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DYNAMIC OF ANTIBODIES AGAINST CANINE DISTEMPER VIRUS AND CANINE PARVOVIRUS IN ROMANIAN CANINE BLOOD DONORS

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Abstract

The objective of the current study was to determine the immunological changes in canine blood donors in Romania. The data of the study is being collected since 2016 and is still in process of research. The samples are represented by serum antibodies IgG - Canine Parvovirus and IgG - Canine Distemper virus, which were analysed at each blood donation of dogs being part of the donation program. They fulfil the eligibility criteria of being clinically healthy with a completed vaccination schedule. ***The study is based on 17 canine blood donors (5 males/12 females), over one-year-old (2-4 years), with owners and living in similar environments.*** *The ELISA VetLine Canine Distemper Virus (CDV) and VetLine Canine Parvovirus (CPV) kits from NovaTec (Immundiagnostica GMBH, Germany) were used following the kits manufacturer's recommendations. The analysis of the results obtained from the serum samples collected showed that no individual presented negative results (0/17-0%) below the protection standard on the two viral strains, all the serum samples having positive results (17/17-100%). The results indicate that repeated blood donation cannot influence the loss of post-vaccine antibodies.*

Key words*: CDV-CPV, IgG - ELISA, canine blood donors.*

INTRODUCTION

The request for blood components associated with better emergency and critical care treatments has increased in the last decade in the veterinary medicine field, mainly for canine patients, leading to the creation of canine blood banks in several countries, thus consequently leading to an increased number of dogs that provide frequent donations (Ferreira et al., 2014).

More concerns have been raised upon the safety and bioethics for frequent blood donations regarding the donor's well-being because of this arising demand for canine blood all over the world.

Until now, studies have been made on how the frequency of donations can affect the canine donor's iron status that can cause iron-deficient erythropoiesis (like in human donors) because of the act of excessive phlebotomies (Giger, 2005; Lewis & Stone, 2012) and on some hematologic variables such as haemoglobin concentration, platelet count, WBC count and reticulocyte count (Ferreira et al., 2014) but no other research was made on other parameters such as immunological ones, especially regarding serum antibody titres evolution following vaccination from one blood donation to another.

The lack of studies on the immunological status of the canine blood donors recommends future research to establish if there is an immunelogical risk and if a specific vaccination program needs to be developed for this group of animals.

The guideline for the vaccination of dogs compiled by the Vaccination Guidelines Group of the World Small Animal Veterinary Association recommends that Canine Parvovirus-2 (CPV), Canine Distemper Virus (CDV), and Canine Adenovirus-2 as core vaccines (vaccines which all dogs should receive), while rabies where required by statue or in areas where the disease is endemic (Day et al., 2016).

No study was yet performed on canine blood donors and the consequences of regular blood donations upon the capacity of the donor's immune system to maintain a protective IgG level against none of the core vaccines.

In this context, our study aims to investigate how does the immune system of canine blood donors responds from one donation to another and if it is able to maintain protective IgG levels against two of highly pathogenic

microorganisms namely CPV and CDV from one donation to another.

MATERIALS AND METHODS

Serum samples (n = 80) were collected from healthy dog donors (n = 17; 5 males and 12 females) that provided frequent donations within a Romanian blood bank. All dogs were client-owned animals and the owner's consent was provided for the participation of their dogs in this study. All dogs were 2 to 6 years old, weighing between 25 and 60 kg, dewormed and with a complete initial vaccination program and yearly boosters administered. Furthermore, at each donation they have been tested and provided negative results for *Anaplasma phagogytophilum*, *Anaplasma platys*, *Ehrlichia canis*, *Ehrlichia ewingii*, *Borrelia burgdorferi*, *Dirofilaria immitis* using the enzyme immunoassay technology (EIA) - SNAP 4Dx Combo Plus® (Idexx Laboratories, Fremont, CA) and negative blood smears for *Babesia canis*. The register code, breed, gender, last vaccination date and the number of blood donations were noted for each animal (Table 1).

