



## Investigation of the effect of bee venom on the immune response of dogs to rabies vaccine

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### ABSTRACT

Aiming to raise the levels of immunity induced in dogs by the cell culture inactivated rabies vaccine, bee venom BV was subjected to investigate its immune stimulant effect in vaccinated dogs. It was found that a dose of 1mg of BV/ dog did not cause any post inoculation reaction showing its safety. Mutual vaccination of dogs with BV inoculation was carried out in different groups of susceptible dogs of about 3-5 months' age. Monitoring of the exhibited rabies antibodies in vaccinated dogs using serum neutralization test (SNT) and indirect Enzyme Linked Immune Sorbent Assay (ELISA) revealed that BV induced the highest levels of antibodies (128 by SNT and 7 log<sub>2</sub> and 6 log<sub>2</sub> by ELISA) when inoculated before and simultaneously with rabies vaccine. Rabies vaccine alone or before inoculation of BV induced lower titers of antibodies (32&64 and 5 & 6log<sub>2</sub> by SNT and ELISA respectively) by the 4<sup>th</sup> week post vaccination. However, BV could be used to initiate the immune response of dogs to rabies vaccine.

**Key words:** bee venom, the immune response, rabies vaccine, ELISA

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### 1. INTRODUCTION

RABIES is an acute viral disease of the nervous system of warm-blooded animals that is caused by a rhabdo virus (species *Rabies virus* of the genus *Lyssa virus*) (National Centre for Disease Control, 2014). The disease transmitted by infected saliva usually through the bite of a rabid animal characterized by increased salivation, abnormal behavior, and eventual paralysis and death when untreated (WHO, 2014). Rabies affects domestic and wild animals, and is spread to people through bites or scratches; usually via saliva. The disease is almost fatal following the onset of clinical signs. Dog vaccination is the most effective strategy for preventing rabies in people. Rabies vaccines induce an active immune response that includes the production of neutralizing antibodies persists for greater than or equal to 2 years. Rabies immune globulin (RIG) provides a rapid, passive immunity that persists for only a short time (half-life of approximately 21 days) (WHO, 2010). Bee venom is a complex mixture of proteins, peptides and low molecular component. The main components are proteins and peptides. BV has numerous polypeptides the main one being melittin (Shkenderov and Ivanov, 1983) It contains several

biochemical or pharmacologically active substances, including at least the following: histamine, dopamine, melittin, apamin (mast cell destroyer - MCD), peptide, minimine, and the enzymes - phospholipase A, and hyaluronidase. Bee venom has antibacterial, anti-parasitic and antiviral properties (Guillaume et al., 2006). Bee venom PLA2 has an immune stimulate effect by the innate immune system and induces a type 2 immune response in mice by inducing a T helper type 2 (Th2) cell-type responses and group 2 innate lymphoid cell activation via the enzymatic cleavage of membrane phospholipids and release of interleukin-33. Furthermore, the IgE response to PLA2 could protect mice from future challenge with a near-lethal dose of PLA2 (Noah et al., 2013).

The present study aims to spot the light on bee venom as immune stimulant agent for rabies vaccination in order to maximize the acquired immunity induced by inactivated rabies vaccine in dogs.

### 2. MATERIALS AND METHODS

#### 2.1. Bee venom:

Honey BV was supplied by Sera plant, The Holding Company for Biological Products and Vaccines (VACSERA). It was obtained at a concentration of 1mg/ml saline

### 2.2. Rabies virus:

Evelyn Rokintniki Abelseth (ERA) strain of rabies virus adapted on BHK cell culture of a titer  $6\log_{10}\text{TCID}_{50}/\text{ml}$  was supplied by the Department of Pet Animal Vaccine Research (DPAVR), Veterinary Serum and Vaccine Research Institute (VSVRI) and used in serum neutralization test to evaluate the immune response of vaccinated dogs.

### 2.3. Rabies vaccine:

Inactivated cell culture rabies vaccine was supplied by DPAVR; VSVRI and used for vaccination of experimental dogs.

### 2.4. Cell culture:

Baby hamster kidney cell line (BHK<sub>13</sub>) was supplied by DPAVR and used in serum neutralization test.

### 2.5. Dogs:

Thirteen native breed dogs of about 3-4 months age were screened using SNT and found to be free from rabies antibodies; free from external and internal parasites were subjected for experimental vaccination with rabies vaccine alone and with honey bee venom. The dogs were divided into 3 groups as follow: Group-1 of 5 dogs was vaccinated with the cell culture inactivated vaccine using a dose of 2ml/ dog inoculated S/C Group- 2 of 5 dogs was vaccinated with the same dose of rabies vaccine through the same route in addition to 50µgm/dog of honey bee venom according to Diez-Gomez et al. (1995). Group-3 of 3 dogs was kept without inoculation as test control. Animal groups were housed under hygienic measures receiving balanced diet and adequate water. Serum samples were obtained from all animals on week intervals up to 4 weeks' post vaccination then on month intervals up to 6 months later.

### 2.6. Serum Neutralization test (SNT):

SNT was carried out using the micro titer technique according to Bass et al. (1982) while the serum neutralizing antibody titer was expressed as the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100 TCID<sub>50</sub> of rabies virus according to Singh et al. (1967).

