Full length paper

Comparison of Antibody Responses after Vaccination with Two Inactivated Rabies Vaccines in Thimphu Dogs

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ABSTRACT

We compared the antibody responses after vaccination with two commercially available inactivated rabies vaccines – Rabisin (Merial, France) and Raksharab (Indian Immunologicals) in dogs in Thimphu city, Bhutan. Fifty puppies were randomly assigned to two groups of 25 each and one group was subcutaneously vaccinated with a single dose of Rabisin and other group with Raksharab rabies vaccines as primary vaccination on day 0. Similarly, 50 adult dogs were also randomly assigned to two groups of 25 each and each group was subcutaneously vaccinated with a single dose of Rabisin and Raksharab vaccines as booster vaccination. Serum samples were collected on day 0 (prior to vaccination), 14 and 28 from all the dogs. Rabies antibodies were measured over a period of 28 days using SERELISA® Rabies Ab Mono Indirect enzyme-linked immunosorbent assay (ELISA).

A total of 198 blood samples from 66 dogs (3 sample each on day 0, 14 and 28) were collected and analyzed. Eight puppies (8/35; 22.86% belonging to Raksharab (n=7) and Rabisin vaccination group (n=1) demonstrated a minimum protective antibody titre (≥0.5 IU/ml) ranging from 0.6 to 2.75 IU/ml while the remaining puppies (n=27) demonstrated an antibody titre ranging from 0.1 to 0.45 IU/ml on day 0 (prior to primary vaccination). The antibody titre level increased after primary vaccination ranging from 0.21 to 4.78 IU/ml and 0.16 to 7.38 IU/ml under Rabisin group on day 14 and 28, respectively, and from 0.59 to 5.80 IU/ml and 1.19 to 6.76 IU/ml under Raksharab group on day 14 and 28, respectively.

In adult dogs under Rabisin booster vaccination group, 56% (9/16) of the dogs had ≥0.5 IU/ml of antibody titre (ranges: 0.25 to 6.45 IU/ml) on day 0 (before vaccination) and all dogs attained protective titre on day 14 (ranges: 1.07 to 6.46 IU/ml) and on day 28 (ranges: 2.22 to 10.26 IU/ml). Similarly, under Raksharab booster vaccination group, 56% (9/16) of the dogs had ≥0.5 IU/ml of antibody titre (ranges: 0.27 to 5.39 IU/ml) on day 0 (before vaccination). Excepting one adult male dog, all other dogs attained protective titre (≥0.5 IU/ml) on day 14 (ranges: 0.62 to 5.59 IU/ml) and on day 28 (ranges: 1.86 to 6.66 IU/ml). The findings showed that both the inactivated vaccines have elicited minimum threshold level for protection (≥0.50 IU/ml) responses as per the recommendation of the World Animal Health Organization (OIE) and World Health Organization (WHO), indicating that the vaccines used in Bhutan are potent and efficient and thus, acceptable for primary and booster vaccination against rabies in dogs.

KEYWORDS
Rabies vaccine
Rabies antibody titer Rabies antibody responses
Dog vaccination
Bhutan

1. INTRODUCTION

Rabies is a fatal viral disease caused by rabies virus of the genus *Lyssavirus*, family *Rhabdoviridae*. It is mainly transmitted through the bite of a rabid animal of which domestic dogs are responsible up to 99% of human rabies deaths in the world (WHO 2013). Globally, rabies kills more than 59,000 humans annually with most deaths occurring in Asia and Africa (Hampson et al. 2015). Rabies in humans can be prevented by bite wound management and administration of postexposure prophylaxis (PEP) whilst source of rabies virus in dogs can be eliminated through mass dog vaccination covering more than 70% of the total dog population (Zinsstag et al. 2009; WHO 2013). Thus, control of rabies in the animal reservoirs –

domestic dogs – is the only means to prevent the transmission cycle of disease and eliminate both dog and human rabies cases in the world (Cleaveland et al. 2006; Zinsstag et al. 2009).

