



## Evaluation of Anti-Rabies Hyperimmune Serum Prepared Using Different Adjuvants

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### ABSTRACT

Anti-rabies virus hyperimmune serum was prepared in horses using both inactivated and live attenuated rabies virus (ERA strain) adjuvanted with three different adjuvants including 20% Alhydro gel, 5% Pet gel-A and 20% calcium phosphate gel. The six vaccine preparations were inoculated subcutaneously in three groups of horses, separately, as each horse group received 4 increased doses of an inactivated vaccine followed by 4 increased doses of a live attenuated vaccine (one dose twice weekly) with the same adjuvant. All prepared vaccine formulae were found to be free from foreign contaminants and safe with no post inoculation abnormal signs in inoculated mice. Monitoring of the levels of exhibited rabies antibodies in the sera of immunized horses using serum neutralization test (SNT) and quantitative ELISA kit revealed that the prepared rabies vaccines with 5% Pet gel A adjuvant induced the highest antibody titers (2048 by SNT and 2.20 by ELISA kit) with expected protection percentage 96.14% followed by those induced by 20% Calcium phosphate gel recording the values of 2048 by SNT and 2.11 by ELISA kit with expected protection percentage 92.2%. Lower values (1024 by SNT; 2.05 by ELISA kit and 89.59% protection percentage) were recorded for the Alhydro gel adjuvanted vaccine. These obtained data reflected the potency of the prepared equine anti-rabies hyperimmune serum (EARHIS) to be used for post exposure treatment in emergency cases and that is the Pet gel A was the best adjuvant to be used.

**Keywords:** ELISA, ERA, hyperimmune serum, Rabies, serum neutralization.

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### INTRODUCTION

Rabies is acute enzootic progressive fatal viral encephalitis, caused by negative-stranded RNA virus belonged to the Genus Lyssavirus (Family Rhabdoviridae, Order Mononegavirales). Rabies virus is mainly transmitted via the saliva of infected domestic and wild animals (**Rupprecht and Shlim, 2014**). It infects domestic and wild animals and is spread to people through close contact with infected saliva via bites or scratches. Once the rabies virus reaches the central nervous system and symptoms begin to appear, the infection is effectively untreatable and usually fatal within days (**Green and Rupprecht, 2006 ; Nigg and Walker, 2009** ).

There is a vaccine and also anti-rabies Serum (ARS) for Rabies disease, but the anti-rabies vaccine

alone may not save lives. Anti-Rabies Serum is prepared from Human origin and Equine origin. Equine Rabies Immunoglobulin (ERIG) obtained from the blood plasma of healthy equines that have been immunized against rabies by vaccination. Equine rabies hyperimmune serum (ERHIS) is cheaper; safe and can be used to reduce the post-exposure hazard. Effective treatment of Rabies is critically dependent on the availability of good-quality antisera (**WHO, 2007**).

Particularly in cases of severe exposure (that defined as category III and Immunocompromised category II patient by the World Health Organization, the victims should undergo wound treatment and administration of vaccine and Rabies Immunoglobulin (RIG). The administration of RIG as soon as possible after exposure is essential in the management of severe bites, to provide passive immunity before the development of host active immunity against rabies

virus (WHO, 2005 and Manning, *et al.*, 2008). If a limited amount of RIG is available, RIG allocation should be prioritized for exposed patients based on the following criteria: Multiple bites, deep wounds, bites to highly innervated parts of the body (such as head, neck and hands), severe immunodeficiency, the biting animal is a confirmed or probable rabies case, and bites, scratches or exposures of mucous membranes caused by a rabid animal (WHO, 2018).

Post-exposure vaccination treatment against rabies along with anti-rabies immunoglobulin prevents the development of the disease. In this respect, Al-Behwar (2009) concluded that the best immunization of farm animals post-exposure is the administration of rabies vaccine and antiserum on the suitable time (1-3 days post-exposure). Also, post-exposure intervention using specific antiserum was carried out in different animal species (Khodeir and Daoud, 2008).

Antibody-based therapies using monoclonal or polyclonal antibodies are emerging as an important therapeutic approach for the treatment of several diseases. The clinical need for polyclonal therapeutics for the treatment of a variety of specific illnesses and infections is often overlooked. Polyclonal antibody therapeutics are today widely used in medicine for viral and toxin neutralization and replacement therapy in patients with immunoglobulin deficiencies. Over the past 20 years, intravenous immunoglobulins have shown beneficial immunomodulatory and anti-inflammatory effects in many illnesses. Hyperimmune antibody preparations have been used over the past century for the treatment of a variety of infectious agents and medical emergencies, including digoxin toxicity, snake envenomation and spider bites.

