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Review Article

Equine Rabies Immunoglobulin: A Review

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ABSTRACT

Rabies is a major cause of human death in many developing countries. There is a worldwide shortage of human and equine rabies immune globulin. The WHO recommends combined administration of rabies vaccine and rabies immune globulin to patients after exposure. The implementation of post exposure prophylaxis by vaccination and specific immunoglobulin therapy are largely hampered by its high cost. The equine rabies immunoglobulin is less expensive than human rabies immunoglobulin, and is used for rabies post-exposure prophylaxis in developing countries.

Equine rabies immunoglobulin is a F(ab)2 with high specific activity, purity, and safety. Healthy horses are immunized with a rabies vaccine. Crude plasma is collected and immunoglobulin is converted into F(ab)2 fragments by pepsin digestion. The F(ab)2 fragments are purified using caprylic acid precipitation followed by ultrafiltration.

Keywords: Rabies, Equine rabies immunoglobulin (ERIG), Human rabies immunoglobulin (HRIG), IgG, Caprylic acid, Pepsin.

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INTRODUCTION

RABIES

Rabies is an acute viral disease of the central nervous system (CNS) that affects all warm-blooded animals including mammals.[1, 2, 3] It is an acute infectious encephalomyelitis, caused by a number of lyssaviruses including: rabies virus and Australian bat lyssavirus.[4] The rabies virus infects the central nervous system, ultimately causing disease in the brain and death. It is almost invariably fatal if post-exposure prophylaxis is not administered prior to the onset of severe symptoms. It is a zoonotic disease, spread most commonly by a bite from an infected animal. The disease is slow and progressive. The average incubation period of rabies is 1 to 2 months, but may vary from one week to greater than one year, depending upon the site of dog bite, virulence of the virus, and the inoculation dose. The incubation period is short if the bite is on face, scalp, or neck because of the proximity to the brain. The prodormal phase comprises fever, nausea, vomiting, headache, fatigue, and other nonspecific symptoms. Some patients experience symptoms like pain, burning, prickling, or tingling sensations at the wound site [5].

Post-bite treatment involves both post-bite vaccination using rabies tissue cultured antigen (active immunization) as well as administration of Antirabies immunoglobulin (passive immunization). Post-bite vaccination would result in development of immunoglobulins, but not earlier than 2-3 weeks and therefore simultaneous administration of rabies immunoglobulin (antirabies serum) constitutes the best approach. Antirabies serum is injected locally in the biting wound area, immediately after dog-bite or preferably within eight days [6, 7, 8].

Epidemiology of rabies

Rabies is endemic in most parts of the world except New Zealand and Antarctica and is the tenth leading cause of death due to infection in humans, predominantly affecting the poor people in the developing countries and remains a serious health problem. Even those countries declared rabies free like United Kingdom, Ireland, areas of Scandinavia, Japan, Australia, New Zealand, Singapore, Brunei, most parts of Malaysia, some islands of Indonesia, Papua New Guinea bear a risk due to the expanding prevalence of the disease.[9]

About 65,000 people die due to rabies annually. This may be an underestimated figure because many cases go unreported

and misdiagnosed. Most cases are observed in Africa (24,000) and Asia (31,000); majority being in the developing countries; 99 % of all the cases are due to dog bites [6, 10]. Every 10 to 15 min. someone dies due to rabies, of which majority of the cases are children under 15 years of age. In children due to their short stature, dog-bites occur on the upper parts of the body, face, neck and hence are dangerous. More than 3.3 billion people from over 85 countries are at risk of rabies and prevalence of rabies is in more than 150 countries and territories. Annually more than 15 million people receive post-exposure preventive treatment. Of 1.5 million dog bite cases, 24,000 to 80,000 are from rabid dogs and require post exposure prophylaxis. [11]

Transmission of rabies

Rabies virus can cause disease in all warm blooded animals, especially mammals including humans. Rabies virus is predominantly neurotropic, killing the host after its growth in neurons. However before death, virus reaches salivary glands and is excreted in saliva. Mere licking of pre-existing scratch in skin is adequate for transmission of virus.