In Bhutan, rabies is endemic in southern parts of the country bordering India (Tenzin et al. 2011). Sporadic occurrences in dogs have been reported in interior rabies free areas as a result of incursion from the bordering towns (Tenzin et al. 2010; Tenzin 2016; Tenzin et al. 2017). Bhutan practice One Health approach to control rabies in animals and humans focusing mainly on mass dog vaccination and sterilization, provision of PEP in humans, awareness education and surveillance (MoH 2014; NCAH 2017). This has resulted in substantial reduction of human rabies deaths from 5 cases in 2011 to zero death in 2017 and 2018, and aims to achieve zero death in human from dog mediated rabies before the global target of 2030 (WHO and OIE 2016; NCAH 2018). Bhutan use commercially available inactivated tissue culture rabies vaccine - Rabisin and Raksharab - to vaccinate dogs and cats against rabies. Both the vaccines contain inactivated rabies virus glycoproteins with a potency of at least 1IU per ml. Although, dog vaccination is carried out annually along with animal birth control campaign, no detailed studies have been conducted to assess the antibody responses after vaccination in Bhutan. The standard method of testing to check whether a dog has adequate immunological protection is by measuring virus neutralizing antibodies (VNA) using the fluorescent antibody virus neutralization test (FAVN), rapid fluorescent focus inhibition test (REFIT) and also by enzyme-linked immunosorbent assay (ELISA) (Smith et al. 1973; Cliquet et al. 1998; Ma et al. 2012). A serum titre of 0.5 IU/ml and above of rabies virus-specific antibodies is considered adequate protection against rabies. A titre below this level is considered a vaccination failure, leaving the dog susceptible to rabies virus infection (Ma et al. 2012; OIE 2013). In this study we conducted a clinical trial and compared two commercially available rabies vaccines and assessed the antibody responses in free-roaming dogs in Thimphu city, Bhutan.

2. MATERIALS AND METHOD

2.1 Study area

The study was conducted at Jangsa Animal Saving Trust (JAST) and at Royal Society for Protection and Care of Animals (RSPCA) dog shelter located at Serbithang in Thimphu from December 2014 to March 2015. These shelters keep sick and weak dogs that were collected from Thimphu street. The animals are treated, dewormed, neutered and vaccinated against rabies at the shelter. Both JAST and RSPCA had 140 and 60 dogs, respectively, during our clinical trial. We have chosen these two shelters because of the availability of different age groups of dogs and also for logistic reasons. Prior to carrying out the study, a meeting was held with the managers and the caretakers of both the shelters to brief them about the research objectives and to get their approval for carrying out the study in their shelters. They have consented and agreed to provide necessary support during the conduct of the study period.

2.2 Study design and sampling of dogs

2.2.1 Primary vaccination group

The study was designed to recruit 50 dogs (puppies below 100 days of age) for primary vaccination on day 0 and then follow up this group for subsequent sampling on day 14 and 28 post vaccination. The puppies were assumed to have not been vaccinated against rabies previously (considered to be naïve). We captured 50 puppies from Changjiji and Dechencholing area and then taken to JAST and RSPCA shelters for vaccination and monitoring. The age of the puppies was determined based on the physical body structure through the experiences of the first author who was engaged in pet animal treatment for many years. The street dogs (puppies) was used for this primary vaccination since there were no unvaccinated dogs at the JAST and RSPCA shelters. Fifty puppies captured from the street were randomly divided into two groups of 25 each. Group 1 (n=25) was vaccinated with 1 ml of RAKSHARAB vaccine and the group 2 (n=25) was vaccinated with 1 ml of RABISIN vaccine subcutaneously. Prior to vaccine administration, a serum sample (5 ml blood) were collected from each dog and then a subsequent serum sample post vaccination was collected on day 14 and 28. The puppies were given neck collar with number tag for identification for monitoring and subsequent sample collection.

