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**Comparison of antibody responses after vaccination with two inactivated rabies vaccines**

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## 22 Abstract

23 Thirty laboratory dogs were randomly assigned to two groups (A and B) of 15 dogs and  
 24 subcutaneously vaccinated with a single dose of one of two commercially available  
 25 monovalent inactivated rabies vaccines: RABISIN<sup>®</sup> (Merial, France)(group A) and  
 26 NOBIVAC<sup>®</sup> Rabies (Intervet International)(group B). Rabies antibodies were measured over  
 27 a period of 4 months using the fluorescent antibody virus neutralization (FAVN) test. The two  
 28 vaccines performed differently in terms of magnitude and persistence of rabies antibodies  
 29 titers in dogs. Two weeks after vaccination, average rabies antibody titers peaked at 2.53  
 30 IU/mL (range, 0.17 – 13.77 IU/mL) and 1.26 IU/mL (range, 0.50 – 4.56 IU/mL) in group A  
 31 and B dogs, respectively. The average FAVN antibody titres against rabies on D28, D56, D84,  
 32 D112 and D120 were significantly higher in group A than in group B. Although all dogs from  
 33 group B serologically responded to vaccination, the proportion of dogs with antibody titres  
 34  $\geq 0.5$  IU/mL dropped significantly after D28 and was statistically significantly lower on D56,  
 35 D84 and D112 compared to group A dogs. In conclusion, in the context of international trade,  
 36 the choice of the vaccine and the timing of blood tests are critical factors in achieving  
 37 successful serological test results after rabies vaccination. RABISIN induces high and  
 38 sustained antibody titres against rabies, increasing the flexibility for the time of blood  
 39 sampling after primo-vaccination.

40

41 Keywords: rabies; dog; neutralizing antibodies; inactivated vaccines

42 <sup>®</sup>RABISIN is a registered trademark of Merial in the United Kingdom and elsewhere

43 <sup>®</sup>NOBIVAC Rabies is a registered trademark of Intervet International in the United Kingdom  
 44 and elsewhere

45

46 The rules for non-commercial movement of pet animals (dog, cat, ferret) are laid down in  
47 directive 998/2003 of the European Community. It requires the identification of the animal by  
48 tattoo and/or microchip, a certificate of vaccination against rabies, and a 21-day waiting  
49 period in case of primary vaccination. In addition, in four countries (United-Kingdom [UK],  
50 Ireland, Sweden and Malta), for a transitional period of 5 years, animals have to be tested for  
51 rabies antibodies within the period specified in their national rules (30 days after vaccination  
52 are recommended for UK, Ireland and Malta, and at least 4 months for Sweden). The Pet  
53 Travel Scheme (PETS) procedure, applied in the UK and Ireland, also requires that the pet  
54 must wait for 6 months after blood sampling before entering those countries. When importing  
55 animals from rabies-infected third countries into the E.U., pets must be tested for rabies  
56 antibodies 30 days after vaccination and wait for 3 additional months after blood sampling  
57 before entering into the E.U. The blood testing has two main objectives. One is to check that  
58 the animal has developed an adequate humoral immune response to vaccination (efficacy); the  
59 other is to ensure that it has been properly vaccinated (compliance).

60 Recently, the European Food Safety Authority (EFSA) was asked to assess the risk of rabies  
61 introduction into the UK, Ireland, Sweden, or Malta, as a consequence of abandoning the  
62 serological test for rabies. EFSA opinion was published in February 2006 (EFSA, 2006).  
63 Based on the known efficacy of authorised rabies vaccines, EFSA recommended testing for  
64 rabies antibodies (or carrying out a second rabies vaccination) only those animals coming  
65 from European countries with a no negligible risk of rabies *i.e.* an annual incidence in the  
66 domestic pet population higher than one infected animal per million.

67

68 To obtain marketing authorisation in European countries, rabies vaccines for veterinary use  
 69 have to fulfil a number of tests in terms of immunogenicity, potency and safety (European  
 70 Pharmacopoeia 2007).