Rabies Antiserum is a preparation containing the specific globulin obtained by purification of hyperimmune serum/plasma of healthy equines having a specific activity of neutralizing the rabies virus and phenol as preservative. The anti-rabies serum is used to provide passive immunity against rabies in post-exposure prophylaxis of individuals, exposed to the disease or virus after contact with a rabid animal or an animal presumed to be rabid. Anti-rabies serum itself does not constitute an anti-rabies treatment and should always be used in conjunction with rabies vaccine (Central Research Institute, Kasauli, 2019).

The antibodies production induced by Montanide Pet Gel A based vaccines was higher than aluminium based vaccines, also Montanide Pet Gel A can be used associated with a wide range of antigenic media and recommended to be used as an adjuvant for sensitive animal's vaccines ( Devillea *et al.*, 2011). The present work aims to prepare rabies hyperimmune

serum of high quality for the post-exposure treatment of animals exposed to biting of a rabid animal and to evaluate different types of adjuvants.

## MATERIALS AND METHODS

### Animals

#### 1.Horses

Eleven native breed male horses of about 3-5 years old without previous history of rabies vaccination were used for preparation of rabies antisera using inactivated and live attenuated rabies vaccine with three different adjuvants (Alhydro gel; Pet gel-A and calcium phosphate gel), where each formula was inoculated in each of three horses while 2 horses were kept without vaccination as test control.

#### 2.Mice

Fifty-six Swiss albino weaned mice of 4 weeks old supplied by Vaccine and Serum Veterinary Research Center (VSVRI) were used to test the safety of the prepared vaccine formulae.

### Virus

BHK-21 cell culture adapted Evelyn Rokintniki Abelseth (ERA) strain of rabies virus with a titter  $10^7$  TCID<sub>50</sub> /ml (Edries, 1994) was supplied by the Department of Pet Animal Vaccine Research (DPAVR), Veterinary Serum and Vaccine Research Institute (VSVRI) Abassia Cairo and used in vaccine preparations and serological tests

### Cell culture

Baby hamster kidney cell line (BHK<sub>13</sub>) was supplied by DPAVR; VSVRI and used for vaccine preparations and serum neutralization test.

### Vaccine preparations

Harvest of rabies virus strain ERA from infected BHK-21 cell culture was inactivated using Binary Ethyleneimine (BEI) working solution 0.01M prepared according to Girard *et al.* (1977). The inactivation process was carried out using 3% of the stock BEI solution at 37°C for 3.5 hours according to Edries (1994). Three formulae of both live and inactivated rabies vaccines were prepared using three different adjuvants including 20% Alhydro gel according to Edries, (1994); 5% Pet gel A according to Edries *et al.* (2017) and 20% calcium phosphate gel according to Relyveld (1980).

### Quality control testing of the prepared vaccines

#### 1.Sterility test

Sterility testing of each prepared vaccine formula was carried out according to the standard procedures of WHO (1973) using thioglycolate;

soybean casein digest, sabouraud, mycoplasma solid and liquid media.

## 2.Safety test

According to the **British Pharmacopeia (1985)**, 0.03 of each vaccine formula were inoculated intraperitoneally in each of 8 weaned Swiss Albino mice. Inoculated mice with another 8 non-inoculated mice were kept under observation for 10 days.

## Preparation of rabies hyperimmune sera (RHIS)

RHIS was prepared following the direction of **WHO (1973)** where each 3 horses were immunized subcutaneously with 8 increased doses (25; 50; 75 & 75ml) of one of the prepared inactivated rabies vaccine twice weekly followed by 4 doses (25 and 50ml where each dose contains  $10^7$ TCID<sub>50</sub>/ml ) of live attenuated rabies virus strain ERA twice weekly so the first horse group was immunized with the Alhydro gel vaccine, the second group was immunized with Pet gel A vaccines and the third group was immunized with calcium phosphate vaccine.

The fourth horse group (2 horses) was kept without immunization as a test control. Serum samples were collected from all horses on 2weeks intervals for monitoring of the induced rabies antibodies in their sera up to 8 weeks. One and two weeks after the last immunization of horses; 500ml serum was obtained from each immunized horse and subjected to the quality control testing including sterility, safety and potency. Sterility and safety tests were carried out as mentioned above while the potency test was carried out serologically using virus neutralization test and rabies virus antibody quantitative ELISA kit.

## Virus neutralization test (VNT)

VNT was carried out using the microtiter technique on BHK-21 cells using 2 fold dilutions according to **Ferreira (1976)** and the endpoint of neutralizing antibody titers was expressed as the reciprocal of the final dilution of serum inhibiting the CPE of 100 TCID<sub>50</sub> of the virus according to **Singh et al. (1967)**.

## Rabies virus antibody quantitate ELISA kit

Zhenrui Rabies virus antibody quantitative ELISA kit, version RBV1805003 was supplied by Shenzhen Zhenrui Biotech Co., Ltd. China where rabies antibody titer was expressed as IU/ml according to the standard curve performed by the kit manufacturer.