Humans are infected primarily through a bite or scratch by an infected animal. Dogs are the main host in transmission of rabies, in 99% of all the human cases the virus is transmitted by dog bites.

Human-to-human transmission by bite is the only theoretical possibility and has never been confirmed. Amongst the important reservoirs, dogs are the major cause of human deaths (99%) along with cats or wild animals (bats, monkeys & foxes). Transmissions of the disease to animals of great ecological interest like cattle and horses have been reported.

As shown in Fig. No. 1 initially after entry into the host through the bite on the skin or mucous membrane, virus replicates in the striated muscle cells. It multiplies here for a week and then spreads to the peripheral nervous system (PNS) at the exposed neuromuscular junction sites and nerve endings at unmyelinated sites such as sensory and motor terminals.[12, 13]

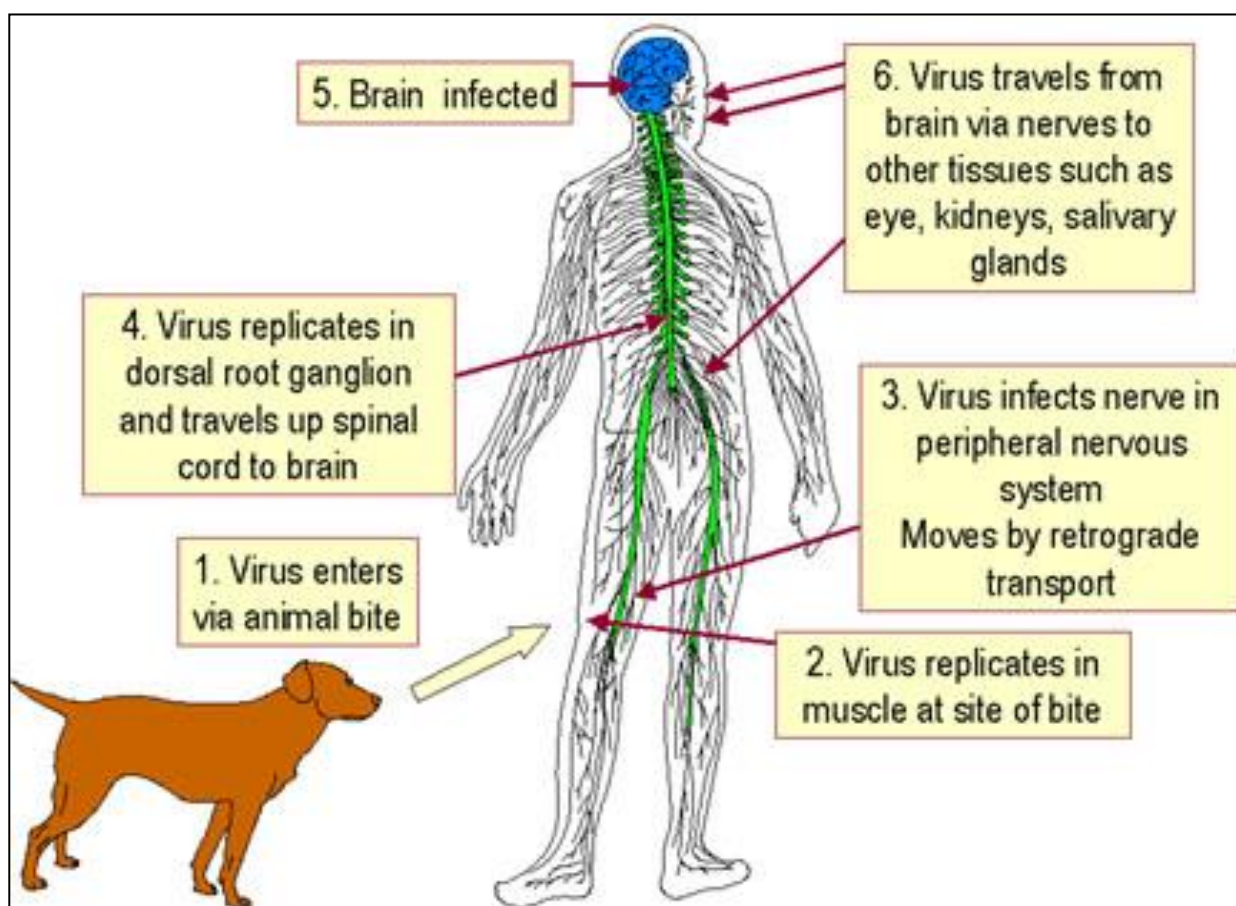

Fig. No. 1 Transmission of rabies

Source: www.microbiologyandimmunology, chapter twenty

ANTIRABIES SERUM

Importance of antirabies serum

In the confirmed information about 'health status' of biting dog, each dog bite case is treated as exposure to rabies virus. In such case post-bite treatment involves both post-bite vaccination using rabies tissue cultured antigen (active immunization) as well as administration of Antirabies immunoglobulin (passive immunization). Post-bite vaccination would result in development of immunoglobulins, but not earlier than 2-3 weeks and therefore simultaneous administration of rabies immunoglobulin (antirabies serum) constitutes the best approach. Antirabies serum is injected locally in the biting wound area, immediately after dog-bite or preferably within eight days. [14] Rabies immunoglobulin not to administer in the same syringe or at the same injection site as rabies vaccine. [15, 16, 17, 18]

Types of antirabies serum

Two types of antirabies serum products are available for post exposure prophylaxis:

Human Rabies Immunoglobulin (HRIG)

Equine Rabies Immunoglobulin (ERIG)

HRIG is prepared from the blood of human volunteers immunized with purified cell culture based rabies vaccine while ERIG is prepared from the blood of horses immunized with rabies vaccine.

Human Rabies Immunoglobulin (HRIG)