71 In this study, the kinetics of neutralizing rabies antibodies were compared in dogs over a  
 72 period of 4 months after the administration of two commercially available inactivated  
 73 adjuvanted monovalent rabies vaccines, tested under the same experimental conditions.

74

75 The following commercially available monovalent inactivated rabies vaccines were used.  
 76 RABISIN<sup>®</sup> (Merial SAS, France, batch No.L185053) containing the G52 fixed virus of the  
 77 Pasteur strain at  $\geq 1$  IU/dose and NOBIVAC<sup>®</sup> Rabies (Intervet Nederland B.V., batch  
 78 No.74120D) containing the Pasteur RIV strain at  $\geq 2$  IU/dose. The two strains have different  
 79 passage histories and were inactivated for vaccine production. Both vaccines were  
 80 administered according to the recommendations of the Manufacturer's, i.e. one dose from the  
 81 age of 3 months. From here on, RABISIN and NOBIVAC Rabies will be referred to as  
 82 vaccine A and B, respectively.

83 Thirty (30) conventional Beagles, 13 to 18 weeks old, were obtained from an accredited  
 84 commercial supplier and randomly assigned to two groups (A and B) of 15 animals each  
 85 according to sex, age and weight. Dogs were conventionally housed and fed a high quality  
 86 commercial dry ration with unlimited access to water. Dogs were identified by a microchip  
 87 implanted subcutaneously. On D0, dogs from group A and B were vaccinated with a single  
 88 dose of vaccine A and B, respectively. All vaccines were administered subcutaneously  
 89 between the shoulder blades.

90 Blood samples were collected from all puppies at regular intervals following vaccination (D0,  
 91 D14, D28, D56, D84, D112, and D120). Rabies antibodies were titrated by the FAVN test

(Cliquet et al. 1998) using a positivity threshold of 0.50 IU/mL. Personnel performing the laboratory analysis were blind to the treatment assignments.

Statistical analyses were performed using SAS<sup>®</sup> (version 9.1) and STATGRAPHICS<sup>®</sup> softwares. Statistical significance was based on two-tailed tests of the null hypothesis resulting in a p-value of 0.05 or less. The immunogenicity of the two vaccines was evaluated by comparison of their respective antibody kinetics for the D14–D120 period, by fitting a general linear mixed model with repeated measures on the log10 transformed titers (IU/mL). Furthermore, the number of dogs with a titer of at least 0.50 IU/ml was compared between groups by fitting a logistic regression model with factors “group” and “day” and the corresponding interaction. For both parameters, due to a group x day interaction, comparison between groups was performed day-by-day for the D14-D120 period by a Fisher’s F-test with adjusted 1<sup>st</sup> error risk (Bonferroni’s method).

All, but two puppies were seronegative for rabies at the start of the study (Table 1). Two puppies from group A (Nos.2 and 9) had a SN titer of 0.66 IU/mL, which most probably represented residual maternal antibodies. Two weeks after vaccination, average rabies antibody titers peaked at 2.53 IU/mL (range, 0.17 – 13.77 IU/mL) and 1.26 IU/mL (range 0.50 – 4.56 IU/mL) in group A and B dogs, respectively (Table 1). A significant “group x day” ( $p = 0.0004$ ) and “group” effect ( $p < 0.0001$ ) was found for the period D14-D120, indicating that the kinetics of antibody responses to vaccination differed between the two groups of dogs. Time-by-time comparison showed that the average FAVN antibody titers against rabies on D28, D56, D84, D112 and D120 were significantly higher in the dogs from group A when compared to the titers in group B (Figure 1). One dog (No.10) from group A did not reach 0.5 IU/mL and two dogs from group B (Nos.19 and 23) developed maximum SN titers just above the WHO threshold value (Table 1). The two dogs with residual

maternally derived antibodies both responded to vaccination. The proportion of dogs in group B with antibody titers  $\geq 0.5$  IU/mL dropped significantly after D28 and was statistically significantly lower on D56, D84 and D112 compared to group A dogs.