### 2.7. Anti-dog horse radish peroxidase conjugate:

Anti-dog immunoglobulin [IgG whole molecule] conjugated with Horse Radish Peroxidase (HRP) was obtained from Sigma Chemical Company(USA) and treated before use by double dilution to cover the expected range and the suitable dilution was selected by using PBS phosphate buffer saline with bovine albumin and tween 20 which showed suitable color with a titer of 1:10000.

### 2.8. Indirect enzyme linked immune sorbent assay (ELISA):

Indirect ELISA was applied on collected serum samples from vaccinated dogs with rabies vaccines according to Hubschle et al. (1981).

## 3. RESULTS

Following up rabies antibodies in vaccinated dogs using serum neutralization test (SNT) revealed that BV induced the highest levels of antibodies (128) when inoculated before and simultaneously with vaccination by rabies vaccine. Rabies vaccine alone or before inoculation of BV induced lower titers of antibodies (32&64 respectively) by the 4<sup>th</sup> week post vaccination as shown in table (1).

Indirect Enzyme Linked Immune Sorbent Assay (ELISA) also revealed that BV induced the highest levels of antibodies (7 and  $6\log_2$  respectively) when inoculated before and simultaneously with vaccination by rabies vaccine. Rabies vaccine alone or before inoculation of BV induced lower ELISA titers ( $5\log_2$ ) by the 4<sup>th</sup> week post vaccination as tabulated in table (2).

Table (1): Mean Rabies serum neutralizing antibody titers in vaccinated dogs

Dog groups	Mean rabies serum neutralizing antibody titer*/ WPV**							
	0 time	1WPV	2WPV	3WPV	4WPV	8WPV	12WPV	16WPV
1	0	2≤	4	16	32	32	32	32
2	0	2≤	8	32	64	64	64	64
3	0	2	16	32	128	128	128	128
4	0	4	16	32	64	128	128	128
5	0	0	0	0	0	0	0	0

\*Rabies serum neutralizing antibody titer= the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100TCID<sub>50</sub> of rabies virus \*\*WPV= week post vaccination

Table (1): Mean Rabies ELISA antibody titers in vaccinated dogs

Dog groups	Mean rabies ELISA antibody titer (log <sub>2</sub> /ml) / WPV*							
	0 time	1WPV	2WPV	3WPV	4WPV	8WPV	12WPV	16WPV
1	0.0	1	2	3	5	5.5	5.5	5.5
2	0.0	1	2	3	5	5	5	5
3	0.0	2	3	5	7	7	7	7
4	0.0	2	3	4.5	5.5	6	6	6
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

\*WPV= week post vaccination

Dog groups 1 vaccinated with rabies vaccine only; 2 vaccinated with rabies vaccine one week before BV inoculation; 3 vaccinated with rabies vaccine one week after BV inoculation; 4 vaccinated with rabies vaccine simultaneously with BV inoculation; 5 non-vaccinated and non-inoculated

#### 4. DISCUSSION

As it is well known that the majority of viral diseases are incurable and vaccination against them is the corner stone in their control and protection of susceptible hosts. Vaccination of dogs with a single dose of the inactivated cell culture rabies vaccine resulted in induction of specific rabies neutralizing antibodies from the first week post vaccination with a mean titer of 3.33 that increased gradually to reach its peak (64-128) by the 4<sup>th</sup> week later then still unchanged till the 16<sup>th</sup> week (Khodier et al., 1998). ELISA results showed similar behavior of its titers as SNT where the obtained mean titers were 7- 6log<sub>2</sub> by the 4<sup>th</sup> week. These results came to be parallel to and confirmed by the findings of Albehwar (2009); Bass et al. (1982); El-Karamany (1986); Khodeir and Daoud (2008); Khodier (1999); Khodier et al. (1998); Larghi and Nebel (1980); Sikes et al. (1971) who obtained similar results and stated that the cell culture inactivated rabies vaccine is safe for all animal species and clarified that the protective neutralizing antibody titer should not be less than 1:5. On the other side it was found that administration of bee venom before or simultaneously with rabies vaccine enhanced the immune response of vaccinated dogs in a safe manner where it did not cause any abnormal signs after its inoculation. In this respect Birnbaum et al. (1993) who stated that bee venom LIKELY SAFE when used by subcutaneous injection Wesselius et al. (2005) reported that BV is approved product. In addition, American Apitherapy Society (2015) showed that some evidence suggests that BV might stimulate immune system activity. It was stated that the Bee venom is used subcutaneously, intra-dermal, and intra-arterially for many possible protocols for immunotherapy. These findings could be attributed

to melittin structure which has the ability to bind the cell membrane giving rise to immunogenicity for IgG response as suggested by Fehlner et al. (1991) or may be due to rapid shift in cytokine expression Th2 to Th1 and induction of IL1 at first by B cells and monocytes. It is well known that IL1 promotes production of IgG and T cell immunity as stated by Akdis et al. (1998); Bellinghausen et al. (1997) Akdis and Blaser (1999) and Mashoor (2013). So, it could be concluded that bee venom is a safe immune stimulant and could be used safely in dogs to enhance.

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