2.2.2 Booster vaccination group

In this group, 50 adult dogs from two dog shelters that had been previously vaccinated against rabies were randomly selected and assigned into two group of 25 each. Group 1 (n=25) was vaccinated with 1 ml of RAKSHARAB vaccine and group 2 (n=25) was vaccinated with 1 ml of RABISIN vaccine subcutaneously as booster vaccination. Prior to vaccine administration, a serum sample (5 ml blood) was collected from each dog and a subsequent serum sample post vaccination were collected on day 14 and 28. However, it is unknown whether these dogs received Raksharab or Rabisin vaccines previously and the date of previous vaccination was also unknown. The dogs were tagged for identification, monitoring and subsequent sample collection.

The blood samples collected from all the study dogs were shipped to National Centre for Animal Health laboratory, Serbithang and then extracted serum on the same day of collection. The serum samples were labelled and preserved at -20 degree Celsius until analysis. The data related to breed, age, sex, date of vaccination, vaccine type and batch, type of management and the number of vaccinations were collected for each study dog. After the study period the dogs captured from the streets were sterilized and vaccinated using DHPPI + L and released back to the place of capture. Dog capturing and sampling were assisted by the staffs of JAST, RSPCA and the three volunteers.

2.3 Serological analysis

The serum samples were subjected to antibody titre measurement using SERIELISA as per the manufacturer's direction (SERILISA™ Rabies Ab Mono Indirect, Synbiotics, France). The SERELISA kit is designed for the quantitative detection of rabies antibodies in dog and cat serum samples. Based on OIE guideline, the post-vaccination antibody titers level ≥0.5 IU/ml or <0.5 IU/ml were considered acceptable or a vaccine failure, respectively (OIE 2013).

2.4 Data analysis

The data were managed in Microsoft excel (Microsoft excel 2013, Redmond, USA) and analyzed using Stata version 14 (StataCorp USA). T-test for two independent samples were performed to determine the differences in mean antibody titer responses between and within the vaccination group.

3. RESULTS

Of the total 100 dogs initially enrolled for the study, only 66 dogs (66%) were available for subsequent sampling. Fourteen puppies had died due to parvovirus infection while the rest (20 adult and puppies) had escaped from the shelter. Therefore, a total of 198 blood samples from 66 dogs (one sample each on day 0, 14 and 28) were collected and analyzed in this study.

3.1 Antibody titer of primary vaccination group

The mean and minimum-maximum antibody titre (IU/ml) of rabies vaccination at day 0 (prior to vaccination) and post vaccination on day 14 and 28 of the two-vaccination group and for individual dog is shown in Table 1 and Figure 1, respectively. Eight puppies (8/35; 22.86%) belonging to Raksharab group (n=7) and Rabisin group (n=1) had demonstrated a protective antibody titre (≥0.5 IU/ml ranging from 0.6 to 2.75 IU/ml) on day 0 while the remaining puppies (n=27) have demonstrated an antibody titre ranging from 0.1 to 0.45 IU/ml. There was a significant difference (p = 0.028) in the mean antibody level on day 0 between the puppies of Raksharab and Rabisin primary vaccination group (see Table 1). The antibody titre level had increased after primary vaccination in both the vaccination group ranging from 0.21 to 4.78 IU/ml