This study shows new serological data obtained in the same study on two commercially available monovalent rabies vaccines (same origin of dogs, same protocol of vaccination, same technique of serology performed blindly in the same conditions by the same technicians on coded samples).

As expected, results confirmed the immunogenicity of both vaccines, because 93% and 100% of the puppies vaccinated with vaccine A and B respectively, developed rabies antibody titers above 0.50 IU/mL after vaccination. Interestingly, both vaccines induced an early seroconversion with antibody titers peaking as early as 14 days post-vaccination. This early peak is consistent with results obtained in other studies on experimental dogs (Minke, personnel observation, Kallel et al. 2006). Peak antibody response in pets is classically reported in the literature between 3 and 6 weeks after vaccination (Sugiyama et al. 1997, National Association of State Public Health Veterinarians 2007, Barth et al. 1985), but laboratory dogs are known to respond better to vaccination than pet dogs (Aubert, 1992). An in-depth review of many experimental studies (Aubert, 1993) has shown a strong correlation between the development of rabies antibodies after vaccination and protection against rabies infection. This correlation is independent of the interval between vaccination and blood sampling (Aubert, 1992). The OIE and WHO (1992) have defined the protective threshold at 0.50 IU/mL in humans in absence of challenge data, and this threshold has been extended to animals. Several studies on the sensitivity of the FAVN test have shown that a threshold of positivity of 0.24 IU/ml could be adopted (Cliquet et al. 1998, Cliquet et al. 2000, Hammami et al. 1999). Therefore, the 0.5 IU threshold gives a comfortable margin in interpreting



serology after vaccination. The seroprotection rates observed in this study are consistent with those reported in the literature. Cliquet et al. (2003) reported that within a total of 17,693 sera analysed from primo- and booster vaccinated pets, 93% of the samples had an antibody titer of at least 0.50 IU/mL. Among 14,035 dog sera tested by the Veterinary Laboratories Agency (VLA) in Weybridge, 96% of the samples taken from owner vaccinated dogs had a rabies antibody titer higher than 0.50 IU/mL (Mansfield et al. 2004). Limited studies in Finland and Alaska showed that 97% and 100% of the vaccinated dogs, respectively, had a seroprotective titer 30-40 days after one injection of rabies vaccine (Sihvonen et al. 1995, Sage et al. 1993). Primo-vaccinated pets had significantly lower rabies antibodies than dogs vaccinated twice or more, and a rapid decrease of rabies antibodies was observed in primo-vaccinated dogs (Cliquet et al., 2003).

An important outcome of this study was that the two vaccines performed differently in terms of magnitude and persistence of rabies antibodies titers in dogs. A strong brand effect was also found in several other studies showing significant differences between mean antibody titers induced by three different rabies vaccines licensed in the UK (Mansfield et al. 2004) and vaccines licensed in Germany (Jakel et al., 2007). Interestingly, differences in immunogenicity apparently do not seem to correlate with the potency of the vaccines as measured by the National Institute of Health (NIH) test, as long as the values are equal or greater than 1 IU/mL (Chappuis and Tixier 1982, Aubert 1992). Other factors such as the quality and quantity of the rabies antigen, choice and quantity of the adjuvant, and blending of the vaccine may explain the observed differences. Our study results also demonstrated that a non-negligible proportion of primo-vaccinated dogs failed to pass the test when sampled 28 or more days after vaccination. This was particularly the case for vaccine B, where antibody titers dropped off significantly 4 weeks after vaccination. This observation has important consequences for the timing of blood testing, leaving a very narrow window of opportunity