Human Rabies Immunoglobulin (HRIG) is a homologous preparation of refined, purified rabies immunoglobulin, i.e. immunoglobulins are human-proteins. To produce this immunoglobulin, human volunteers are immunized with purified cell culture based vaccines. HRIG is expensive (five times that of ERIG) and available in only limited quantities, because of involvement of human volunteers. Being homologous in nature, they do not cause any sensitization. The use of homologous immunoglobulins for human post-exposure treatment virtually eliminates the risk of anaphylaxis and serum sickness associated with heterologous serum products. To avoid such reactions, human rabies immunoglobulin (HRIG) preparations have been developed and used for post exposure treatment in most industrialized countries. Also HRIG being of human origin are eliminated from the human body at much reduced rate.

Equine rabies immunoglobulin (ERIG) becomes the immunoglobulin of choice, especially for the developing countries, where the animal bite cases are very much higher leading to greater requirement of immunoglobulins[6, 19, 20,21].

Equine Rabies Immunoglobulin (ERIG)

Equine rabies immunoglobulin assumes great importance in treatment of post-bite cases with suspected exposure to rabies virus. ERIG can be made available in much larger quantities and also at much lesser cost. The heterogeneous nature can cause allergic reactions but the technological developments in processing of horse immunoglobulins have rendered them less allergic and more stable. Purification techniques can be used to reduce the risk of sensitization to ERIG. Their objective of purification is to maximize the specific activity and to minimize the allergenic substances in the product. These techniques are implemented with careful adherence to the recommendations of the WHO Expert Committee on Biological Standardization. The clinical safety of Equine Rabies Immunoglobulin is also well established. [9, 22] In developing countries the use of highly purified horse immunoglobulin, are safer than the heterologous products of the previous generation. In the past few years, purified equine immunoglobulins have become available, and in recent studies, the incidence of serum sickness among recipients was reported to be as low as 1-6.2%. In India, manufacturers of Equine Rabies Immunoglobulin follow CPCSEA guidelines and cGMP norms.