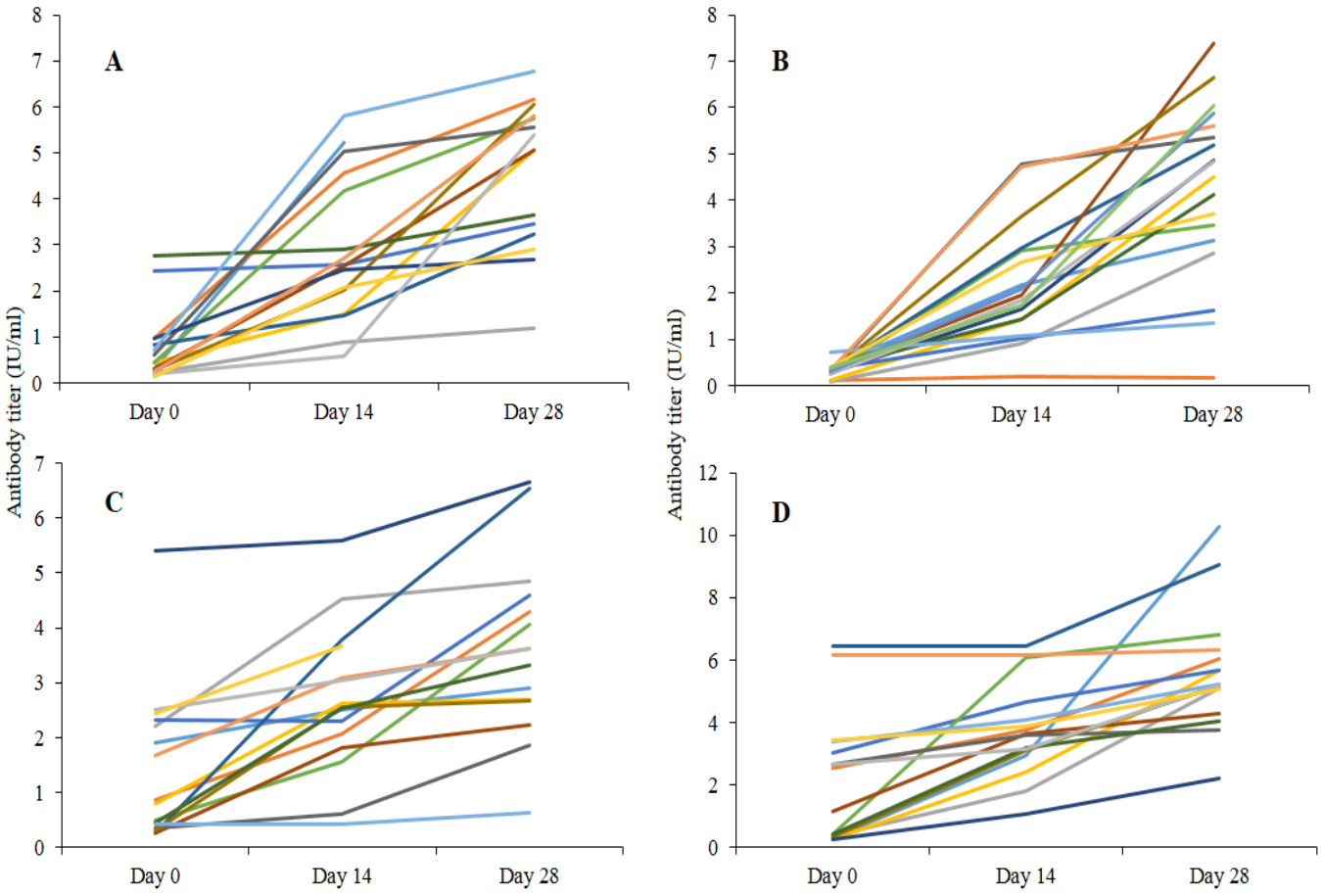

Figure 1: Rabies antibody titre (IU/ml) responses before and after vaccination in experimental dogs (n = 66). (A) primary vaccination group in puppies using Raksharab vaccine; (B) primary vaccination group in puppies using Rabisin vaccine; (C) booster vaccination group in adult dogs using Raksharab vaccine; (D) booster vaccination group in adult dogs using Rabisin vaccine.

and 0.16 to 7.38 IU/ml under Rabisin group on day 14 and 28, respectively and from 0.59 to 5.80 IU/ml and 1.19 to 6.76 IU/ml under Raksharab group on day 14 and 28, respectively (Table 1, Figures 1 and 2). However, no significant difference in the mean antibody responses were observed between the dogs vaccinated with Rabisin and Raksharab vaccine on day 14 (p=0.148) and 28 (p=0.609) (Table 1). One female puppy under Rabisin vaccination group that elicited an antibody titre of 0.12 IU/ml on day 0 had failed to respond to vaccination and have not attained protective titre on day 14 (0.21IU/ml). The titre dropped to 0.16 IU/ml on day 28. Overall, there was significance differences (p=0.281) in the mean antibody titre level of Rabisin vaccination group (mean Ab titre =2.25 IU/ml) and Raksharab vaccination group (mean Ab titre =2.27 IU/m).

