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CANINE HERPESVIRUS-1 SPECIFIC SEROCONVERSION AND CLINICAL ASPECTS IN KENNEL DOGS FROM ROMANIA

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Abstract

The Canine herpesvirus-1 (CHV-1) is causing in dogs a wide range of reproductive problems: infertility, foetal resorption, abortion, weak puppies, stillborn, low conception rate, small litter size and neonatal mortality, according to the age and pregnancy stage. The aims of the study where to assess the status of CHV-1 infection and to investigate the clinical pattern of the disease, in three Romanian kennel dogs. Blood samples from 44 subjects, aged from 1 to 5.5 years (20 dogs from kennel A, 16 dogs from kennel B, and 8 from kennel C), without history of vaccination against CHV-1 where submitted to study. The serum samples were analysed for the detection of antibodies to CHV-1 by immunofluorescence assays. In this survey, the average of seropositive animals were being 86.36%, but ranged from 100% in kennel A and B, to 25.00% in kennel C. Registered reproductive disorders were represented by neonatal mortality (70%) and infertility (30%). Our study emphasizes the widespread of CHV-1 infection and strengthens the recommendation for the animals' immune status assessment before their breeding season.

Key words*: CHV-1, immunofluorescence assay, canine infectious diseases, reproductive pathology.*

INTRODUCTION

Canine herpes virus infection (CHI) is an acute disease reported in dogs, wolves and coyotes, clinically characterized by respiratory, ocular and genital/reproductive disorders (Carmichael et al., 1965; Poste and King, 1971; Carmichael and Greene, 1998).

The first description of CHI was done by Carmichael et al. (1965) as a fatal septicaemia disease of puppies. Since then, numerous studies have been carried out, enabling the complete characterization of the etiological agent and the worldwide spread of Canine herpesvirus-1 (CHV-1) (Spertzel et al., 1965; Lundgren et Clapper, 1969; Huxtable and Farrow, 1970; Delisle, 1982; Takumi et al., 1990; Gaskell and Willoughby, 1999; Carmichael and Greene, 1998).

CHV-1 is a virus belonging to family *Herpesviridae*, subfamily. *Alphaherpesvirinae*, genus *Varicellovirus*. CHV-1, Feline herpes virus-1 (FVH-1) and Phocine herpesvirus-1 (PhHV-1) are closely related genetically (Gaskell and Willoughby, 1999). Most viruses range in size from 115 to 175 nanometres (nm). The virus is replicating in Dog Kidney Cells, producing cytopathic effect in 2-3 days (Spertzel et al., 1965; Carmichael and Greene, 1998).

The highest prevalence of CHI is obvious mainly in animal clusters without specific surveillance programs. It has been reported in the USA (Carmichael et al., 1965; Lundgren et Clapper, 1969), Europe (Delisle, 1982), Australia (Huxtable and Farrow, 1970), Asia and Oceania (Takumi et al., 1990).

CHV-1 can be transmitted horizontally through direct contact with infected material (e.g., uterine secretions, oronasal secretions) and transplacental (Hashimoto et al., 1982).

The infection is prevalent in many countries and produces significant losses due to reproductive pathology and neonatal death (Carmichael and Greene, 1998).

The reproductive pathology is represented by low conception rate, embryonic and foetal death followed by resorption or abortion or stillborn puppies and small litter size (Poste and King, 1971).

Also, CHV-1 is one of the etiological agents of the canine infectious respiratory disease complex, alongside several other canine viruses, such as canine adenovirus type 2, canine

respiratory coronavirus, canine influenza virus, and canine parainfluenza virus (Buonavoglia and Martella, 2007), as well as bacteria *Bordetella bronchiseptica, Streptococcus equi* subsp. *Zooepidemicus*, and *Mycoplasma cynos* (Zeugswetter et al., 2007; Priestnall et al., 2010; Singh et al., 2015).

Respiratory disorders are described in older dogs and the clinical signs are mild, usually restricted to the upper respiratory tract (e.g. nasal discharge, coughing); the pneumonia is rare (Appel et al., 1969).

Diagnosis of any suspicion of CHI, followed by correct management and a cautious attitude towards animals with clinical signs are crucial in the fight against this pathogen. These state of art make Ronsse et al. (2003) to conclude that "*A good collaboration between breeders, veterinarians and laboratories will allow a rapid intervention, able to limit the often considerable economic losses*" (Ronsse et al., 2003).

In Romania, CHI is underdiagnosed and frequently the symptomatology is associated with other causes.

Also, the confirmation diagnosis is not applied on a regular basis. This led to the lack of knowledge of the prevalence of CHV-1 infections in Romania.

Since dog breeding increased in Romania in the recent years then it has become an imperative requirement to confirm the status of breeding animals - kennel animals, versus CHV using appropriate diagnostic methods.

