

## Comparison of Immunogenic Efficacy of Mono- and Polyvalent Rabies Vaccines in Dogs

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**Abstract.-** Monovalent and polyvalent vaccines are commonly used rabies cell culture vaccines that can vary in their immunogenic efficacy in dogs. In this study, immunogenic efficacy of two monovalent vaccines (Rabisin and Rabisyva-VP13) and a polyvalent vaccine (Hexadog DHP-LR) was compared. Sixteen adult, healthy, non-vaccinated dogs were randomly assigned to 4 groups (A to D). Dogs in groups A, B, and C were inoculated with Rabisin (1 dose S/C), Hexadog DHP-LR (1 dose S/C), and Rabisyva-VP13 (1 dose S/C), respectively, at day 0 and 21. Dogs in group D were maintained as negative controls (non-vaccinated). Serum samples were collected on days of vaccination (0 and 21) and every 30 days for up to 300 days. Rabies virus neutralizing antibody (RVNA) titers were determined by Rapid Florescent Focus Inhibition Test (RFFIT). The monovalent vaccines were found to generate higher RVNA titers than the polyvalent vaccine. Vaccine type and post vaccination intervals significantly affected the RVNA titers. Gender of dogs did not affect vaccine efficacy except that gender had some effect on RVNA titers generated by polyvalent vaccinated dogs.

**Key words:** Hexadog DHP-LR, monovalent vaccines, polyvalent vaccines, Rabisin, Rabisyva-VP13, vaccine efficacy.

### INTRODUCTION

**R**abies is an important public health hazard that causes up to 100,000 human deaths per year worldwide (Burki, 2008). This disease is endemic in many developing countries particularly in Asia and Africa (Leung *et al.*, 2007; Frymus *et al.*, 2009; Nizishono, 2009). Vaccination is the most effective protection tool against rabies virus infection. In dogs, monovalent and polyvalent vaccines of cell culture origin are available (Kamrani *et al.*, 2004). Rabies virus neutralizing antibody (RVNA) titers as determined by Rapid Florescent Focus Inhibition Test (RFFIT) can indicate vaccine efficacy. An RVNA titer  $\geq 0.5$  IU/ml is considered protective against rabies (Fooks *et al.*, 2002; Burr and Snodgrass, 2004). Several factors can potentially affect post vaccination RVNA titers in dogs

including vaccine strain, sampling schedule, health status, sex and age (Mansfield *et al.*, 2004; Debenedictis *et al.*, 2009; Rashid *et al.*, 2009). To avoid vaccine failures, periodic evaluation of immunogenic efficacy of rabies vaccines is important, especially in rabies endemic areas.

The RFFIT is a gold standard technique for the determination of RVNA titers (Bahloul *et al.*, 2002). The test has good specificity (100%) and reproducibility ( $P > 0.05$ ) and is highly reliable in detecting rabies status of dogs (Yu *et al.*, 2009). This study was carried out to determine the comparative immunogenic efficacy of Rabisin, Hexadog DHP-LR and Rabisyva-VP13 vaccines in dogs under controlled experimental conditions. In addition, the effect of sex and health status on RVNA titers was also evaluated.

### MATERIALS AND METHODS

#### *Source of dogs*

A total of 16 adult, healthy, stray dogs were captured and quarantined for two months. Dogs

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included were 8 males and 8 females, approximately 1-2 years old. All dogs were subjected to general physical and laboratory examination to evaluate their health status and were de-wormed 11 days prior to vaccination. The animals that were positive for helminth infestation were de-wormed two more times at one and two weeks after initial de-worming. Dogs were divided into four groups (A, B, C and D) each group containing 2 male and 2 female dogs. Groups A, B, and C were vaccinated with different vaccines while group D served as negative control. All the dogs were maintained for 10 months under controlled conditions in indoor kennels (each 4×7 ft). Daily health record of each dog was maintained and displayed in the kennels.

