THE IMMUNE RESPONSE AGAINST RABIES VIRUS AMONG CANINE DONORS IN A ROMANIAN BLOOD BANK

Teodor-Stefan Ionescu¹, Marius Dan², Dragos Cobzariu³,
Stelian Baraitareanu⁴, Doina Danes⁵

Abstract:

INTRODUCTION: Seventy-six samples were collected from sixteen canine blood donors at every blood donation performed in accord with the canine donor selection guidelines. Statistical analysis of ELISA OD values revealed no significant variation of anti-rabies antibodies values during repeat donations. Our results support the statement that blood donation does not affect the immune status of donors.

OBJECTIVES: The study aimed to assess the immune status against rabies among the canine blood donors in a Romanian canine blood bank

METHODS: Serum samples (n=76) were collected from pet owned dogs (n=16) recorded in the blood bank register of a Romanian blood bank were performed with the immunological status of the investigated canine blood donors was evaluated using the indirect ELISA commercial kit PLATELIA RABIES II (BIO-RAD, France) as per the manufacturer's instructions

RESULTS: The overall picture of ELISA OD results revealed non-significant variation between and within D1-D6 groups of values ($p>0.05$, $F<F_{crit}$).

CONCLUSION: The next studies topic would be to compare the results provided by the ELISA technology with FAVN.

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Keywords: blood transfusion; blood donation; canine blood bank; ELISA OD; rabies

Introduction

The request for blood and blood products increased in the last decades. The transfusion technology initially developed for human health became more easily available for companion animals, which led to the design and set up of veterinary blood banks Radulescu et al (2009). Like in human transfusion medicine, the canine blood bank use pretransfusion testing for canine erythrocyte antigens Yagi and Bean (2016); Ionescu et al. (2018a) and blood-borne pathogens Reine (2004); Ionescu et al. (2018b) to minimize the risks associated with blood transfusion. In this regard, our previous studies established that canine blood donors of Romania blood banks were 56.19% (59/105) DEA1 positive [37.29% (22/59) male, and 88.13% (52/59) purebred] and 43.81% (46/105) DEA1 negative [56.52% (26/46) male, and 93.48% (43/46) purebred] Ionescu et al. (2018a), provided negative results for *B. burgdorferi* and *E.canis/E. ewingii* (0/1253), while 0.87% (11/1253) were positive for *A. phagocytophilum/A. platys* and 6.94% (87/1253) positive for *D. immitis* Ionescu et al. (2018b). All these tests are in accord with the current canine donor selection rules Yagi and Bean (2016) that guarantee transfusion safety for both donor and transfused dogs.

However, questions about the quality of the vaccine immunological response in dogs with several blood donations need to receive more clear responses. The lack of studies considering the immunological status of the canine blood donors recommends future research to establish an immunological risk and if a specific vaccination program needs to be developed for this particular group of animals. Guidelines for the vaccination of dogs compiled by the Vaccination Guidelines Group of the World Small Animal Veterinary Association recommend Canine Parvovirus-2, Canine Distemper Virus, 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). However, vaccinating dogs against

¹ Faculty of Veterinary Medicine, University of Agronomic Science and Veterinary Medicine of Bucharest, Romania; ionescuteodorstefan@gmail.com

² Faculty of Veterinary Medicine, University of Agronomic Science and Veterinary Medicine of Bucharest, Romania; dan.fmaris@yahoo.com

³ Faculty of Veterinary Medicine, University of Agronomic Science and Veterinary Medicine of Bucharest, Romania; dragoscobzariu@gmail.com

⁴ Faculty of Veterinary Medicine, University of Agronomic Science and Veterinary Medicine of Bucharest, Romania; doruvet@gmail.com

⁵ Faculty of Veterinary Medicine, University of Agronomic Science and Veterinary Medicine of Bucharest, Romania; danes.doina@gmail.com

rabies aims to establish pre-exposure immunity and protect animals from contracting rabies and stop the further spread to humans or other animals (Cox et al (1977)).

In Romania, the history and epidemiological evolution of rabies among wild animals between 2011 and 2016 shows us that the peak of rabies cases were reported from 2011 until 2013, while a significant decrease in positive cases was recorded between 2014 and 2016, reaching the year of 2017 with no positive results detected in the wild animal population EC DG SANTE (2017). In Romania, vaccinating dogs against rabies every year is mandatory.

In light of those data, the study aimed to assess the immune status against rabies among the canine blood donors in a Romanian canine blood bank.

Material and Methods

Animals, management, and experimental design

Serum samples (n=76) were collected from pet-owned dogs (n=16) recorded in the blood bank register of a Romanian blood bank. Register Code, breed, gender, last vaccination dates, and the number of blood donations were noted for each animal (Table 1).