The primary objective of this research was to design a comprehensive protocol for the diagnosis of CHV-1 infection, according to the Romanian particularities.

The second objective was to identify among canines with reproductive disorders the ones who, based on the anamnesis provided by the owner and on the clinical signs expressed, match to the specific pattern of the CHV infection suspicion.

The third objective was to propose an easy, fast, highly specific and reasonably priced method, in order to confirm the diagnosis suspected/presumed. The last objective was to associate clinical symptomatology with the animal's immune status in relation to the presence of CHV-1 infection.

MATERIALS AND METHODS

For this survey, three Romanian breeding kennels were chosen, below identified as A, B and C. The size and structure of populations are listed as fallow.

Table 1. The size and structure of Romanian breeding kennels

Population	Kennel A	Kennel B	Kennel C	Total
Male	5	4	2	1
Female	15	12	6	33
Total	20	16	8	44

In total, investigated kennels owned 44 dogs (Table 1) of different ages, breeds, number of pregnancies, and performance in reproduction and without history of vaccination against CHV-1.

Blood samples from each subject, where collected from the cephalic vein, in vacutainer blood collection tubes without additives, Samples were stored in the refrigerator until centrifugation (15 min at 2,200 rpm) and the sera has been collected in sterile Eppendorf tubes (1.5 ml). The serum was stored at −20°C until serological testing.

The serum samples were analysed with a commercial immunofluorescence assay (IFA) designed to detect antibodies to CHV-1 (FluoHERPESVIRUS canine, Agrolabo, Italy). IFA method was performed as recommended by the manufacturer. Briefly, all reagents were brought to room temperature (20-25°C) before testing and each serum has been diluted 1:80 in buffered saline.

For each serum to be tested and for the Negative and Positive controls were used 20 µl in the individual slide wells, pre-coated with inactivated cells infected with CHV antigens. Incubation was done in humid chamber for 30 minutes at 37°C. The conjugate anti-Dog-IgG-FITC was added in each well, in the same volume (20 µl/well) and incubated in the same conditions in the dark. The lecture of stained substrate slides was performed at 400X magnification. The samples providing negative results at 1:80 screening dilution were considered negative for CHV-IgG antibodies, and the ones providing positive test results at 1:80 screening dilution were considered positive for CHV-IgG antibodies.

RESULTS AND DISCUSSIONS

In the first investigated kennel, the serological assessment of CHV-1 circulation was based on the history of the infertile mating and on the neonatal mortality (Table 1).

Table 1. Reproductive pathology associated with immune status in kennel A

No. #	Breed	Gender	IFA result	No of infertile matings	No of litters	Litter size	Neonatal mortality
1.	Rottweiler	F	+	1	1	4	2
2.	Rottweiler	F	+	0	0	0	0
3.	Rottweiler	F	+	0	1	4	4
4.	Rottweiler	F	+	0	1	4	0
5.	Rottweiler	F	+	0	1	5	0
6.	Rottweiler	F	+	0	1	4	0
7.	Rottweiler	F	+	0	1	5	2
8.	Rottweiler	F	+	1	0	0	0
9.	Rottweiler	F	+	0	0	0	0
10.	Rottweiler	F	+	0	1	6	0
11.	Rottweiler	F	+	0	1	5	5
12.	Rottweiler	F	+	0	0	0	0
13.	Rottweiler	F	+	0	1	5	5
14.	Rottweiler	F	+	1	0	0	0
15.	Rottweiler	F	+	0	1	7	2
16.	Rottweiler	M	+	-	-	-	-
17.	Rottweiler	M	+	-	-	-	-
18.	Rottweiler	M	+	-	-	-	-
19.	Rottweiler	M	+	-	-	-	-
20.	Rottweiler	M	+	-	-	-	-
TOTAL				**3**			**20**

In the kennel A, the reproductive disorders suddenly appeared, with several cases in a short period of time. The intensity of signs was different in affected animals: 100% neonatal mortality in bitches' litters #3, #11, and #13, while bitch #7 had 60.00% neonatal mortality, bitch #1 had 50.00%, bitch #15 had 28.57%, and bitches #4, #5, #6, and #10 had 0.00% neonatal mortality. Bitch #1 seems to have been the most affected, expressing both, infertile matings and neonatal mortality.

Overall, in the kennel A was 20 cases of neonatal mortality (death in first 72 hours of life), in a total of nine calving with 49 newborne puppies.

In second investigated kennel, the serological evaluation of CHV-1 circulation started after several multiple cases of neonatal mortality (Table 2).