In developing countries, in general and India in particular, the need of RIG exceeds the production. In addition, the animal protection groups that are becoming more and more influential in developing countries, and they condemn animal rearing for serum production. [4, 9, 10].

PRODUCTION OF EQUINE RABIES IMMUNOGLOBULIN

Hyper immune antibody preparation produced from animals serum have been used over the past century for the treatment of a variety of infectious agent and medical emergencies, including dioxin of toxicity, snake envenomation and spider bites. [13, 24] Traditionally equine IgG has been purified by pepsin digestion and Caprylic acid precipitation. [19]Equine rabies immunoglobulin (ERIG) has been produced using various immunogenic preparations, consisting usually of a combination of inactivated and fixed strains of rabies virus. The animals are given a series of injections of the vaccine in increasing quantities. All the injections are given subcutaneously into the lateral side of the neck. The general process of preparing equine rabies immunoglobulin as follows (Fig. No.2)

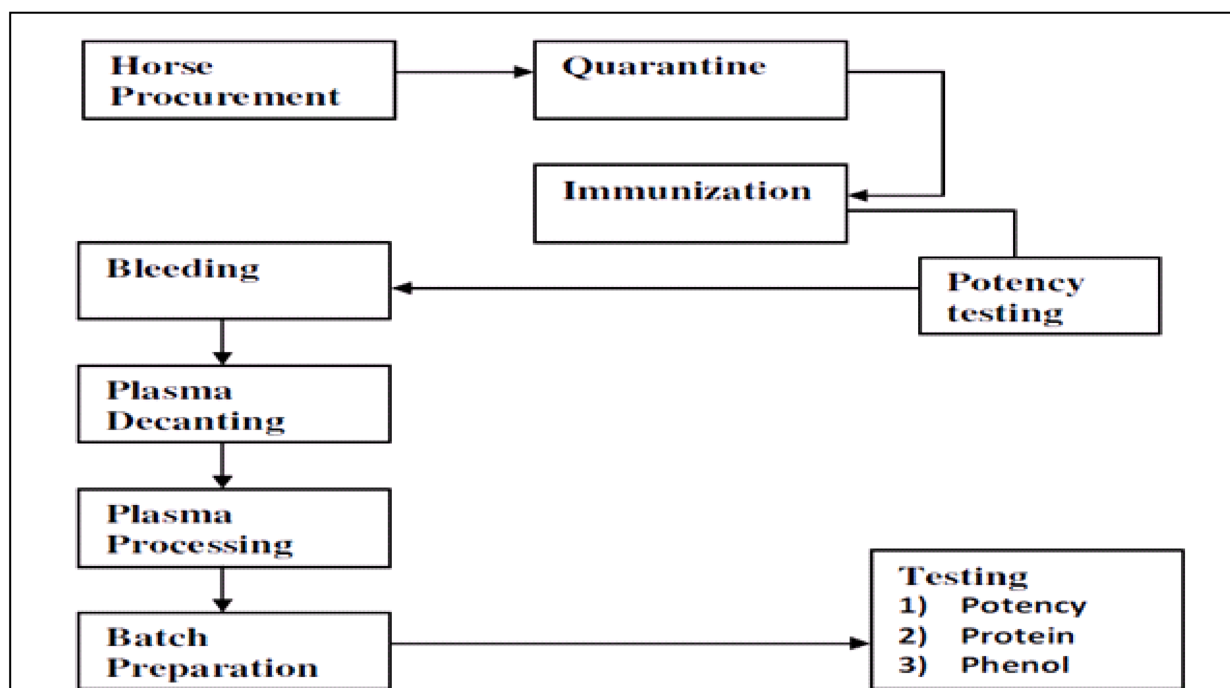

Fig. No. 2Antirabies serum manufacturing process

Source: WHO Technical Report Series No. 1004, 2017, 252

Immunization of horses

Numerous animal species have been used on various scales in antiserum production (horse, sheep, donkey, goat and rabbit) or for experimental purposes (camel, llama, dog and hen). However, the production of large volumes of antiserum from large animals such as equines is an advantage compared to the smaller species. The selection of the animal species should be based on several considerations, such as locally prevalent diseases, availability in the region, adaptation to the local environment, and cost of maintenance. The information in these guidelines refers mostly to horse-derived immunoglobulins. The horse is the animal of choice for commercial antivenom production. Horses are docile, thrive in most climates and yield a large volume of plasma. [25]