Table 1. Donors' identification by register number, age, gender, and number of donations

No	Donor	Breed	Gender	Age	Number of donations
1	BNA 04MP	American Staffordshire Terrier	M	4	6
2	BNA 05FN	American Staffordshire Terrier	F	4	6
3	AMN 16MN	Cane Corso	M	2	6
4	HER 12FN	American Staffordshire Terrier	F	6	6
5	BRD 16FP	Crossbred	F	3	5
6	CRV 02F-	Cane Corso	F	2	5
7	CRV 06M-	Cane Corso	M	3	5
8	BNA 11FP	German Shepherd	F	6	5
9	DRS 14FP	Golden Retriever	F	5	4
10	CRV 07F-	Cane Corso	F	3	4
11	CRV 15MP	Cane Corso	M	3	4
12	BNA 03FP	American Staffordshire Terrier	F	4	4
13	BNA 13M-	Crossbred	M	4	4
14	BNA 14F-	Crossbred	F	4	4
15	BNA 15F-	Crossbred	F	4	4
16	IMDB13 FN	Doberman	F	4	4
17	IMGR05 FP	Golden Retriever	F	6	4

Study protocol

The dog donors were set up in three groups: the first group providing 6 donations, the second group providing 5 donations and the last one providing 4 donations. All the dogs donated 450 mL of whole blood at an interval of 2 to 4 months apart (starting in October 2016 and ending in December 2018) to fulfil the minimum "resting" time, as described in veterinary literature (Schneider, 1995; Ford & Mazzaferro, 2006; Mathews et al., 2006; Gibson & Abrams-Ogg, 2012), but considering also the maximum period agreed in human medicine standards (Europe Council, 2011).

Blood donations

All blood collections were performed by the same operator. Each donor dog has undergone a complete physical examination before each donation.

Dogs were placed in right lateral recumbency and the puncture area over the left jugular vein was aseptically prepared using 70% alcohol. The hair was not clipped as most of them were show dogs. Jugular venepuncture was then performed, and blood was collected by gravity into the collection bag.

Sample collection

All blood samples (9 ml per sample) were collected on clot activator vacutainers directly from the blood bag's tube (containing whole blood with no anticoagulant) at the end of the blood collection after clamping the tube. After 30 minutes at room temperature, the vacutainers were centrifuged at 3500 rpm for 10 minutes. Serum samples were then separated and stored at – 20°C for 2 years until being analysed.

Qualitative ELISA assay technique

The immunological status of the investigated canine blood donors was evaluated using the qualitative ELISA commercial kits: VetLine Canine Parvovirus (CPV) and VetLine Canine Distemper Virus (CDV) kits from NovaTec (Immundiagnostica GMBH, Germany).

Working protocols were followed as indicated by the manufacturer for both test methods and the interpretation of the results.

Briefly, all reagents and the microtiter strip wells precoated with Canine Parvovirus

/Morbillivirus antigens to bind corresponding antibodies of the specimen were brought to room temperature (20-25ºC). The serum samples, the positive and negative controls were diluted 1:50 in sample diluent, and 100 µl were dispensed into the appropriate wells of the microtiter plate.

The microtiter plate was sealed with adhesive film and incubated for 60 minutes at 37ºC and washed three times with 300 µl of washing solution. Afterwards, 100 µl Vet Line Canine Parvovirus/Morbillivirus Protein A/G Conjugate was dispensed into each well; the microtiter plate was sealed with adhesive film and incubated 30 minutes at room temperature (20-25ºC) and washed four times with 300 µl of washing solution.

Then, 100 µl tetramethylbenzidine substrate (TMB 0.25%) prepared just before use was dispensed into each well and incubated in the dark at room temperature (20-25ºC) for 15 minutes resulting in the immune complex formed by the bound conjugate which gives a blue reaction in the specimen.

In the end, 100 µl of the Stop solution (1N sulphuric acid solution) were added to each well to stop the reaction producing a yellow endpoint colour.

The results were read at dual wavelength mode of 450-620 nm and recorded for statistical analysis.