#### *Vaccines*

Three different vaccines were used; two were monovalent (Rabisin and Rabisyva-VP13) and one was polyvalent (Hexadog DHP-LR). Rabisin (Merial, France) is an inactivated GS-57 wistar strain vaccine. Rabisyva-VP13 (Fort Dodge, USA) is inactivated Pasteur strain 13. Hexadog (*laboratorios Syva s.a.u.*, Spain) is freeze dried Trivirovax DHP (attenuated canine adenovirus, canine distemper virus, canine parvovirus) and liquid Leptorabisin (inactivated rabies virus PW-G52 strain and *Leptospira canicola* and *L. icterohaemorrhagiae*).

#### *Vaccination*

On day 0, groups A, B and C were subcutaneously (scruff region) administered a single dose of Rabisin, Hexadog DHP-LR, and Rabisyva-VP 13, respectively. A second dose was given 21 days after first vaccination.

#### *Serum samples*

Blood samples were collected from all dogs prior to vaccination and at day 21 post vaccination. Thereafter, 4 ml blood sample was drawn every 30 day until 300 days. Sera from these blood samples were obtained and shipped to Rabies Testing Laboratory at Kansas State University, Manhattan, KS. The RFFIT was done at this laboratory.

#### *RFFIT procedure*

Serum samples were placed in a water bath at 56°C for 1 hour to inactivate complement (Wager

*et al.*, 1968; Samuelsson and Herlitz, 2008; Kramer *et al.*, 2009; Moore and Hanlon, 2010). The eppendorf tubes, 96-well microtiter plates, and 8-well chamber slides were labeled appropriately for sample identification. Serum samples were diluted in RFFIT media at 1:2.5, 1:12.5, 1:62.5 and 1:312.5. After mixing with equal amounts of virus, these dilutions became 1:5, 1:25, 1:125, and 1:625. All dilutions were mixed using Precision 2000 mixer and transferred to 8-well Lab Tek chamber slides. A pre-diluted rabies virus (strain CVS-11) was added to all serum dilutions followed by the addition of 200 µl of baby hamster kidney cells (BHK-21). After 24 hours of incubation at 37°C, the chamber slides were fixed in cold acetone followed by a rinse in phosphate buffer saline (PBS) for three min. After drying in a bio safety cabinet for 10 min, 156 µl of conjugate was added all wells. The slides were incubated at 37°C for 45 min, washed once in PBS and then dried. Under a fluorescent microscope, 20 microscopic fields were examined in each well of the slide at 160X and 200X magnification. Number of fields containing virus infected cells was counted starting at the same corner of the slide and taking four rows of the five fields. Control slides were read first and the number of positive fields in each serum dilution was recorded.

#### *Interpretation of results*

There were two serum samples per slide and end point titer of the test serum samples was calculated on the basis of number of positive fields in corresponding dilution using Reed and Muench (1938) calculation chart. RVNA titer (IU/ml) for serum samples was calculated using the following formula

$$\frac{\text{End-point titer of test serum}}{\text{End-point titer of reference serum}} \times 2.0 \text{ IU/ml of reference serum}$$

#### *Statistical analysis*

Data were statistically analyzed using Student's t-test and One Way Analysis of variance (ANOVA) and multiple comparisons were carried out using Least Significant Difference test (Snedecor *et al.*, 1967).

**RESULTS**

All dogs were negative for rabies antibody on day 0 of the study. Dogs started showing RVNA titers at day 21 post vaccination. There was no significant difference among titers of different vaccinated groups on days 21, 30, and 60. Large variations in post vaccination RVNA titers were observed during the 300 days of the study (Fig. 1). There was a significant difference ( $P < 0.05$ ) between day 30 and 90. RVNA titer of group C was significantly higher than those of groups A and B on day 90. There was a decline in RVNA titers of groups A and C but an increase in those of group B on day 120. No significant difference ( $P > 0.05$ ) was found between RVNA titers of groups A, B, and C on day 120. On day 150, 210, 240, 270, and 300 the mean RVNA titer of group C was significantly higher than those of groups A and B. On day 240, an abrupt drop of titer was observed in group B. In general, RVNA titers at days 90 to 300 were higher than those at days 0 to 60 post vaccination. Dogs in group D were never positive for RVNA titer.