Horses are purchased and kept isolated for a period of one month under observation and only healthy horses are taken for production. During this period, equines are immunized with tetanus toxoid. Primary immunization with Rabipur vaccines begins after the completion of quarantine period. [27]Horses are injected with gradually increasing doses of rabies antigen. Amount of antirabies antibodies in serum is determined using the ELISA test. After the required antirabies titer is obtained, the horses are taken for bleeding. The animals are bled from a jugular vein. Usually, 6-9 months are required for immunization.

Collection of hyper immune plasma

Plasma as a starting material is preferred to serum largely because red blood cells can be returned to the animal, thus preventing anaemia and hypovolaemia in the donor animal and allowing more frequent bleeding. Some laboratories have found that using plasma enables higher recovery of antibodies per donation and it is less contaminated with haemoglobin (Hb) than serum. Separation of plasma from anticoagulated blood is much faster than separation of serum from clotted blood. Plasma for fractionation can be obtained either from the collection of whole blood or by the apheresis procedure. [25]

From healthy immunized horses with respect to immunization and bleeding schedule withdraw 1.5% of body weight blood quantity from each animal in the sterilized glass bottles which contains anticoagulant solution, and kept the bottles in cold room at for settling of the cells at (5±3) °C overnight. The supernatant plasma is siphoned into another sterile bottle and stored in cold room till processed. Diethyl ether and phenol are added as preservative.

Enzymatic digestion of plasma

Antirabies prepared from the starting plasma pool to obtain one of the active substance F(ab)2 fragments. In general, fractionation procedures should not impair the neutralizing activity of antibodies; they should yield a product of acceptable physicochemical characteristics and purity with a low content of protein aggregates, which is non-pyrogenic and which should provide good recovery of antibody activity. [25]

The method of pepsin digestion involves the digestion of horse plasma proteins by pepsin, leading to the degradation of many non-IgG proteins, and to the cleavage of IgG into bivalent F(ab.)2 fragments by removal and digestion of the Fc fragment into small peptides.[26]

The hyper immunized plasma is maintained at room temperature (22°C to 25°C) and diluted using WFI (Water for Injection). The pH was adjusted to 3.2± 0.2 with5M hydrochloric acid. At this pH and temperature, the proteolytic treatment with pepsin is carried out. The 0.1% w/v pepsin added for digestion under constant stirring for 3 hours at 30-37°C for complete action of pepsin on IgG. The digestion was stopped by adding 10% v/v solution of sodium hydroxide and pH adjusted to 5.5± 0.3.

Caprylic acid precipitation

Caprylic acid fractionation allows the production of antivenoms of relatively high purity and with a low protein aggregate content, because the immunoglobulins are not precipitated during the process. The yield may reach up to 60–75% of the activity in the starting plasma, depending upon the particular procedure and/or the equipment used. The effectiveness and safety profiles of caprylic acid-fractionated antivenom immunoglobulins have been demonstrated in clinical trials. [28, 29, 30]

The caprylic acid was added very carefully and slowly with constant stirring for 1 hour at 22° C to 25° C at variable strength to reach final concentration of 3% v/v and the mixture was centrifuged at 4400 rpm for 30 minute.

Ultrafiltration

The slurry is micro filtered and the filtrate thus obtained is subjected to ultrafiltration. This results in removal of ammonium sulphate and also in concentration of filtrate by a factor of ten. The ultra filtered antirabies serum thus obtained is described as one Lot. Based on the appropriate titer requirements in the finished product, lots are pooled together, diluted, and sterile filtered.

