote about greater susceptibility of some pure breeds, i. e. Setters (4), Pointers (4), Doberman Pinschers (4, 16, 17) and Rottweilers (4, 16). Pospischill and Yamaho (14) and Houston et al. (17) established that German Shepherds are the most susceptible breed for canine parvovirus. According to statistical data from the year 1996, we noticed that German Shepherds and Rottweilers, followed by Labrador Retrievers and Poodles were the most numerous amongst pure breed's offspring in Slovenian dog's population. In our study we found out that these are breeds with the highest percentage of death due to canine parvovirus.

Most of Slovenian dogs died due to canine parvovirus from August to January. The highest percentage of deaths was reported in August, which is one

of the warmest months in Slovenia, followed by September. High daily temperatures induce loss of appetite, decrease function of the immune system and increase susceptibility to bacterial, parasitic and viral diseases. In the literature we found out that the highest titer of the antibodies against canine parvovirus was determined during summer months (4). Pospischil and Yamaho (14) reported the highest incidence of canine parvovirus in November, December and January and the lowest number in June, July and September. Increased number of deaths during winter could be related to low temperatures which also suppress immune system. Using statistical analysis we demonstrated that the difference between months and the number of deaths due to canine parvovirus was statistically significant. We can conclude that very warm and very cold months have influence on the death due to canine parvovirus. Ernst et al. (4, 5) also demonstrated that the difference between months and morbidity due to canine parvovirus is statistically significant.

References

1. Kelly WR. An enteric disease of dogs resembling feline panleukopenia. *Aust Vet J* 1978; 54: 593.
2. Kelly WR, Atwell RB. Diffuse subacute myocarditis of possible viral aetiology: a cause of sudden death in pups. *Aust Vet J* 1979; 55: 36-7.
3. Osterhaus AD, van Steenis G, de Kreek P. Isolation of a virus closely related to feline panleukopenia virus from dogs with diarrhea. *Zentralbl Veterinarmed B* 1980; 271: 11-21.
4. Ernst S, Montes S, Martin R. Estudio epidemiológico retrospectivo de los factores de riesgo asociados a la ocurrencia de parvovirus en una población canina hospitalaria. *Arch Med Vet* 1988; 20: 38-43.
5. Ernst S, Cid L, Martin R, Thinbaut J. Distribution temporal de la parvovirus clinica en una población canina hospitalaria de Valdivia, Chile (1981 - 1990). *Arch Med Vet* 1992; 24: 157-61.
6. Barker IK, Van Dreumel AA, Palmer N. The alimentary system. In: Jubb KV, Kennedy PC, Palmer N, eds. *Pathology of domestic animals*. Vol.2. San Diego: Academic Press, 1993: 475-84.
7. Šenk L, Pogačnik M. Virusni enteritis psov. *Zb Biotehn Fak Univ E Kardelja Vet* 1982; 19: 17-26.
8. Carman PS, Povey RC. Pathogenesis of canine parvovirus-2 in dogs: haemathology, serology and virus recovery. *Res Vet Sci* 1985; 38: 134-40.
9. Carman PS, Povey RC. Pathogenesis of canine parvovirus-2 in dogs: histopathology and antigen identification in tissues. *Res Vet Sci* 1985; 38: 141-50.
10. Sherding RH. Diseases of the small bowel. In: Ettinger SJ, ed. *Textbook of veterinary internal medicine*. 3rd ed. Philadelphia: WB Saunders Company, 1989: 1894-915.
11. Švara T, Juntos P, Pogačnik M, Gombač M, Hostnik P. Imunohistokemično dokazovanje virusnega antigena v organih psov pri parvovirozi. *Slov Vet Res* 2003; 40 (2): 83-92.
12. Martella V, Cavalli A, Pratelli A, et al. A canine parvovirus mutant is spreading in Italy. *J Clin Microbiol* 2004; 42: 1333-6.
13. Twedt DC, Magne ML. Diseases of the stomach. In: Ettinger SJ, ed. *Textbook of veterinary internal medicine*. 3rd ed. Philadelphia: WB Saunders Company, 1989: 1351-3.
14. Pospischil A, Yamaho H. Parvovirus enteritis in dogs based on autopsy statistics 1978 - 1985. *Tierarztl Prax* 1987; 15: 67-71.
15. Meunier PC, Cooper BJ, Appel MJG, Slauson DO. Pathogenesis of canine parvovirus enteritis: importance of viremia. *Vet Pathol* 1985; 22: 60-71.
16. Glickman LT, Domanski LM, Patronek GJ, Visintainer F. Breed-related risk factors for canine parvovirus enteritis. *J Am Vet Med Assoc* 1985; 187: 589-94.
17. Houston DM, Ribble CS, Head LL. Risk factors associated with parvovirus enteritis in dogs: 283 cases (1982-1991). *J Am Vet Med Assoc* 1996; 208: 542-6.
18. Macartney L, McCandlish IAP, Thompson H, Cornwell HJC. Canine parvovirus enteritis 1: clinical, haematological and pathological features of experimental infection. *Vet Rec* 1984; 115: 201-10.
19. Ruiz Romero RA, Aranda EC, Godoy FS, Watty AD. Immunohistochemical diagnosis of canine parvovirus-2 (cpv-2) in domestic dogs. *Rev Vet Mexico* 2007; 38: 41-53.

RETROSPEKTIVNA ŠTUDIJA PARVOVIROZE PRI PSIH V SLOVENIJI

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Povzetek: Retrospektivno študijo parvoviroze pri slovenskih psih smo opravili z namenom, da bi ugotovili morebitno povezavo med spolom, starostjo, pasmo psov in mesecem, v katerem so psi poginili, ter parvovirozo. Anamnestične podatke o raztelesenih psih, poginulih od januarja 1987 do januarja 2005, ter podatke o vzrokih njihovega poginov ali usmrtilcev smo vzeli iz arhiva Inštituta za patologijo, sodno in upravno veterinarstvo Veterinarske fakultete v Ljubljani. Parvoviroza je bila diagnosticirana s patohistološko preiskavo črevesja, v katerem parvovirusi povzročajo za to bolezen patognomonične spremembe, pri 18 psih pa smo bolezen potrdili tudi z imunohistokemično preiskavo črevesja in nekaterih parenhimskih organov.

Od januarja 1987 do januarja 2005 je bila na Inštitutu opravljena raztelesba 2486 psov, od katerih jih je 84 (3,4 %) poginilo zaradi parvoviroze. Največ poginov zaradi parvoviroze je bilo zabeleženih v avgustu (16,7 %) in septembru (11,9 %), v oktobru, decembru in januarju pa je bilo poginov nekoliko manj (10,7 %). Najmanj psov je zaradi parvoviroze poginilo v februarju. 67,6 % psov je bilo ob poginu starih do 6 mesecev, le 6,7 % psov pa je bilo starejših od enega leta. 83,3 % psov je bilo moškega spola, med čistokrvnimi psi pa je bila parvoviroza najpogosteje diagnosticirana pri nemških ovčarjih, rotvajlerjih in labradorcih.

Na osnovi statističnih analiz podatkov smo ugotovili, da so starost psov in meseci v letu v povezavi s poginom zaradi parvoviroze, spol in pasma pa ne.

Ključne besede: veterinarska medicina, patologija; parvoviroza; parvovirusni enteritis, psi