3.2 Antibody titer of booster vaccination group

The mean and minimum-maximum antibody titre (IU/ml) of rabies vaccination at day 0 (prior to vaccination) and post vaccination on day 14 and 28 of the two-booster vaccination group and for individual dog is shown in Table 1 and Figure 1, respectively. Under Rabisin booster vaccination group, 56% (9/16) of the dogs had ≥ 0.5 IU/ml of antibody titre (ranges: 0.25 to 6.45 IU/ml) on day 0 (before vaccination) and all dogs attained protective titre on day 14 (ranges: 1.07 to 6.46 IU/ml) and on day 28 (ranges: 2.22 to 10.26 IU/ml). Similarly, under Raksharab booster vaccination group, 56% (9/16) of the dogs had ≥ 0.5 IU/ml of antibody titre (ranges: 0.27 to 5.39 IU/ml) on day 0 (before vaccination) and excepting one adult male dog (0.42 IU/ml) all other dogs attained protective titre on day 14 (ranges: 0.62 to 5.59 IU/ml) and on day 28 (ranges: 0.63 to 6.66 IU/ml). No significance differences in the mean antibody titre (IU/ml) were observed between Rabisin and Raksharab vaccination group on day 0 (p=0.266). The dogs that were vaccinated (booster vaccination) with Rabisin vaccine showed comparatively higher mean antibody titer when compared to Raksharab vaccine on both day 14 (p=0.039) and 28 (p=0.004) post vaccinations and the difference were significant (Table 1, Figures 1 and 2). Overall, there was significance differences (p=0.002) in the mean antibody titre level of Rabisin vaccination group (mean Ab titre =3.829 IU/ml) and Raksharab vaccination group (mean Ab titre =2.552 IU/m).

Table 1: Mean and minimum-maximum antibody titer (IU/ml) for primary and booster rabies vaccination group before vaccination (day 0) and after vaccination (day 14 and 28) in 66 dogs.

	Primary Rabisin group			**Primary Raksharab group**		
	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28
Mean	0.315	2.178	4.259	0.739	2.902	4.581
Minimum	0.1	0.21	0.16	0.139	0.59	1.19
Maximum	0.72	4.78	7.38	2.75	5.8	6.76
	Booster Rabisin group			**Booster Raksharab group**		
	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28
Mean	2.119	3.751	5.62	1.419	2.667	3.636
Minimum	0.255	1.07	2.22	0.27	0.42	0.63
Maximum	6.45	6.46	10.26	5.39	5.59	6.66