For the interpretation of the results the following values were considered as a guideline: For Canine Parvovirus: Positive at > 11 NTU (NTU = NovaTec Units calculated like indicated in Table 2); Equivocal at 9-11 NTU; Negative < 9 NTU. For Canine Distemper Virus: Positive at > 7 NTU; Equivocal at 6-7 NTU; Negative at < 6 NTU.

Table 2. Results in units [NTU] – method of calculation

$\frac{\text{Sample (mean)absorbance value x 10}}{Cut-off}$ = [NovaTec Units = NTU]
Example: $\frac{1.591 \times 10}{0.43} = 37$ NTU (units)
Calculated Cut-off for Canine Parvovirus = 10 NTU Calculated Cut-off for Canine Distemper Virus = 6.5 NTU

Statistical analysis

All NTU values for each individual (for both Canine Parvovirus and Canine Distemper Virus) were recorded and analysed in Excel application of Microsoft Office 365 suite and One-Way ANOVA Analysis Tool pack; $p < 0.01$ was considered significant.

RESULTS AND DISCUSSIONS

There are studies for human donors that observed the effect of blood donations on the profile of lymphocytic cells stating that following ordinary blood donations, no change in Ig levels and peripheral lymphocyte populations was found (Ieromnimon et al., 1981; Lewis et al., 1992).

Some more recent studies from human transfusion medicine suggest that there are some transient changes in lymphocyte subsets following a single blood donation in male subjects (Borai et al., 2017). On the other hand, veterinary transfusion studies from the past decades have led to the development of general guidelines for donor's selection to increase their safety (Yagi & Bean, 2016) but there is no data available at the moment on the effects of blood donation neither on the dog's immune system nor on the vaccine-induced antibody levels.

Enrolled donors

Seventeen dogs enrolled in the elected canine blood banks' donation program were included in this study.

The median age of included dogs was 3.9 years. There were 5 (29.41%) male dogs (MC 1/5; MI 4/5) and 12 (70.59%) female dogs (FC 8/12; FI 4/12).

Breeds included were represented by American Staffordshire Terrier (4), Cane Corso (5), German Shepherd (1), Dobermann (1), Golden Retriever (2), Crossbreed (4). From the 17 dogs tested, 4 were crossbreeds and 13 were purebred.

The small number of tested animals did not allow a breed analysis; therefore, the statistical analysis and interpretation of the results were done across the group for both the Canine Parvovirus group and Canine Distemper Virus group.

It is to be mentioned that breed, age, and gender frequency distributions were generally representative of the canine blood banks' donors.

NTU for Canine Parvovirus

For the Canine Parvovirus, the overall picture of NTU results revealed non-significant variation between and within D1-D6 groups of values ($p<0.01$, $F<F$ crit) as showed in Table 3. NTU values were above the minimum value used as a guideline (>9 NTU) in all groups, ranging between 10.03 NTU and 23.55 NTU (Table 4, Table 5, and Table 6), but only one dog (BNA11FP) had equivocal NTU values (9-11 NTU) at 3 of 5 blood donations (10.03; 10.78; 10.55) with a booster of 12.78 NTU following the annual booster vaccine, but the NTU value did not drop under 9 NTU for the dog to be considered not protected by the vaccine-induced antibodies (Table 5 and Figure 2), thus we recommend that the canine blood donors should be vaccinated yearly in order to maintain protecting antibody levels for Parvovirus. In all three groups, the dynamic between donations tends to be the same for each tested donor (Figure 1, Figure 2, and Figure 3)

Table 3. Statistical analysis – ANOVA Single Factor (alpha = 0.01) – NTU values for Canine Parvovirus for all donors

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	14.31435	5	2.86287	0.449675	0.812220825	3.275224
Within Groups	471.1231	74	6.366528			
Total	485.4374	79				

Table 4. Results as NovaTec Units (NTU) for Canine Parvovirus in the first group (dogs with 6 blood donations)