No.	Blood Bank Animal Code	Breed	Gender	Age (years)	No of Blood Donations
#1	BNA04MP	American Staffordshire Terrier	M	4	6
#2	BNA05FN	American Staffordshire Terrier	F	4	6
#3	AMN16MN	Cane Corso	M	2	6
#4	HER12FN	American Staffordshire Terrier	F	6	6
#5	BRD16FP	Crossbred	F	3	5
#6	CRV02F-	Cane Corso	F	2	5
#7	CRV06M-	Cane Corso	M	3	5
#8	BNA11FP	German Shepherd Dog	F	6	5
#9	DRS14FP	Golden Retriever	F	5	4
#10	CRV07F-	Cane Corso	F	3	4
#11	CRV15MP	Cane Corso	M	3	4
#12	BNA03FP	American Staffordshire Terrier	F	4	4
#13	BNA14F-	Crossbred	F	4	4
#14	BNA15F-	Crossbred	F	4	4
#15	IMDB13FN	Doberman	F	4	4
#16	IMGR05FP	Golden Retriever	F	6	4

Source: Author

ELISA for anti-rabies antibodies

The immunological status of the investigated canine blood donors was evaluated using the indirect ELISA commercial kit PLATELIA RABIES II (BIO-RAD, France) as per the manufacturer's instructions. Briefly, all reagents and the pre-coated ELISA microplate coated with rabies glycoprotein extracted from the inactivated and purified virus membrane were brought to room temperature (18-26°C). 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. Controls and serum samples were run in duplicate. Six serial dilutions made standard curve drawing of the R4b reagent (run in duplicate). The microtiter plate was sealed with adhesive film, incubated 60 minutes at 37°C, and washed three times with 300 µl wash solution. Afterward, 100 µl conjugate (a protein A from *Staphylococcus aureus* coupled with peroxidase) was dispensed into each well; the microtiter plate was sealed with adhesive film and incubated 60 minutes at 37°C and washed five times with 300 µl wash solution. Then, 100 µl tetramethylbenzidine (TMB 0.25%) substrate (prepared just before use) was dispensed into each

well and incubated in darkness at room temperature (18-26°C) for 30 min. In the end, 100 µl of the stop solution (1N sulphuric acid solution) were added to each well. The results were read at dual-wavelength mode of 450–620 nm and recorded for statistical analysis. The OD of the negative, positive, and standard curve dilutions validated the results (Table 2).

Table 2. Optic densities of the negative, positive, and standard curve dilutions used in ELISA (PLATELIA Rabies II kit, BIO-RAD).						
ELISA Control Means	Rate of dilution*					
	1×10 ⁻²	1×10 ⁻⁴	1×10 ⁻⁸	1×10 ⁻¹⁶	1×10 ⁻³²	1×10 ⁻⁶⁴
C̄-	0.007	-	-	-	-	-
C̄+0.5	0.789	-	-	-	-	-
C̄+4	2.160	1.778	1.288	0.755	0.325	0.131
*dilutions of the stock solutions supplied by the manufacturer						
Source: Author						

Statistical analysis

All data were recorded and analyzed (one-way analysis of variance) in the Excel 2010 spreadsheet application of the Microsoft Office 2010 suite and the One-Way ANOVA Analysis Tool pack.

Results and Discussion

Of the 16 dogs tested, 3 were mongrels, and 16 were purebred. Twenty-five percent of the dogs (4/16) were male, and seventy-five percent of the dogs (12/16) were female (Table 1). Of the purebred dogs, 50 % (6/12) tested OD >DOC+0.5 in all tests, while mongrels 33 % (1/3) tested OD >DOC+0.5 (Table 3). The small number of animals tested did not allow a breed analysis, and the statistical interpretation of the results was done across the group. However, the breed and gender frequency distributions were generally representatives of the canine blood bank donors. The overall picture of ELISA OD results revealed non-significant variation between and within D1-D6 groups of values ($p > 0.05$, $F < F_{crit}$).

Table 3. Optic densities of the samples used in ELISA (PLATELIA Rabies II kit, BIO-RAD)						
No.	Donation					
	D1	D2	D3	D4	D5	D6
#1	1.730	1.883	1.524	1.411	1.627	1.605
#2	1.475	1.501	1.525	1.068	1.301	1.156
#3	0.685	1.36	2.18	2.232	2.195	2.012
#4	2.425	2.353	2.096	1.840	2.195	1.948
#5	0.345	1.241	1.416	1.733	1.295	
#6	0.199	0.07	0.074	0.103	0.051	
#7	0.072	0.174	0.400	0.507	0.140	
#8	1.823	1.931	1.701	1.855	1.472	
#9	1.269	1.612	1.351	1.765		
#10	1.189	0.953	1.236	1.077		
#11	0.073	0.084	0.184	0.108		
#12	0.399	1.252	1.189	0.968		
#13	1.345	1.358	1.712	1.615		
#14	0.175	0.230	0.173	0.413		
#15	0.067	0.024	0.048	0.058		
#16	0.279	0.200	0.099	0.125		
Source: Author						