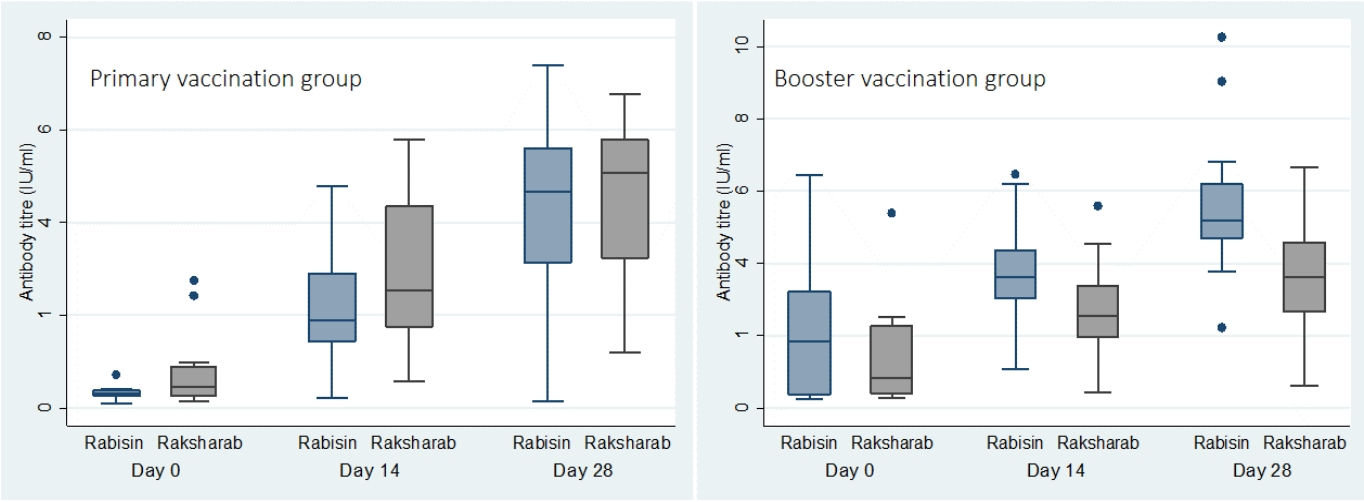

Figure 2: Comparison of antibody titer (IU/ml) between primary and booster vaccination group using Raksharab and Rabisin vaccine before vaccination (day 0) and after vaccination (day 14 and 28).

3.3 Antibody titer difference between sexes

No significant differences in the mean antibody titres were found in male and female dogs vaccinated with Rabisin and Raksharab vaccine in both primary vaccination group on day 0 (p=0.761), day 14 (p=0.943) and day 28 (p=0.548) and booster vaccination group on day 0 (p=0.812), day 14 (p=0.655) and day 28 (p=0.609) (Figure 3).

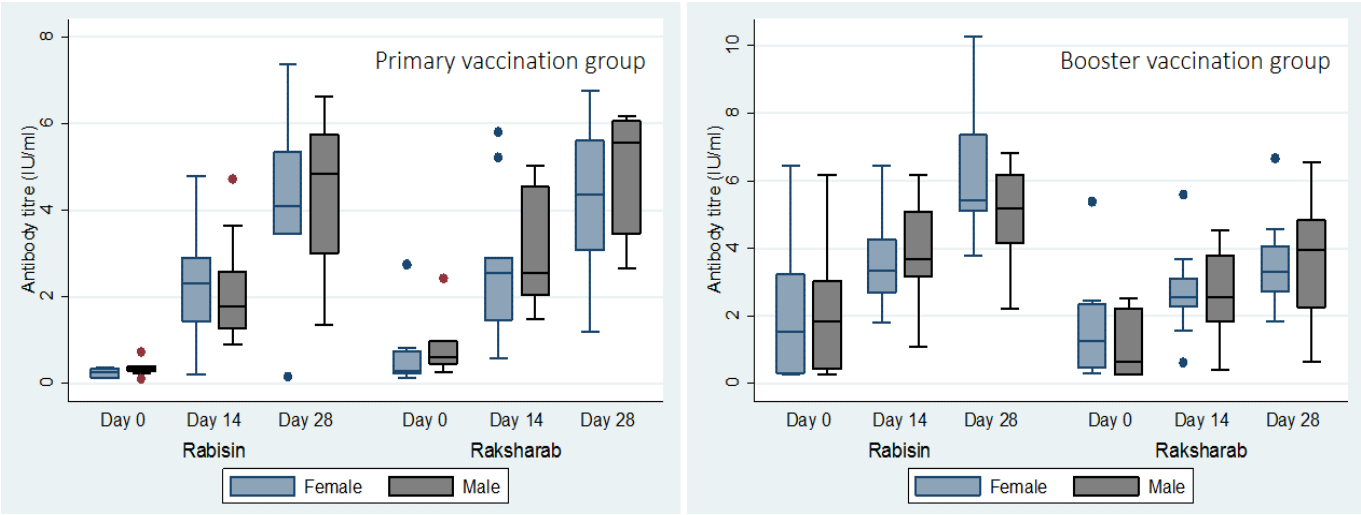

Figure 3: Rabies antibody titer of male and female dogs at day 0, 14 and 28 between primary (in puppies) and booster (in adult dogs) vaccination group using both Rabisin and Raksharab vaccine.