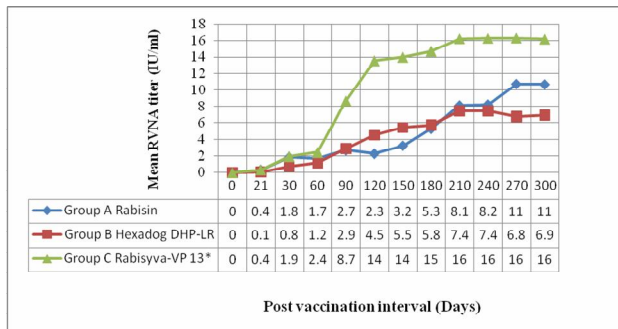
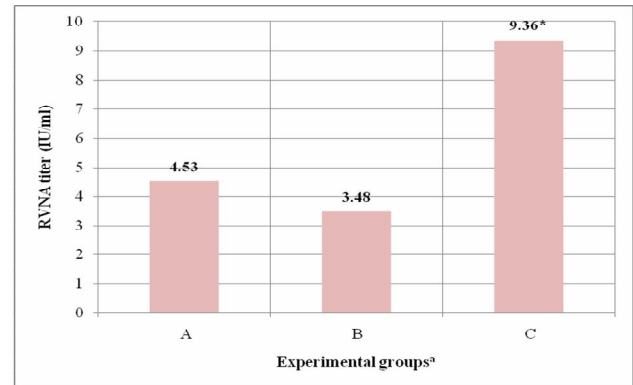


Fig. 1. RVNA responses of dogs in groups A, B, C vaccinated with Rabisin, Hexadog DHP-LR and Rabisyva-VP13 under experimental conditions. The figure represents the mean RVNA titers (in IU/ml) of vaccinated dogs from day 0-300. Mean RVNA titers of Rabisyva-VP 13 were significant at days 90 and then days 150-300. RVNA data of different experimental groups was found significantly different ( $P < 0.05$ ) at various post vaccination intervals. Statistical analysis was carried out at 5% alpha level.

*Effect of vaccines on RVNA titers*

Periodic variations were observed in mean RVNA titers of dogs vaccinated with Rabisin,

Hexadog DHP-LR and Rabisyva-VP 13 vaccines (Fig. 2). RVNA titer of group C was significantly higher than those of groups A and B while there was no significant difference between groups A and B ( $P > 0.05$ ).



<sup>a</sup> A, Rabisin; B, Hexadog DHP-LR; C, Rabisyva-VP13.

Fig. 2. Effect of vaccine on RVNA titers. A significant statistical difference ( $P < 0.05$ ) was revealed among mean RVNA data extracted from different experimental protocols at alpha level 5%.

*Effect of health status on RVNA titers*

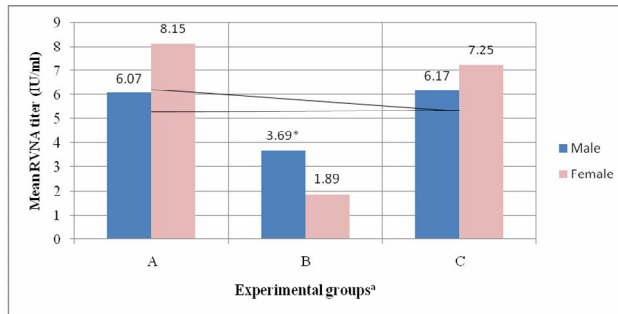
During the course of this study, dogs in different experimental groups contracted various infectious diseases. Statistical analysis did not reveal a significant difference ( $P > 0.05$ ) among the RVNA titers of healthy and diseased dogs in groups A, B and C at various intervals of the study.

*Effect of gender on RVNA titers*

In groups A and C there was no significant difference between the RVNA titers of males and females while in group B mean RVNA titer of males was significantly higher than the females. Groups A, B, C were not significantly different ( $P > 0.05$ ) from each other (Fig. 3).