Transfusion studies conducted in the last decades have led to writing comprehensive donor selection guides for an increased blood-donor safety Yagi and Bean (2016). However, the effects of blood donation on the immune system are still unclear Lange et al. (1996). In this context, any study on blood donation and the immune system is useful by bringing new data into the field. In our study, the PLATELIA RABIES II (BIO-RAD, France) ELISA kit allowed dogs to detect and titrate IgG anti-rabies virus glycoprotein. Despite of the usefulness for international animal movement or trade (non-validated methods by OIE), indirect ELISA proved to be a valuable tool for monitoring rabies vaccination OIE (2018). Also, by using the calibrated positive controls provided by manufacturer (0.5 EU/ml Positive control and 4 EU/ml Positive control), there was possible the comparison of the anti-rabies antibodies

titers and the interpretation of the ODs dynamic during the periodical blood donations in 16 canine donors.

Four groups of blood donors have been detected in our study:

(1) dogs with $OD > DOC + 0.5$ in all tests: #1, #2, #4, #8, #9, #10, #13.

Figure 1: The anti-rabies antibodies optic density (OD) for dogs with $OD > ODC(+0.5UI)$ in all tests

(2) dogs with $OD < ODC + 0.5$: #6, #7, #11, #14, #15, #16.

Figure 2: The anti-rabies antibodies optic density (OD) for dogs with $OD < ODC + 0.5$

(3) dogs with arising DO after vaccination: #3, #5, #12.

Figure 3: The anti-rabies antibodies optic density (OD) for dogs with arising OD after vaccination

Source: Author

(4) optic density (OD) curves analysis in 16 dogs (#1 – #16).

Figure 4. The anti-rabies antibodies optic density (OD) curves analysis in 16 dogs (#1 – #16). C+0.5(M) – mean of two ODs 0.5 EU/ml Positive control; C+4(M) – mean of two ODs 4 EU/ml Positive control; C-(M) – mean of two ODs Negative control

Source: Author

The comparison of all these three groups with the vaccinations scheme received by all dogs proved a close correlation of immunological status and the day of vaccination. All dogs included in the group (1) received the annual booster vaccination, while the dogs of group (3) provided a titer of anti-rabies antibodies after a delayed vaccination.

Even if our results did not show statistically significant differences, we could not ignore the OD differences, conducting us to identify three groups of protection and dynamic.

We are strengthened to consider the data published by Bahloul and col notifying on the protective value considered to be 0.5 IU, which may be unsatisfactory in relation to the dog's living conditions and which therefore recommends a higher threshold associated with epidemiological conditions specific to each region Bahloul et al. (2005).

The dogs we submitted to investigation are satisfying the donor criteria, but they belong to different breeding systems, some of them being pets and others belonging to a kennel.

We should also consider the results of studies published by Wasniewsky and col (2014), on the specificity and sensibility of ELISA Plateliaut Rabies kit: they noticed a problem of underestimated values in ELISA compared to FAVN, the ELISA identifying as values over the threshold, less than half from the positive sera in FAVN. In this respect, our ELISA negative samples could be expected to test in FAVN over the threshold Wasniewsky et al (2014).

Zhang's 2009, studies, which compares the results of the investigation of more than four thousand sera using a C-ELISA kit versus FAVN, concludes that at FAVN titers greater than 8 IU, C-ELISA no longer provides a quantitative method for determinations rabies neutralizing antibody level. This could be due to a limit of saturation of the coating antigen, a phenomenon that Wasniewsky's work also faced. In the case of the samples used in our study, the aim was to prove the maintenance of the anti-rabies immune status of blood donors: considering the donor group (2), it would also be necessary to investigate them by FAVN Zhang's et al. (2009).

Our results considering no significant variation of anti-rabies antibodies values during repeated donations can be added to the studies made by Lange et al. (1996) regarding no significant differences of cytokines in the different groups or during repeat autologous donations Lange et al. (1996) but it is to be considered if the new approach of longer intervals between the administration of rabies vaccine will affect the immune response in frequent blood donors as the latest studies prove that the immunity to rabies in vaccinated dogs extends beyond 3 years and anti-rabies antibodies can persist up to 6 years when using a recombinant or a killed rabies vaccine Dodds et al. (2020).

In conclusion, the anti-rabies antibodies ODs in a group of 16 canine blood donors in a Romanian blood bank weren't influenced by blood donations. However, the results show that not all dogs benefit from a proper vaccination program and regular vaccination of dogs is of utmost importance for the protection of animals throughout their life.

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