4. DISCUSSION

In this study we compared two commercially available rabies vaccine - Rabisin and Raksharab -vaccination responses in dogs. Our findings showed that both the inactivated vaccines have elicited minimum threshold level for protection (≥0.5 IU/ml) responses as per the recommendation of OIE and WHO indicating that these vaccines used in Bhutan are potent and efficient and thus acceptable for primary and booster vaccination against rabies in dogs.

We have selected puppies from the streets and enrolled for primary vaccination based on physical body condition that are deemed to be below 3 months of age. We assumed that they would be naïve against rabies vaccine antibodies. However, our results showed that all puppies (n=34) had antibodies against rabies prior to primary vaccination (day 0) and 20% (7/34) had protective titer (≥0.5 IU/ml) indicating the presence of maternally derived antibodies. The dam of those puppies must have been vaccinated previously against rabies and transferred maternally derived immunity to the puppies. In addition, we have observed the protective antibody titer level at day 0 (prior to primary vaccination) in those puppies that received Raksharab vaccination group when compared to Rabisin vaccination group (Figure 2). Therefore, the detection of comparatively higher antibody titer level on day 14 and 28 post vaccination in the Raksharab vaccination group when compared to Rabisin vaccination group could be explained by the presence of already higher antibody titer level in Raksharab group prior to the primary vaccination (Figure 2). Thus, the result should be interpreted cautiously since a completely naïve animals was not available for comparison in this study. Nevertheless, both vaccines have induced protective titre (≥0.50IU/ml) post vaccination when measured at day 14 and 28 suggestive of an anamnestic response to vaccination (Figure 2). Similarly, in the booster vaccination group (adult dogs that have been previously vaccinated against rabies and have already developed protective titer), both Rabisin and Raksharab vaccines have induced production of higher antibody titer after vaccination when measured at day 14 and 28. However, the dogs under Rabisin group had comparatively higher pre-vaccination antibody titer (day 0) which might have surged production of higher antibody titre after booster vaccination when compared to those dogs under Raksharab group. Thus, it is difficult to conclude in this study that Rabisin vaccine performed better than Raksharab vaccine in terms of antibody responses. Similar findings were observed in the primary vaccination group (as discussed above) in which those puppies that had higher pre-vaccination titer against rabies virus (day 0) produced higher antibody titre level after vaccination. Therefore, we can conclude that both the vaccines have induced production of protective antibody against rabies virus in this clinical trial after vaccination (by day 14) and the titre increased by third week of vaccination. Study conducted by Lhamo (2012) in free-roaming dogs in Samdrup Jongkhar, Bhutan also demonstrated development of protective titre (≥0.5 IU/ml) by day 14 after vaccination which may be due to an anamnestic response to the vaccine. Other studies have showed development of protective titre by day 14 and peaked by day 21 and 30 post vaccination (Morters et al. 2015; Moore et al. 2016). Our study also demonstrates no significant difference between the sexes in antibody responses using both Rabisin and Raksharab vaccines in either primary and booster vaccination group (Figure 3) which is in agreement with studies conducted elsewhere indicating that the primary immune response is not associated with sex (Delgado and Carmenes 1997; Jekel et al. 2008; Berndtsson et al. 2011; Wallace et al. 2017).