No.	Donor ID	Donation					
		D1	D2	D3	D4	D5	D6
1.	BNA04MP	17.41	16.66	16.98	17.03	15.96	15.41
2.	BNA05FN	17.61	15.71	16.05	16.95	17.31	16.83
3.	AMN16MN	17.66	17.75	21.63	18.16	23.01	20.68
4.	HER12FN	15.18	15.11	19.26	13.96	15.45	11.68

*the values after the yearly vaccine booster are highlighted in blue
*Positive > 11 NTU; Equivocal 9-11 NTU; Negative < 9 NTU

Table 5. Results as NovaTec Units (NTU) for Canine Parvovirus in the second group (dogs with 5 blood donations)

No.	Donor ID	Donation				
		D1	D2	D3	D4	D5
5.	BRD16FP	14.78	16.06	15.68	17.73	16.48
6.	CRV02F-	19.66	14.68	14.23	12.95	12.90
7.	CRV06M-	17.76	17.65	17.41	18.33	16.88
8.	BNA11FP	10.03	12.78	10.78	11.71	10.55

*the values after the yearly vaccine booster are highlighted in blue
*Positive > 11 NTU; Equivocal 9-11 NTU; Negative < 9 NTU

Table 6. Results as NovaTec Units (NTU) for Canine Parvovirus in the third group (dogs with 4 blood donations)

No.	Donor ID	Donation			
		D1	D2	D3	D4
9.	DRS14FP	19.23	18.11	16.58	15.06
10.	CRV07F-	15.60	19.91	18.91	16.98
11.	CRV15MP	17.01	23.55	16.85	16.21
12.	BNA03FP	14.53	17.38	15.70	16.31
13.	BNA13M-	17.48	17.00	16.68	15.83
14.	BNA14F-	20.36	17.83	18.26	18.43
15.	BNA15F-	18.90	18.91	18.46	18.38
16.	IMDB13FN	17.73	17.11	15.33	17.50
17.	IMGR05FP	16.73	16.50	15.55	13.51

*the values after the yearly vaccine booster are highlighted in blue
*Positive > 11 NTU; Equivocal 9-11 NTU; Negative < 9 NTU

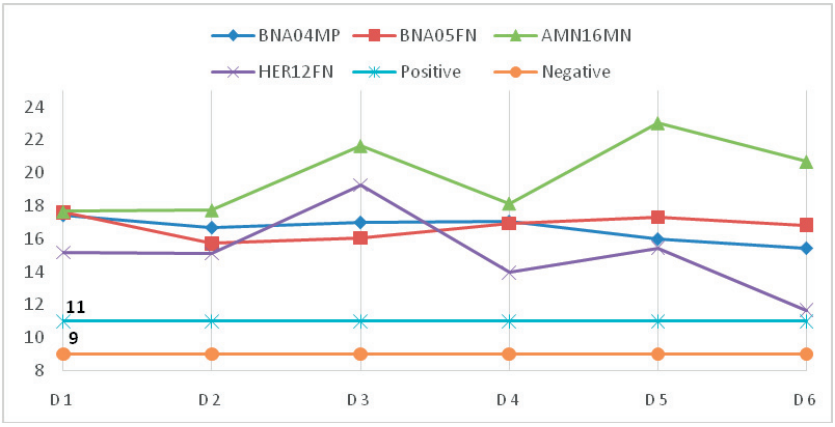

Figure 1. Graphic representation of NTU for Canine Parvovirus in the first group (dogs with 6 blood donations)

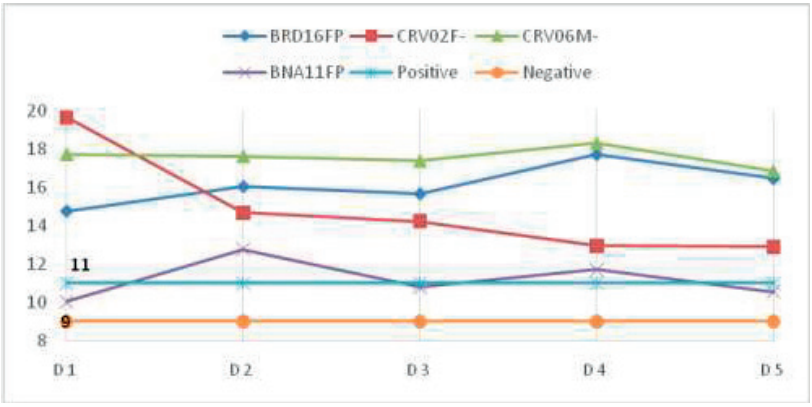

Figure 2. Graphic representation of NTU for Canine Parvovirus in the first group (dogs with 5 blood donations)

