Failure of monovalent and polyvalent rabies vaccines to induce anti-rabies IgG in dogs as measured using an indirect ELISA

Zinnurine S.[1]; Sarkar E.R.[2]; Sikder S.[1*]

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Abstract

We aimed to investigate the efficacy of current practice of rabies vaccination in dogs of Bangladesh. Pet dogs (n=20) visited Teaching Veterinary Hospital of Chattogram Veterinary and Animal Sciences University were injected with commercially available monovalent or polyvalent vaccines containing inactivated adjuvanted rabies virus. Group of dogs were administered with single dose of monovalent (n=5) or polyvalent (n=5) rabies vaccine. Separate group of animals were injected with a second dose (monovalent n=5, polyvalent n=5) 14-days after the primary injection. Blood samples were collected at day-28 after the initial injection. Baseline sera were collected before starting injections. Indirect enzyme-linked immunosorbent assay was performed with sera samples to detect anti-rabies IgG. We were unable to detect any anti-rabies IgG titer in the dogs vaccinated with either monovalent or polyvalent vaccines irrespective of frequency of vaccination. The results indicate that the current commercial rabies vaccines are doubtful in generating protective antibody titer in dogs of this area. The results predict public health risk and demand for extensive investigation of vaccine quality and transportation.

Keywords: Rabies, Vaccination, Dog, ELISA, IgG

1-Department of Medicine & Surgery, Chattogram Veterinary and Animal Sciences University, Khulshi, Chattogram, Bangladesh-4225, Bangladesh.
2-Department of Biochemistry and Biotechnology, Faculty of Basic Medical and Pharmaceutical Sciences, University of Science and Technology Chittagong, Chattogram-4225, Bangladesh.
*Corresponding author's Email: suchandan@cvasu.ac.bd

Introduction

Rabies is one of the most fatal viral diseases of dogs (Thomsett, 1963; Nikitiuk, 1970; Dodet *et al.,* 2008; Hercules *et al.,* 2018; Eze *et al.,* 2018) but is preventable by proper vaccination. To minimize the risk of rabies and secure One Health, vaccination of dogs is practiced worldwide. The vaccination schedules recommended by the World Small Animal Veterinary Association (WSAVA) does not have exclusive suggestions on Bangladesh (Day *et al.,* 2016). The WSAVA Vaccination Guidelines Group (VGG) recognizes that the keeping of pet small animals is subject to significant variation in practice and associated economics throughout the world. The VGG also suggested that vaccination recommendations that might apply to a developed country may not be appropriate for a developing country. Bangladesh is a place where most of the dogs are stray, have no registration, freely roam ubiquitously with other dogs (and probably foxes) from neighboring areas making them unique and at risk of infectious and contagious diseases compared to the developed countries. Therefore, routine mass vaccination, sterilization and depopulation approaches are employed to reduce unwanted breeding and overpopulation in South-East Asia (WHO, 2022).

Furthermore, Department of Livestock Services (DLS) of Bangladesh produces rabies vaccine through Livestock Research Institute (LRI) that has limited supply and information on efficacy (LRI, 2023). Veterinarians of this region recommend commercially available vaccines imported from abroad and follow different vaccination schedules. Due to inadequate sero-monitoring, there is a chance of emergence of this disease even after vaccination. A well-designed study could solve this dilemma. Therefore, the current pilot study is designed to monitor serum anti-rabies IgG antibody generation in dogs following monovalent or polyvalent rabies vaccinations.

Materials and methods

Study area, and population

The study was conducted on the dogs visited Teaching Veterinary Hospital of Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram, Bangladesh. Total 20 clinically healthy dogs of either sex and more than three-months old with no history of rabies vaccination or exposure to dog or fox bites were included.

Ethics approval

The study strictly followed the institutional guidelines for research set by Chattogram Veterinary and Animal Sciences University (CVASU) Animal Ethics Committee (approval no. CVASU/Dir(R&E) EC/2020/165(5)). The dog restraining, health examination and sample collection was performed in accordance with the current legislation Cruelty to Animals Act 1920, Act No. 1 of 1920 of the Government of the People's Republic of Bangladesh. Written informed consent was taken from all the owners before experimentation.