Our findings showed that puppies responded well to a standard dose of commercially available inactivated rabies vaccine without any apparent adverse reactions. Generally, puppies less than three months of age are excluded from rabies vaccination programmes (also recommended by the vaccine manufacturers) assuming that they have immature immune systems and the presence of maternal antibodies may limit the immune response to rabies vaccine (Day 2007, Hodgins and Shewen 2012; Morters et al. 2015). On the contrary, excepting one, all the puppies sampled following primary vaccination in this study generated antibody titre ≥0.5 IU/ml and our findings is in concordance with other studies that had

seroconverted to protective titer ≥0.5 IU/ml post vaccination without any adverse reactions (Morters et al. 2015). Therefore, puppies below 3 months of age should be vaccinated against rabies on the basis of this findings, WHO recommendations (WHO 2013) and also as per our national rabies prevention and control plan (NCAH 2017) since there is higher risk of human beings contracting rabies from young puppies (Mitmoonpitak et al. 1997, 1998; Taiwo et al. 1998; Widdowson et al. 2002). However, since antibody titre tended to decrease in young dogs after primary vaccination, it is recommended to provide a second dose a few weeks apart followed by annual vaccination as booster (Tasioudi et al. 2018). However, this will not be practically feasible in the free-roaming dog population, and thus recommended for annual vaccination.

It is to be noted that one puppy (female) under primary vaccination group and one adult dog have not responded well to the vaccination. It is apparent that not all dogs respond equally to vaccination, leaving them susceptible to disease. It has been demonstrated that approximately 10% of rabies vaccine naïve dogs fail to reach the recommended threshold of ≥0.5 IU/ml after primary vaccination (Aubert 1992; Kennedy et al. 2007; Moore and Hanlon 2010; Wallace et al. 2017). Failure to produce an adequate antibody response is related to intrinsic factors such as genetics (breed of dogs) and various extrinsic factors such as health status of dogs, stress during vaccination, vaccine type, age, timing of vaccination and subsequent blood sampling (Cliquet et al. 2003; Mansfield et al. 2004; Kennedy et al. 2007; Jakel et al. 2008). During this clinical trial period, there was an outbreak of parvo virus in the shelter which leads to death of puppies (n=14), which could have affected immune responses. On other hand, some animals also appear to over respond to vaccines potentially leading to adverse reactions, such as hypersensitivity reactions when re-vaccinated, but was not observed in our study (Kennedy et al. 2007).

There are few limitations to be noted in this study. First, no naïve puppies (with zero antibody titer at day 0) were available in the primary vaccination for making effective comparison and measuring antibody responses over time. Obtaining naïve puppies for vaccine clinical trial is difficult since majority of the dogs (adults) have received one or more vaccine injection during annual vaccination campaigns conducted in the country and thus must have transferred maternal immunity to the puppies. Second, the adults' dogs were randomly selected and captured from the dog shelter that does not have individual record of previous vaccination date. Ideally, all dogs (both primary and booster vaccination group) should have proper record of vaccination history to understand the development of antibody over time. Third, we could not recruit control group for both primary and booster vaccination group due to logistic reasons. Fourth, the neutralization assays such as rapid fluorescence focus inhibition test (RFFIT) and fluorescent antibody virus neutralization test (FAVN) are considered the gold standard and has been used to assess the titer of rabies virus neutralizing antibodies for more than three decades (Ma et al., 2012), but in this study we used ELISA (SERILISA) to estimate the level of rabies virus antibody. Although, the ELISA appears simpler, safer and more efficient, the assay is less sensitive in detecting low values of rabies virus neutralizing antibodies than neutralization tests (Cliquet et al. 2004; Servat and Cliquet 2006; Ma et al. 2012). Fifth, in this study, we could not continue sampling beyond 28 days due to logistic constraints. Ideally, the clinical trial should have been followed for up to one year or even two year to understand the duration of immunity so that vaccination schedule can be recommended based on the duration of immunity. Nevertheless, our study has demonstrated good antibody responses in dogs following vaccination with two commercially available rabies vaccines without any adverse reaction.

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