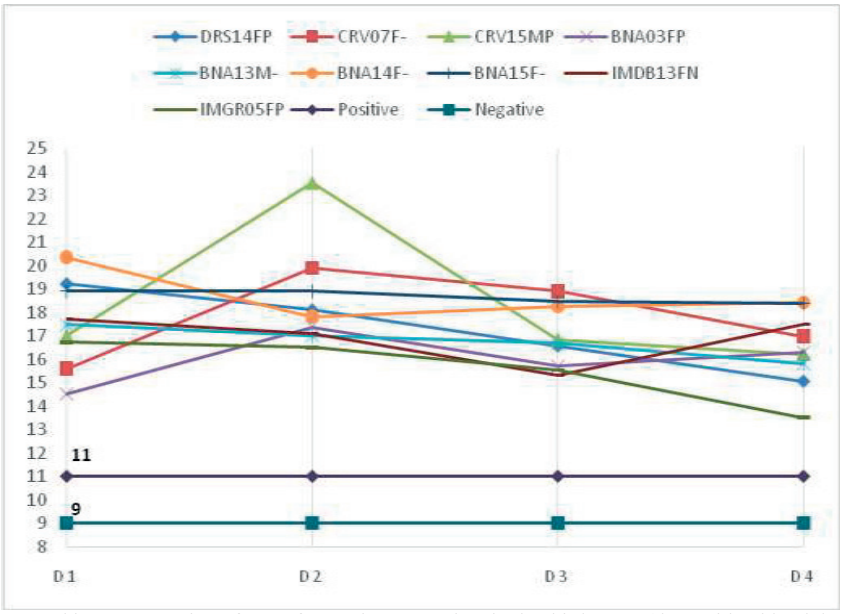

Figure 3. Graphic representation of NTU for Canine Parvovirus in the third group (dogs with 4 blood donations)

NTU for Canine Distemper Virus

As for the Canine Parvovirus, for the Canine Distemper Virus, the overall picture of NTU results revealed also a non-significant variation of values between and within D1-D6 groups ($p<0.01$, $F<F\ crit$) as showed in Table 7. NTU values were above the minimum value used as a guideline (>6 NTU) in all groups, ranging between 8.39 NTU and 44.45 NTU (Table 8, Table 9, and Table 10). No donor dog had equivocal NTU values (6-7 NTU) at any donation in all the three groups analysed, thus suggesting an optimal immunization of canine blood donors even after frequent donations. We can also observe the rhythmicity of antibodies between donors and that the dynamic between donations tends to be the same for each tested donor (Figure 4, Figure 5, and Figure 6).

Because of the low number of donors, a correlation between age/gender and antibody levels could not be determined.

Table 7. Statistical analysis – ANOVA Single Factor (alpha = 0.01) – NTU values for Canine Distemper Virus for all donors

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	88.873	5	17.7746	0.31162	0,904508387	3.27522
Within Groups	4220.9	74	57.0392			
Total	4309.77	79				

Table 8. Results as NovaTec Units (NTU) for Canine Distemper Virus in the first group (dogs with 6 blood donations)

No.	Donor ID	Donation					
		D1	D2	D3	D4	D5	D6
1.	BNA04MP	13.90	12.14	15.08	12.82	15.87	10.27
2.	BNA05FN	26.64	28.09	35.33	32.61	31.69	24.01
3.	AMN16MN	19.38	22.90	44.45	41.33	33.13	36.43
4.	HER12FN	13.06	10.99	18.75	14.41	8.64	13.27