Vaccination and sample collection

The puppies were divided into four-groups; i. Single-dose monovalent rabies vaccine n=5 (Rabisin®, Med-Vet Biolink Pvt. Ltd, India), ii. Two-dose monovalent rabies vaccine n=5, iii. Single-dose polyvalent rabies vaccine n=5 (Nobivac® RL, MSD Animal Health, India), and iv. Two-dose polyvalent rabies vaccine n=5. The vaccines were from local veterinary pharmacy and have different lot numbers throughout the duration of experiments. The monovalent rabies vaccine contains inactivated adjuvanted rabies virus whereas the polyvalent vaccine contains additional *Leptospira canicola* and *L. icterohaemorrhagiae*. The injections were performed sub-cutaneous at day 0 only to the single-dose recipients, and at day 0 and day 14 to the two-dose recipients following standard procedures of injections. Under physical restraint, blood samples from vaccinated dogs were collected at day 28 as a dog is considered to get immunized within 28 days after initial vaccination (CDC, 2011). Baseline sera samples were collected at day 0 before vaccination. The blood samples were kept undisturbed for 30 minutes followed by centrifugation at 1500 rpm for 10 minutes to separate sera and stored at -20ºC until analysis.

Serum anti-rabies IgG detection by ELISA

Serum IgG against rabies virus was detected using indirect enzyme linked immunosorbent assay (ELISA). The ELISA kit used in the current study was from Sunlong Biotech Co. Ltd. (Cat. #SL0086Ca). The procedure of ELISA described by Salvi *et al.* (2010) was followed. Briefly, sera samples were diluted 1:5 using sample diluent and 50 µl added to microtiter plate in duplicates. Negative and positive controls provided with the kit were added in a volume of 50 µL to the negative and positive control wells respectively. The microplate was incubated at 37°C for 30 minutes followed by two wash using wash buffer. Fifty microliter of HRP-conjugated anti-dog IgG was added and incubated for 30 minutes at 37°C. After wash, equal volume of chromogen A and B were mixed and 100 µL were added to each well, and plates were incubated at 37°C for 15 minutes. Fifty microliters of stop solution was added to stop further color development. Optical density (OD) was measured immediately using an automated microtiter plate reader at 450 nm wavelengths. The OD value of the blank control well was set as zero. Blocking percentage (BP) of the samples were calculated as:

BP=(OD of sample–OD of negative control) /(OD of positive control–OD of negative control)×100

Statistical analysis

Data from the ELISA reader was transported to Microsoft Excel. The graphs were made using GraphPad Prism 8.0 software. There was no positive data to perform statistical analysis.

Results

Demographic data of experimental animals

We recruited 20 unvaccinated dogs with no history of exposure to fox or stray dog bite earlier, and no history of vaccination and neurobehavioral disease. Among the dogs, 70% (n=14) were male and 80% (n=16) were aged between 3 to 6 months. Twenty five percent (n=5) of the dogs were local non-descriptive, 25% (n=5) were cross-bred and rest were purebred.

Anti-rabies IgG analysis

We analyzed the sera samples for anti-rabies IgG by ELISA and found that the OD values and blocking percentages (BP) are similar to Baseline sera (Fig. 1). Positive titer for anti-rabies IgG is equal or greater than 70% BP (Wasniewski and Cliquet, 2012). The higher OD and BP values of the positive control indicated that the ELISA procedure and kit worked as expected. However, the sera from dogs vaccinated with either single or two dose of monovalent or polyvalent rabies vaccines did not induce expected levels of anti-rabies IgG.

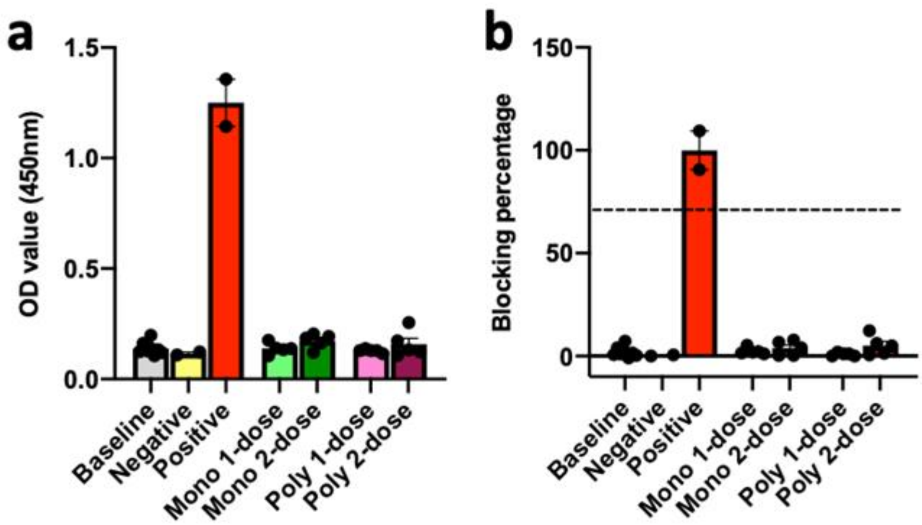

Figure 1: Analysis of anti-rabies IgG data. a) Optical density (OD) values of the controls and samples from experimental dogs. c) Blocking percentages of the samples and controls determined from OD values. Positive titer for rabies neutralizing IgG is indicated by the signal equal or greater than 70%, indicated by dotted line.