During formulation after diafiltration steps the addition of salts to adjust the osmolality, addition of preservatives, other excipients, if needed for protein stability, and the adjustment of pH7.0± 0.5 to improve stability and/or to reduce aggregate formation. Formulation at a pH higher than 7.5 may not be recommended, since the stability of immunoglobulins and their fragments at alkaline pH may be poor, and the formation of aggregates may be favoured.[25]

QUALITY CONTROL

Potency of antirabies serum

The different methods by which the antirabies antibodies in antirabies serum can be quantified are described as under:

Virus neutralization test using mice

This test is a WHO approved test for antirabies serum because it uses a large number of mice, it is not encouraged either by WHO or OIE. This test forms a part of Pharmacopoeial requirement for antirabies serum testing in countries like India. A constant dose of challenge virus (CVS) is mixed with a series of different dilutions of the antirabies serum under test and also with a reference serum standard which can be either a national or international standard antirabies serum. This antigen-antibody mixture is inoculated intracerebrally, in a set of mice (Swiss albino). The basis of the test is that after neutralization with the serum, the remaining free virus (if any) causes the death of the mice. [31, 32]

Enzyme-linked Immunosorbent assay (ELISA)

Enzyme-linked Immunosorbent assay is a diagnostic method used in detecting the presence of antibody or an antigen in a particular sample. The basic principle of an ELISA is to use an enzyme-tagged antibody to detect the antigen (Ag) antibody (Ab) reaction. The use of corresponding chromogenic substrate easily indicates antigen (Ag) antibody (Ab) reaction. An ELISA can be used to detect either the presence of antigens or antibodies in a sample, depending on design of the test. Commercial kits for testing of antirabies serum antibodies in human and dogs or cats are available. The ELISA test is acceptable as a prescribed test by OIE, provided that it has been validated and adopted on the OIE register. Virus neutralization tests may be used as confirmatory tests if desired. [33, 34]

Protein content

The protein concentration of sample determined UV-spectrometrically at 550nm using standard Biuret method. The Biuret reaction, in which protein forms a complex with copper (Cu^{2+}) in alkaline solution, has become the standard chemical test for total serum or plasma protein. This complex, which is dependent on the presence of peptide bonds, is blue-purple in color. The Biuret method is highly accurate for the range of total protein found in serum (1 to 10 g/dl, 10 to 100 g/liter). [35, 36, 37]

Phenol content

Phenol content of samples determined UV-Vis spectrometrically at 450nm using 4-aminoantipyrine and dilute potassium ferricyanide in alkaline condition. [38]

CONCLUSION

Since rabies frequently occurring and the disease is life threatening, affects all warm blooded mammals, and its occurs is worldwide mostly in developing country.Post-bite treatment involves both vaccination using rabies tissue cultured antigen (active immunization) as well as administration of Antirabies immunoglobulin (passive immunization). Simultaneous administration of rabies immunoglobulin (antirabies serum) constitutes the best approach. Human rabies immunoglobulin is expensive five times that of equine rabies immunoglobulin and available in only limited quantities, because of involvement of human volunteers.

Equine rabies immunoglobulin becomes the immunoglobulin of choice, especially for the developing countries, where the animal bite cases are very much higher leading to greater requirement of immunoglobulins. Equine rabies immunoglobulin can be made available in much larger quantities and also at much lesser cost. The technological developments in the processing of horse immunoglobulins, purification, and proteolytic treatment to immunoglobulins have rendered Equine rabies immunoglobulin less allergic and more stable.

FUTURE CHALLENGES

Rabies antiserum available in liquid form, it has shelf life only for two years and also requires storage condition 2°C to 8°C so it is also expensive to maintain storage condition. So the future challenge is to develop Lyophilized Rabies antiserum to maximize stability, to maintain biological activity, safety, and marketability.

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