## RESULTS

Table 1: Titers of prepared equine antirabies hyperimmune sera using VNT

Used vaccine	Mean rabies serum neutralizing antibody titers**					
	2WPI*	4WPI	6WPI	8WPI	9WPI	10WPI
Reh. G	64	512	512	1024	1024	1024
Pet gel	32	512	1024	2048	2048	2048
CaPh gel	32	512	512	1024	2048	2048

\*WPI= week post-immunization

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

Table 2: Titers of prepared equine antirabies hyperimmune sera using ELISA

Used vaccine	Mean ELISA rabies antibody titers					
	2WPI*	4WPI	6WPI	8WPI	9WPI	10WPI
Reh. gel	1.0	1.3	1.8	2.04	2.05	2.05
Pet gel	1.0	1.5	1.8	2.15	2.20	2.20
CaPh gel	1.1	1.5	1.7	2.10	2.11	2.11

\*WPV= week post-immunization

**N.B:** ELISA results were expressed as IU/ml where the protective value should not be less than 0.5IU/ml representing protection value of 58.4%.

## DISCUSSION

Antiserum is blood serum containing polyclonal antibodies and is used for passive immunization of animals against many infectious diseases especially in emergency cases as exposure to biting of a rabid animal. **WHO (2018)** recommended pre-exposure prophylaxis (PrEP) and post-exposure prophylaxis (PEP) for rabies. These updated recommendations are based on new evidence and directed by public health needs that are cost, dose and time-sparing while assuring safety and clinical effectiveness.

The present work deals with the preparation of equine anti-rabies hyperimmune serum to be provided

as a local product on request aiming to protect animals exposed to rabies infection through biting by a rabid animal. Different types of equine anti-rabies hyperimmune serum (ERHIS) have been produced using various immunogenic preparations consisting usually of a combination of inactivated and fixed strains of rabies virus (**Lepinc, 1973**). Through the present work; the followed up schedule for preparation of anti-rabies hyperimmune serum was in agreement with that described by **Liiekralang et al., (1973)** who reported that the used animals are given a series of injections of the vaccine in increasing volumes and all the injections are given subcutaneously into the lateral aspect of the neck. The immunization period lasts 105 days and the first bleeding is made 14 days later.

The prepared rabies vaccine formulae were found to be free from foreign contaminants, safe and potent showing no post-vaccination abnormal signs in mice and resulting in high levels of rabies neutralizing antibody titers in agreement with the recommendation of **WHO (1973)** and **OIE (2012)** concerning the purity and safety of veterinary biologics. It is known that antiserum is a serum containing antibody (ies) specific for one or more antigens obtained from an animal immunized either by injection of antigen or by infection with microorganisms containing antigen (**Anon, 2012**). It is used to confer passive immunity to that disease. Antisera do not provoke the production of antibodies but contain IgG against specified antigens and could be used therapeutically. The present obtained results revealed the success of the preparation of equine anti-rabies hyperimmune serum (EARHIS).

As shown in table (1 and 2); application of serum neutralization test (SNT) and Rabies virus antibody quantitative ELISA kit on horse sera revealed that rabies vaccines with 5% Pet gel A adjuvant induced the highest antibody titers (2048 by SNT and 2.20 by ELISA kit) with suggested protection percentage 96.14% followed by those induced by 20% Calcium phosphate gel recording the values of 2048 by SNT and 2.11 by ELISA kit with suggested protection percentage 92.2%. Lower values (1024 by SNT; 2.05 by ELISA kit and 89.59% protection percentage) were recorded for the Rehydra gel adjuvanted vaccine. These obtained data reflect the safety and potency of the prepared EARHIS to be used for post-exposure treatment in emergency cases. The polymeric technology of Montanide Pet Gel A has already been used in several vaccine models, including pet's vaccines, with a promising safety and efficacy profile. Our findings highlight the safety and efficacy profile of this polymer-based adjuvant dedicated to species where vaccine safety is sometimes of higher importance than efficacy, which agree with that results obtained by **Edries et al., (2017)** and **Parkera et al., (2009)**.

Depending on these results it could be stated that the prepared EARHIS is sufficient to be used in emergency cases inducing passive immunity as post-exposure treatment in animals similarly as concluded by **Mupapa et al., (1999)** who stated that antiserum is blood serum containing polyclonal antibodies used to pass on passive immunity to many diseases where antibodies in the antiserum bind the infectious agent or antigen. The immune system then recognizes foreign agents bound to antibodies and triggers a more robust immune response. The use of antiserum is particularly effective against pathogens which are capable of evading the immune system in the unstimulated state but which are not robust enough to evade the stimulated immune system.

## CONCLUSION

From the obtained results, it can be concluded that, Equine Anti-Rabies Hyperimmune Serum (EARHIS) is of significant importance and can help to protect animals against rabies infections in case of exposure to biting of rabid animals the case that needs rapid management.

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