for vaccine B. The importance of the interval between vaccination and antibody testing was also highlighted by Cliquet et al. (2003) and Mansfield et al. (2004), showing that the risk of test failure significantly increased when dogs were tested beyond 6 weeks after vaccination. In contrast, for multiple vaccinated dogs, antibody titers did not depend on the time elapsed since the last vaccination (Cliquet et al. 2003). As a consequence, the use of two doses of vaccine for primo-vaccination is currently recommended to obtain high and sustained antibody titers (Toma, 1994). To the best of our knowledge there is no comparative published data on the use of two doses of vaccine. It is now generally accepted that, in order to increase the success of passing the serological test, monovalent rabies vaccines should be used (Cliquet et al. 2003). Several other factors such as age, reproductive status, and immunosuppression play an important role as well (Aubert 1992, Mansfield et al. 2004). In conclusion, in the context of international trade, the choice of the vaccine and the timing of blood test are critical factors in achieving a successful serological test result after rabies vaccination. Vaccine A induces high and sustained antibody titers, increasing the flexibility for the time of blood sampling after primo-vaccination.

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Table 1: Individual rabies virus neutralizing antibody titer per group after one-dose vaccination on day 0. Rabies antibodies were measured using the fluorescent antibody virus neutralization (FAVN) test. Titers are expressed in international units per mL. Geometric mean titer (GMT) and standard deviation (SD) are presented per group. The proportion of animals with rabies antibody titer of at least 0.50 IU/mL is presented also. Grey cells correspond to animals with rabies antibody titer  $<0.50$  IU/mL after vaccination. NT= Not Tested.

Figure 1: Mean rabies virus neutralizing antibody titer per group after one-dose vaccination on day 0. Titers are expressed in log international units per mL.

Group number	Animal No.	Rabies virus neutralizing antibodies (IU/mL)						
		Day 0	Day 14	Day 28	Day 56	Day 84	Day 112	Day 120
<b>Group A</b> <b>Vaccine A</b>	1	0.06	3.46	2.62	1.15	1.15	1.51	0.29
	2	0.66	1.99	0.66	1.15	0.87	0.50	1.15
	3	0.06	1.15	1.51	0.66	1.15	0.66	0.39
	4	0.06	3.46	7.92	3.46	2.62	2.62	2.62
	5	0.06	2.62	1.51	0.66	0.66	0.50	0.5
	6	< 0.17	7.92	3.46	1.51	0.39	0.29	0.17
	7	0.06	1.99	4.56	2.62	1.15	1.51	1.51
	8	0.06	10.45	3.46	1.15	0.50	0.87	0.5
	9	0.66	0.87	1.15	1.51	0.66	0.50	0.39
	10	0.06	0.17	0.29	0.22	0.10	0.10	0.07
	11	0.06	1.51	0.50	0.29	0.17	0.29	0.17
	12	0.06	13.77	7.92	1.51	1.99	1.51	1.51
	13	0.06	4.56	1.99	0.89	0.29	0.17	0.17
	14	< 0.10	2.62	4.56	1.51	0.66	0.22	0.17
	15	0.06	2.62	1.99	1.51	0.29	0.22	0.22
<b>GMT</b>		<b>0.09</b>	<b>2.53</b>	<b>2.03</b>	<b>1.07</b>	<b>0.61</b>	<b>0.51</b>	<b>0.40</b>
<b>SD</b>		<b>0.21</b>	<b>3.83</b>	<b>2.43</b>	<b>0.84</b>	<b>0.70</b>	<b>0.72</b>	<b>0.73</b>
<b>% dogs with titer <math>\geq 0.5</math> IU/mL</b>		<b>0%</b>	<b>93%</b>	<b>93%</b>	<b>87%</b>	<b>67%</b>	<b>60%</b>	<b>40%</b>
<b>Group B</b> <b>Vaccine B</b>	16	0.06	2.62	1.15	0.13	0.17	NT	0.13
	17	0.06	1.51	0.87	0.10	0.07	0.39	0.39
	18	0.06	1.99	0.39	0.17	0.06	0.07	0.07
	19	0.06	0.51	0.39	0.06	0.07	0.10	0.10
	20	0.06	0.87	1.51	0.07	0.17	0.17	0.07
	21	0.06	1.99	0.66	0.17	0.07	0.07	0.07
	22	< 0.13	0.51	0.66	0.13	0.29	0.29	0.1
	23	0.06	0.51	0.13	0.06	0.07	0.10	0.13
	24	0.06	1.51	0.29	0.07	0.13	0.13	0.06
	25	0.06	0.87	0.39	0.06	0.06	0.06	0.06
	26	0.06	1.51	1.99	0.22	0.10	0.13	0.1
	27	0.06	1.51	1.51	0.07	0.10	0.50	0.07
	28	0.06	4.56	1.51	0.22	0.29	0.17	0.17
	29	0.06	2.62	1.15	0.17	0.29	0.39	0.5
	30	0.06	0.50	1.51	0.17	0.07	0.17	0.06
<b>GMT</b>		<b>0.06</b>	<b>1.26</b>	<b>0.74</b>	<b>0.11</b>	<b>0.11</b>	<b>0.16</b>	<b>0.11</b>
<b>SD</b>		<b>0.02</b>	<b>1.10</b>	<b>0.58</b>	<b>0.06</b>	<b>0.09</b>	<b>0.14</b>	<b>0.13</b>
<b>% dogs with titer <math>\geq 0.5</math> IU/mL</b>		<b>0%</b>	<b>100%</b>	<b>67%</b>	<b>0%</b>	<b>0%</b>	<b>7%</b>	<b>7%</b>