Table 2. Reproductive pathology associated with immune status in kennel B

No. #	Breed	Gender	IFA result	No of infertile matings	No of litters	Litter size	Neonatal mortality
1.	Cane Corso	F	+	0	1	7	3
2.	Cane Corso	F	+	0	2	6	2
						8	1
3.	Cane Corso	F	+	0	2	10	3
						10	1
4.	American Staffordshire Terrier	F	+	0	2	7	2
						7	2
5.	American Staffordshire Terrier	F	+	1	1	6	6
6.	American Staffordshire Terrier	M	+	-	-	-	-
7.	American Staffordshire Terrier	M	+	-	-	-	-
8.	American Bully	F	+	0	0	0	0
9.	American Bully	F	+	0	0	0	0
10.	American Staffordshire Terrier	F	+	1	0	0	0
11.	American Bully	M	+	-	-	-	-
12.	American Staffordshire Terrier	F	+	0	2	10	2
						8	1
13.	Cane Corso	F	+	1	1	8	8
14.	Cane Corso	M	+	-	-	-	-
15.	Cane Corso	F	+	0	2	16	0
16.	American Staffordshire Terrier	F	+	0	1	5	5
TOTAL				**3**			**36**

As in the previous described kennel, in the kennel B, the main reproductive disorder was the neonatal mortality. The intensity of clinical signs registered variation from one case to another, with 100% neonatal mortality in litter of bitches #5, #13, and #16, 30.00% in litter of bitch #1, 28.57% in litter of bitch #4, 21.43% in litter of bitch #2, 20.00% in litter of bitch #3, 16.67% in litter of bitch #12, and 0% in litter of bitch #15.

Bitch #13 have been the most affected: she suffered one infertile mating and a calving with 100% neonatal mortality. It must be also emphasized the persistence of neonatal mortality in all bitches with two litters. In this kennel, all breeds (Cane Corso, American Staffordshire Terrier, and American Bully) were affected.

Generally, in the kennel B was 36 cases of neonatal mortality from the 13 calving, with 108 new-borne puppies.

In the kennel C, the serological evaluation of CHV-1 circulation started after three cases of major neonatal mortality (Table 3).

Table 3. Reproductive pathology associated with immune status in kennel C

No. #	Breed	Gender	IFA result	No of infertile matings	No of litters	Litter size	Neonatal mortality
1.	Havanese Cuban Bichon	F	–	0	3	15	0
2.	Yorkshire Terrier	F	–	0	2	12	6
3.	Yorkshire Terrier	M	–	-	-	-	-
4.	Havanese Cuban Bichon	F	–	1	0	0	0
5.	Havanese Cuban Bichon	F	–	1	0	0	0
6.	Bichon Maltese	F	+	0	2	10	4
7.	Yorkshire Terrier	F	+	0	2	10	6
8.	Havanese Cuban Bichon	M	–	-	-	-	-
TOTAL				2			16

In the kennel C, they are eight breeding animals from three breeds (Bichon Maltese, Havanese Cuban Bichon, and Yorkshire Terrier). Despite the limited number of animals, the reproductive disorders were considerable, with 16 cases of neonatal mortality from the 47 new-borne puppies. However, serological evaluation for CHV-1 revealed the presence of specific antibodies only in two bitches with neonatal mortality (#6 and # 7). Neonatal mortality in bitch #6 (Bichon Maltese breed) was 40% and in bitch #7 (Yorkshire Terrier breed) was 60%. The intensity of signs was quite similar in both cases. However, the negative result obtained in bitch #2 cannot exclude the involvement of CHV-1 in neonatal mortality. Also, here cannot be excluded the CHV-1 infection in all the negative dogs of the contaminated kennel. Previous studies have shown that CHV-1 is a weak immunogenic virus and detectable antibodies can be recorded within 2-3 weeks after infection (Takumi et al. 1990). Even more, antibodies decrease quite rapidly and cannot be detected after few months (Carmichael and Greene 1998).

The reproductive disorders recorded in kennels A, B and C are graphically represented in figures 1, 2 and 3.

