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

2

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21

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[®] (Merial, France)(group A) and
26 NOBIVAC[®] 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 ≥ 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 [®]RABISIN is a registered trademark of Merial in the United Kingdom and elsewhere43 [®]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[®] (Merial SAS, France, batch No.L185053) containing the G52 fixed virus of the
77 Pasteur strain at ≥ 1 IU/dose and NOBIVAC[®] Rabies (Intervet Nederland B.V., batch
78 No.74120D) containing the Pasteur RIV strain at ≥ 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

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

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

104

105 All, but two puppies were seronegative for rabies at the start of the study (Table 1). Two
106 puppies from group A (Nos.2 and 9) had a SN titer of 0.66 IU/mL, which most probably
107 represented residual maternal antibodies. Two weeks after vaccination, average rabies
108 antibody titers peaked at 2.53 IU/mL (range, 0.17 – 13.77 IU/mL) and 1.26 IU/mL (range
109 0.50 – 4.56 IU/mL) in group A and B dogs, respectively (Table 1). A significant “group x
110 day” ($p = 0.0004$) and “group” effect ($p < 0.0001$) was found for the period D14-D120,
111 indicating that the kinetics of antibody responses to vaccination differed between the two
112 groups of dogs. Time-by-time comparison showed that the average FAVN antibody titers
113 against rabies on D28, D56, D84, D112 and D120 were significantly higher in the dogs from
114 group A when compared to the titers in group B (Figure 1). One dog (No.10) from group A
115 did not reach 0.5 IU/mL and two dogs from group B (Nos.19 and 23) developed maximum
116 SN titers just above the WHO threshold value (Table 1). The two dogs with residual

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

120

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

125 As expected, results confirmed the immunogenicity of both vaccines, because 93% and 100%
126 of the puppies vaccinated with vaccine A and B respectively, developed rabies antibody titers
127 above 0.50 IU/mL after vaccination. Interestingly, both vaccines induced an early
128 seroconversion with antibody titers peaking as early as 14 days post-vaccination. This early
129 peak is consistent with results obtained in other studies on experimental dogs (Minke,
130 personnel observation, Kallel et al. 2006). Peak antibody response in pets is classically
131 reported in the literature between 3 and 6 weeks after vaccination (Sugiyama et al. 1997,
132 National Association of State Public Health Veterinarians 2007, Barth et al. 1985), but
133 laboratory dogs are known to respond better to vaccination than pet dogs (Aubert, 1992). An
134 in-depth review of many experimental studies (Aubert, 1993) has shown a strong correlation
135 between the development of rabies antibodies after vaccination and protection against rabies
136 infection. This correlation is independent of the interval between vaccination and blood
137 sampling (Aubert, 1992). The OIE and WHO (1992) have defined the protective threshold at
138 0.50 IU/mL in humans in absence of challenge data, and this threshold has been extended to
139 animals. Several studies on the sensitivity of the FAVN test have shown that a threshold of
140 positivity of 0.24 IU/ml could be adopted (Cliquet et al. 1998, Cliquet et al. 2000, Hammami
141 et al. 1999). Therefore, the 0.5 IU threshold gives a comfortable margin in interpreting

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

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

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

182

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185

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188 elsewhere; STATGRAPHICS is a registered trademark of Statistical Graphics Corporation in
189 the United States of America.

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

260

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

263

Group number	Animal No.	Rabies virus neutralizing antibodies (IU/mL)							
		Day 0	Day 14	Day 28	Day 56	Day 84	Day 112	Day 120	
Group A Vaccine A	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
	GMT	0.09	2.53	2.03	1.07	0.61	0.51	0.40	
	SD	0.21	3.83	2.43	0.84	0.70	0.72	0.73	
	% dogs with titer ≥ 0.5 IU/mL	0%	93%	93%	87%	67%	60%	40%	
Group B Vaccine 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
	GMT	0.06	1.26	0.74	0.11	0.11	0.16	0.11	
	SD	0.02	1.10	0.58	0.06	0.09	0.14	0.13	
	% dogs with titer ≥ 0.5 IU/mL	0%	100%	67%	0%	0%	7%	7%	

Table 1

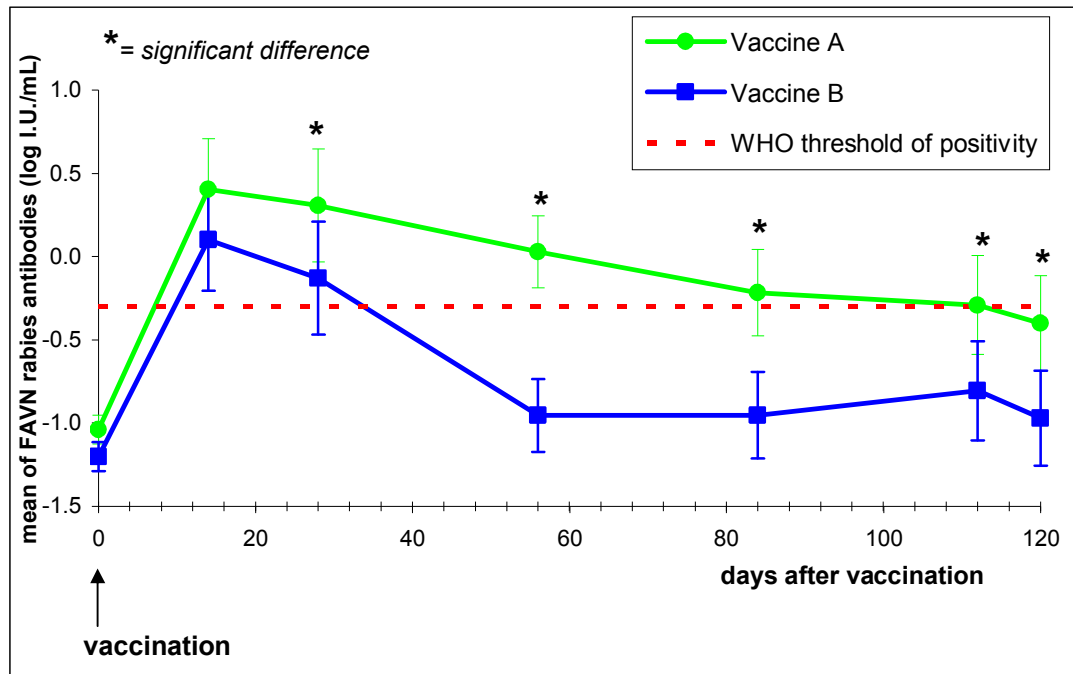


Figure 1