Table 1

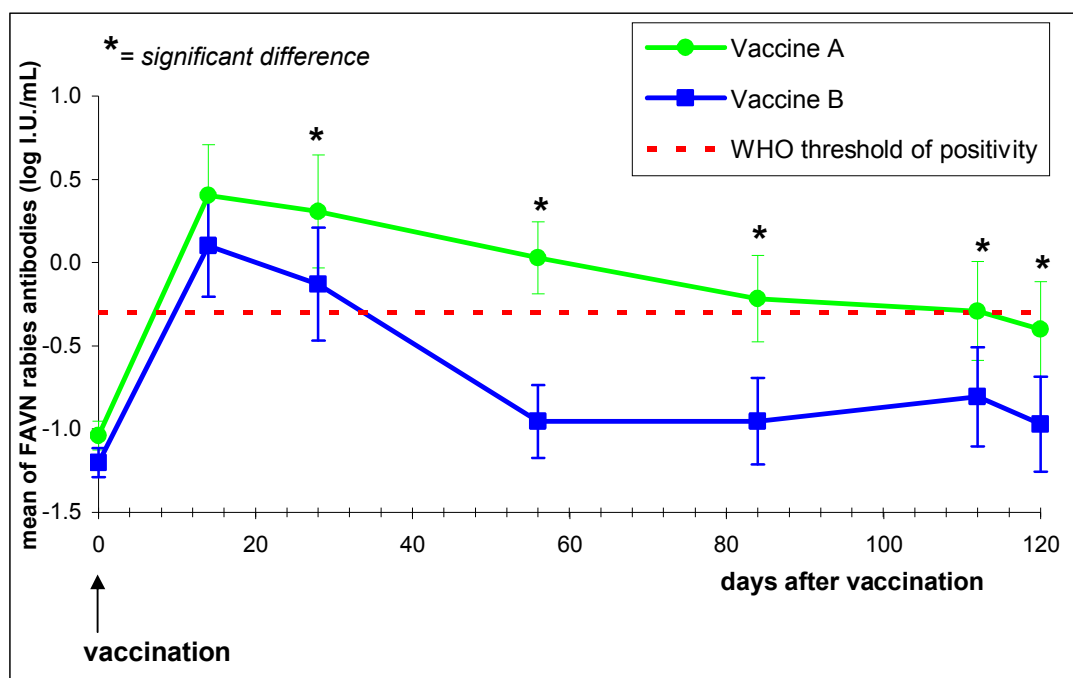


Figure 1