*the values after the yearly vaccine booster are highlighted in blue
*Positive > 7 NTU; Equivocal 6-7 NTU; Negative < 6 NTU

Table 9. Results as NovaTecUnits (NTU) for Canine Distemper Virus in the second group (dogs with 5 blood donations)

No.	Donor ID	Donation				
		D1	D2	D3	D4	D5
5.	BRD16FP	8.39	10.16	11.35	10.27	13.47
6.	CRV02F-	19.60	19.78	24	21	17.33
7.	CRV06M-	27.06	21.35	20.81	21.11	20.37
8.	BNA11FP	12.99	17.29	14.41	14.93	14.55

*the values after the yearly vaccine booster are highlighted in blue
*Positive > 7 NTU; Equivocal 6-7 NTU; Negative < 6 NTU

Table 10. Results as NovaTecUnits (NTU) for Canine Distemper Virus in the third group (dogs with 4 blood donations)

No.	Donor ID	Donation			
		D1	D2	D3	D4
9.	DRS14FP	14.27	13.80	15.80	12.90
10.	CRV07F-	15.31	20	18.68	15.74
11.	CRV15MP	26.25	25.85	26.41	25.40
12.	BNA03FP	16.70	19.33	17	13.67
13.	BNA13M-	21.81	19.83	22.19	16.73
14.	BNA14F-	31.53	29.18	26.70	20.18
15.	BNA15F-	18.70	20.41	19.74	17.89
16.	IMDB13FN	17.58	15.49	17.09	13.29
17.	IMGR05FP	18.93	15.45	14.21	11.44

*the values after the yearly vaccine booster are highlighted in blue
*Positive > 7 NTU; Equivocal 6-7 NTU; Negative < 6 NTU

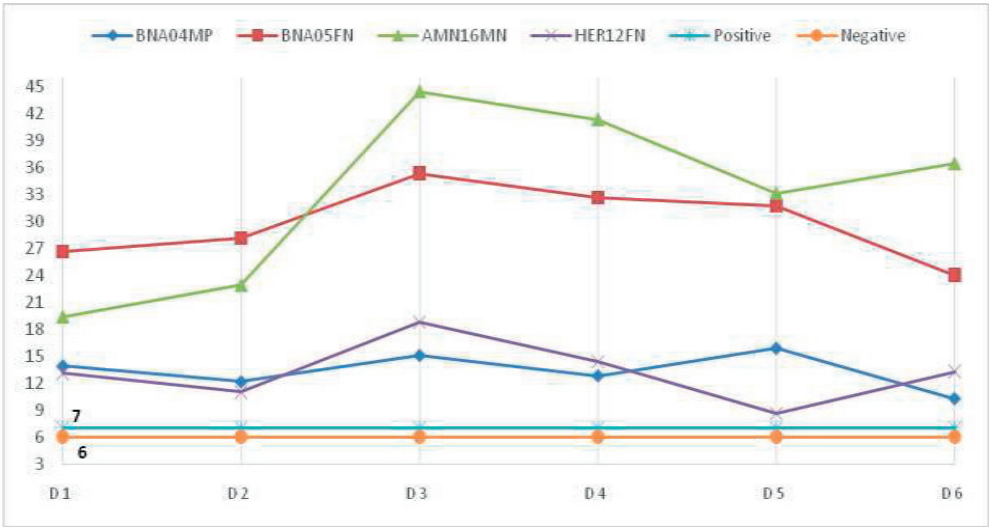

Figure 4. Graphic representation of NTU for Canine Distemper Virus in the first group (dogs with 6 blood donations)