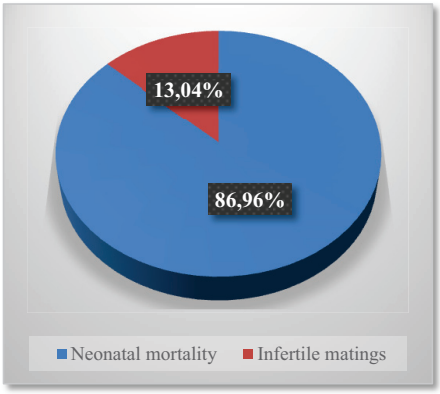

Figure 1. Reproductive disorders recorded in kennel A

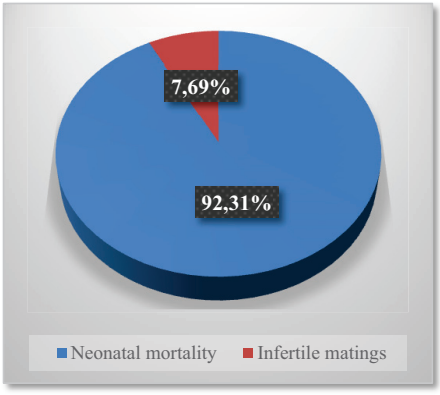

Figure 2. Reproductive disorders recorded in kennel B

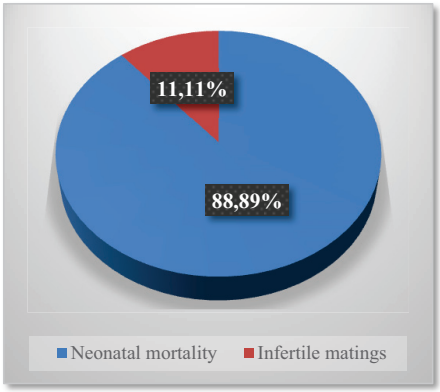

Figure 3. Reproductive disorders recorded in kennel C

In all kennels, neonatal mortality covered 90% (72/80) of reproductive disorders, and infertility 10% (8/80).

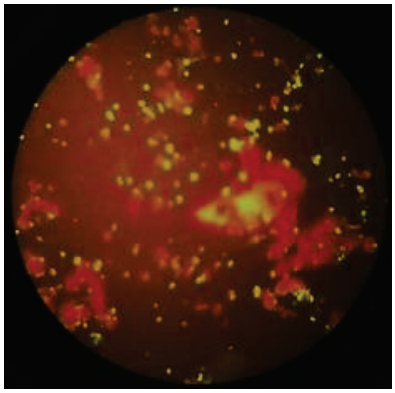

Figure 4. Positive IFA-CHV-IgG result in a female dog with a recent history of neonatal mortality (dilution 1:80; magnification 400X)

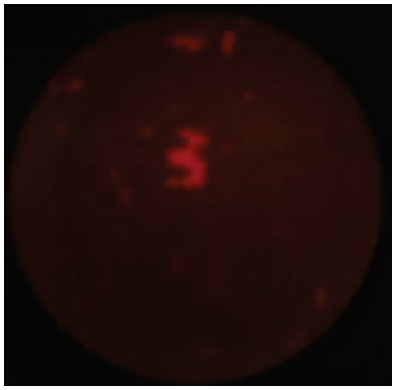

Figure 5. Negative IFA-CHV-IgG result in a female dog without a history of reproductive disorders (dilution 1:80; magnification 400X)

This study was the first approach in order to assess the presence of CHV-1 infection in dog breeding kennels from the Bucharest-Ilfov area. As resulting following the serologic assays and, as to be expected, in this area too, the dogs have been exposed to CHV-1. In Romania, similar studies have been done by Chielaru (2014) in five Northeast counties: Bacau, Galati, Iasi, Suceava, and Vaslui. Research on seroprevalence of canine herpesvirus-1 infection in northwest Romania revealed 20.55% (37/180) seropositive animals. The highest seroprevalences were in Iasi (27.77%) and Vaslui (26.86%) and lowest in Bacau (15.38%), Galati (11.11%) and Suceava (5.00%) (Chielaru, 2014). In our survey, the average of seropositive animals are being 86.36%, ranging from 100% in kennel A (Table 1) and B (Table 2) to 25% in kennel C (Table 3). Previous Romanian CHV-1 seroprevalence data are much lower than those obtained in this study, but it is not relevant to compare those data because the subjects investigated have different status. In the Chelaru (2014) survey were investigated several solitary lifestyle animals and in our study were investigated only breeding animals in kennels. Dog populations with 100% positive titres were reported in Finnish breeding kennels, facing reproductive problems, while lower values were obtained in breeding kennels without reproductive problems (65%) (Dahlbom et al., 2009). Our data are quite similar with other European serological surveys that shown high prevalence in households or breeding kennels in Belgium (45.80%) (Ronsse et al., 2002), England (88.00%) (Reading and Field, 1999), Italy (27.9%) (Sagazio et al., 1998), Lithuania (26.88%) (Musayeva et al., 2013), the Netherlands (39.30%) (Rijsewijk et al, 1999), and Turkish (62.1%) (Yesilbag et al., 2012).

In our survey, reproductive disorders were reported mainly in females that provided a positive result in the serological test for CHV-1. There were also serologically positive female CHV-1 who did not show reproductive disorders, as did serologically negative female CHV-1 who had reproductive disorders. These cases, even if singular, require the extension of investigations to other aetiologies of reproductive disorders but also to the protective effect on gestation of anti-CHV-1 antibodies.

CONCLUSION

Our study emphasizes the widespread of CHV-1 infection. This recommends the assessment of the immune status of the animals before their breeding season. The breeding kennels should benefit from a protocol of surveillance and prophylaxis for those infectious diseases impairing their health status and reproductive performance.

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