**DISCUSSION**

According to reports from different parts of the World, many animals develop rabies in spite of rabies vaccination. Laboratory investigations have suggested that, in many cases, the post vaccination RVNA titers are lower than 0.1 IU/ml, which is not adequate for protection (Rashid *et al.*, 2009). In the



<sup>a</sup> A, Rabisin; B, Hexadog DHP-LR; C, Rabisyva-VP13

Fig. 3. Effect of gender on RVNA titers. No significant statistical difference ( $P > 0.05$ ) was found between experimental groups and within groups A and C while a significant difference ( $P < 0.05$ ) was found within group B at alpha level 5%.

present study, an abrupt change of RVNA titers at various intervals was recorded after vaccination with the three vaccines. Previous studies have shown that, after primary vaccination, there is a gradual decline of RVNA titers with the passage of time (Sage *et al.*, 1993). In a few healthy and diseased dogs, the RVNA titers dropped slightly but recovered to existing levels after some time.

It is hazardous to fully rely on the past vaccination history of a biting dog especially when planning post-exposure immunization in humans. Poor immunogenicity of a vaccine can cause a poor humoral immune response (Aubert, 1992; Sage *et al.*, 1993; Thomas *et al.*, 1994; Rigo and Honer, 2006). No case of vaccine failure or low immunity was observed in this study with all dogs exhibiting protective RVNA titers of  $\geq 0.5$  IU/ml. Rabisyva-VP13 engandered the highest RVNA titer while Hexadog DHP-LR and Rabisin were ranked second and third, respectively. Post vaccination antibody response in a dog may vary with the type and strain of vaccine (Kamrani *et al.*, 2004). Also, a single dose of an inactivated cell culture rabies vaccine has been shown to induce low humoral immune response as compared to multiple vaccination (Singh *et al.*, 2007). Post vaccination RVNA titer depends on the correlation between immunogenic and antigenic activities of vaccines (Minke *et al.*, 2009).

In this study, a few samples collected at various post vaccination intervals were found to be

toxic for BHK cells and could not be processed further. This toxicity may be due to haemolysis, fluid and electrolyte imbalance, poisoning, renal disorders, and infectious organisms. These pathological conditions can interfere with serological/hematological assays and can make the interpretations difficult or impossible. Immunosuppressive conditions can interfere with the vaccination response and consequently with protection of animals against infectious diseases. According to Centers for Disease Control (CDC) and National Advisory Committee on Immunization (NACI), ailments before, after, or along with rabies vaccination can interfere with the development of active immunity and that vaccination should be avoided in diseased/immunosuppressed animals. However, immunization of such subjects should be followed by periodic RVNA titer determination for sake of safety (Burr and Snodgrass, 2004; NACI, 2005).

In the present study some dogs underwent severe infectious and parasitic diseases at various instances but no correlation could be established between the immunity status of healthy and diseased dogs involved in the study. On the basis of these observations, RVNA titer could not be related to the health status. This is in contrast to the previously documented findings that support a relationship between RVNA titer and the health status of the vaccine recipients (Chakraborty and Chatterjee, 1998; CDC, 1999; Tizard, 2000; Hornby, 2001; NACI, 2005; Mojžišová *et al.*, 2007).

There was no gender based difference between RVNA titers of Rabisin and Rabisyva-VP13 vaccinated dogs. However, mean RVNA titers of Hexadog DHP-LR vaccinated male dogs were significantly higher than the female dogs. These findings are in line with the finding of Mansfield *et al.* (2004) but are in contrast to those of Kennedy *et al.* (2007).

## CONCLUSIONS

From the findings of this present study it is concluded that monovalent rabies vaccines elicited higher RVNA titers than polyvalent vaccines and on this basis Rabisyva-VP13, Rabisin and Hexadog DHP-LR are ranked at 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> position,

respectively. It is also derived that vaccine type and post immunization intervals significantly affect the RVNA titers while health status and gender of vaccinated dog do not have significant effect on RVNA titers except that in Hexadog DHP-LR vaccinated dogs that exhibited significant difference of male and female mean RVNA titers.

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