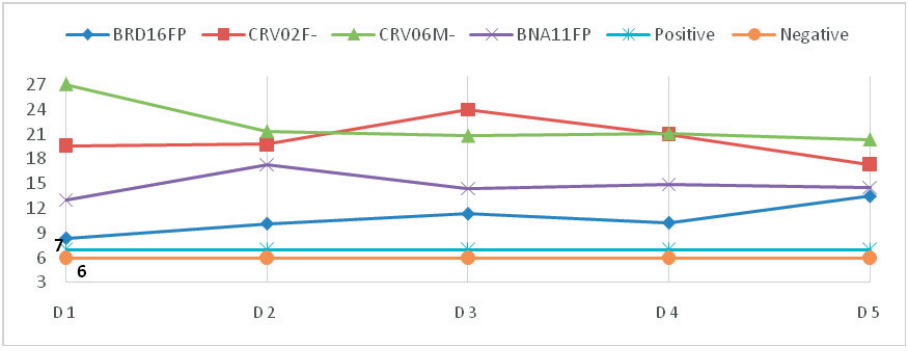

Figure 5. Graphic representation of NTU for Canine Distemper Virus in the third group (dogs with 5 blood donations)

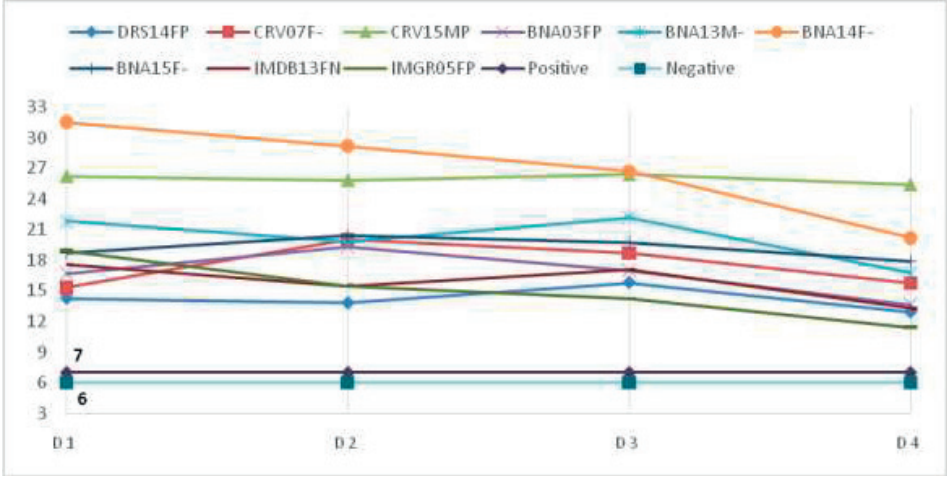

Figure 6. Graphic representation of NTU for Canine Distemper Virus in the third group (dogs with 4 blood donations

Immunology studies show that vaccination stimulates both humoral responses via antibody production and cellular responses via B and T lymphocytes (Day, 2012). How long the post-vaccine immune response is maintained at a protective level is mainly dependent on the immunological memory developed (Day et al., 2016).

However, it is unclear whether a vaccinated dog is fully protected throughout its life or whether revaccination is always necessary (Abdelmagid et al., 2004) and, moreover, if a canine blood donor's immune system can maintain a protective serum antibody titre between blood donations. To clarify these issues, it is important to quantify the rate by which vaccinated canine blood donors become serological-negative again, the so-called seroconversion rate.

There is no data available at this moment for assessing the dynamics of serum antibodies against Parvovirus and Distemper Virus for canine blood donors and if the antibody titre for both CPV and CDV suffers any changes between blood donations, so the present studies results cannot be compared with other studies results.

These preliminary data will be completed with more samples for more than 17 blood donors (different breeds and ages) in order to avoid the individual influence on the results. Also, we will compare in a further study the donors' results with a control group (dogs of the same age and sex, clinically healthy but not enrolled in any blood donation program).

CONCLUSIONS

In conclusion, no significant differences were observed between the average values of serum antibodies against Canine Parvovirus and Canine Distemper Virus, thus the frequency of blood donations does not influence the protective antibody titre against CPV and CDV. The comparison of all three experimental groups with a complete vaccination schedule received by each dog proved a close correlation of immunological status and the time-lapse between annual vaccine boosters.

Further studies are needed in order to assess the real impact of frequent blood donation on the immune system of canine blood donors and its ability to maintain protective antibody levels.

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