Discussion

We investigated whether the current practice of rabies vaccination in dogs generate protective levels of anti-rabies IgG. We recruited 20 unvaccinated dogs with no history of exposure to fox or stray dog bite before. Foxes are natural reservoir of rabies virus and fighting with these animals or exposure of saliva of these animals to open wounds can cause rabies, and hence generate natural anti-rabies antibodies (Singh *et al.,* 2017; Nadin-Davis *et al.*, 2021). We vaccinated healthy puppies of older than three-months with commercially available monovalent Rabisin® or polyvalent Nobivac LR® that contains inactivated

Leptospira sp. additional to the inactivated rabies virus.

Demographic data of the experimental animals revealed that male dogs are predominantly preferred by the residents of this area. Male dogs are believed to be of superior in guarding or protecting the owners and have less risk of unnecessary breeding. In the current study, 80% of the dogs were between three to six months old as expected, as older dogs might get natural immunity due to exposure to non-lethal rabies virus from other dogs or foxes (Gold *et al.,* 2021). We excluded the ownerless street dogs as they may have natural infection and immunity as well.

Findings of the current study revealed that the dogs vaccinated with single or two-dose of monovalent or polyvalent rabies vaccines did not induce anti-rabies IgG (Fig. 1). Although vaccination is performed with an expectation of protective immunity, inherent characteristics of the vaccine may cause variation in inducing immunity. Rabies vaccination in dogs may not induce full protection (Murray *et al.,* 2009). The realistic expectations for rabies vaccination are rather to protect 70% of the population that constitutes herd immunity later. Our results were partially supported by a study in Italy (Nodari *et al.,* 2017), who reported higher rabies vaccination failure in imported dogs than in those vaccinated locally. Another study in Indonesia reported that there was loss of binding antibodies against rabies in dogs vaccinated with locally produced or imported vaccine (Wera *et al.,* 2021). Loss of efficacy is considered as an unexpected outcome of vaccination. Although there is less chance of 100% efficacy with any vaccine, true loss of efficacy is rare and failure to immunize is more likely to result from inappropriate administration, pre-existing disease, immunosuppression, or genetic non-responsiveness than to a problem with the vaccine itself (Day, 2006).

The current loss of efficacy in vaccination might be due to inappropriate inactivation of virus during production as reported in other vaccine such as Severe Acute Respiratory Syndrome Corona virus (Darnell and Taylor, 2006). Cold chain maintenance is another major challenge of vaccine transportation from laboratory to dogs associated with vaccine failure (Pambudi *et al.*, 2022). However, there are ample evidence that rabies vaccine is very stable at room temperatures for extended periods for several months (Smith *et al.*, 2015; Lankester *et al.*, 2016). Vaccinated in sick animals was not a possibility as we carefully included the healthy puppies only and a systematic clinical examination was performed before vaccination. Some breeds of dogs are more resistant or susceptible to rabies. However, in our study, none of the vaccinated dogs generated anti-rabies IgG disclaiming effects of the breed difference. Interference due to maternal immunity is another potential reason of vaccination failure (Arega *et al.*, 2020). Duration of maternal immunity in the puppies depends on colostrum feeding in the first few hours of age (Chastant and Mila, 2019). Neutralization of the virus due to maternal antibody is disagreed as we carefully included puppies of more than 60-days old. Other possibilities of vaccine failure could be fraudulence by the vaccine supplier with supply of blank vaccine or long-term storage of vaccines at ambient temperature as in Bangladesh most of the pharmacies do not have facilities to maintain room temperature in-store.

In summary, our ELISA data suggest that in this very small number of dogs checked that two commercial rabies vaccines appear to fail to elicit an immune response. This finding requires extensive investigation with a properly designed study.

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Conflict of Interest

We declare no conflicts of interests.

Authors contributions

The study was designed by Suchandan SIKDER. Animal injections, sample collection and analysis was performed by Saida ZINNURINE. Suchandan SIKDER, Saida ZINNURINE and Eti Rani SARKAR performed data analysis and written the manuscript.

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