CHAPTER 44

Production of antirabies serum of equine origin

T. Luksrijang,1 J. Wangsa2 & P. Phanuphak3

Introduction

Different types of equine antirabies immunoglobulin (ERG) have been produced using various immunogenic preparations, consisting usually of a combination of inactivated and fixed strains of rabies virus (1). A therapeutic antirabies immunoglobulin for human use is produced at the Queen Saovabha Memorial Institute (QMS), Bangkok, Thailand, by immunizing horses with a purified Vero cell rabies (PVR) vaccine. The animals are given a series of injections of the vaccine in increasing volumes. 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.

Method

Immunization schedule

Healthy male or female horses aged 4–12 years and weighing 350–450 kg are immunized as follows:

Day 0: 0.5 ml (one human dose) of PVR vaccine in 0.5 ml of complete Freund's adjuvant subcutaneously at one site.

Day 21, 28, 35 and 42: 1 ml (two human doses) of PVR vaccine adsorbed with 1 ml of 2%, bentonite subcutaneously at one site (4 injections).

Day 63, 70, 77, 84, 91, 98 and 105: 2 ml (four human doses) of PVR vaccine without adjuvant subcutaneously on both sides of the neck (14 injections).

Day 119: first bleeding.

From day 21 to day 56, serum samples are taken at weekly intervals and the antibody titre is determined by the rapid fluorescent focus inhibition test (RFFIT) or the mouse neutralization test (MNT) (see Chapters 15 and 47). Animals showing a titre of less than 70 IU/ml by day 56 are withdrawn from the donor population. With the above immunization schedule, it is usually possible to obtain antibody titres of 150 IU/ml or higher.

Bleeding of horses

The animals are bled from a jugular vein by plasmapheresis (3000 ml of whole blood is collected at each session) on days 119, 121 and 123. After 5 weeks, they

---

1 Assistant Director, Queen Saovabha Memorial Institute, Bangkok, Thailand.
2 Scientist, Serum Section, Queen Saovabha Memorial Institute, Bangkok, Thailand.
3 Deputy Director, Queen Saovabha Memorial Institute, Bangkok, Thailand.
LABORATORY TECHNIQUES IN RABIES

are given four booster injections of 2 ml (four human doses) of PVR vaccine at weekly intervals. The bleedings are repeated 14, 15 and 18 days after the last booster injection.

Concentration and purification of antirabies serum

The relatively large amounts of antirabies serum necessary for the protection of persons exposed to rabbits, as well as the risk of anaphylactic accidents and other reactions, have led to the development of a number of methods for preparing a purified, concentrated serum.

Protein fractionation was first attempted by Halbe in 1945 (2) using ammonium sulfate, and various other methods have since been described (3, 4). The method of fractionation and purification adopted at the Pasteur Institute, Paris, consists of two stages:

(i) enzymatic digestion of the proteins followed by precipitation with ammonium sulfate;
(ii) removal of the excess proteins by thermocoagulation (see also Chapters 45–47).

Whichever method is adopted, it is advisable to determine the final protein content of the purified serum and relate this to its protective titre (see Chapter 47). Paper electrophoresis should also be performed to check the fractionation of the proteins. In general, a concentrated purified serum with a titre of 120 IRU/ml should not contain more than 5% of total serum proteins.

Equivore hyperimmune plasma or serum with an antibody titre of 150 IRU/ml or higher can be easily concentrated and purified to give an ERIG with a titre of 200 IRU/ml (see Chapter 45).

Factors affecting the production of ERIG

Donor animals

The animals used must be carefully selected, as even for the same breed of horse the suitability of any particular animal for serum production varies according to its age, nutritional status, general health and immunization history. Antibody titres should be checked in donor animals at appropriate intervals (see above), and any animals that do not produce consistently high titres should be withdrawn from the donor population on day 56.

Type of rabies vaccine

Different techniques for the production of ERIG have used a variety of rabies vaccines to induce high antibody levels, including vaccines prepared from nerve tissue as well as those prepared from tissue culture. The former have been associated with the occurrence of anaphylactic and neuroparalytic reactions in vaccinated horses (T. Luukrajang et al., personal communication). No such reactions have been reported following immunization with PVR vaccine prepared according to the requirements for human rabies vaccines published by WHO (5, 6). Indeed, recent data indicate that PVR vaccine is probably more immuno-
genic in humans than other cell-culture vaccines of equal potency (P. Khawplod et al., personal communication).

**Use of vaccines containing adjuvants**

Vaccine adjuvants of various types have been used in different institutions. It has not yet been established whether adjuvants are necessary. Studies are also needed to determine whether non-ulcerogenic adjuvants such as aluminium hydroxide or bemontite are as effective in inducing high antibody titres as complete Freund's adjuvant, which often causes severe local reactions. Studies have shown that aluminium hydroxide mixed with PVR vaccine and used for the intramuscular vaccination of humans will enhance the antibody response significantly (7).

**Vaccination schedules**

Similarly, controlled studies are also required to determine the optimal schedule for vaccinating horses in order to produce high titres of ERIG.

**Potency**

The RFFIT and the MNT are the most widely used potency tests for antirabies serum and immunoglobulin. They are described in Chapters 15 and 47.

When the potency of several lots of ERIG from different manufacturers was assayed at the Queen Saovabha Memorial Institute, wide variations in potency were found, ranging from 122% to 312% of the stated amount (H. Wilde et al., personal communication). Since high doses of ERIG may suppress the rabies virus-neutralizing antibody response in vaccinees, manufacturers must ensure that ERIG preparations are calibrated against the International Standard for Rabies Immunoglobulin (8) or a national reference preparation, and that the potency (in IU per ml) is clearly stated on each ampoule or vial. The leaflet accompanying the package should include information about the storage conditions and specify the expiry date of the product.

**Safety**

ERIG preparations from various manufacturers have been shown to induce serum sickness in some recipients. In several prospective studies of recipients, the incidence of serum sickness ranged from 0.32% to 6.19%, depending on the protein content of the ERIG (9, 10). Most cases were relatively minor and did not require alteration of the post-exposure treatment regimen. Nevertheless, the purification procedures used by different manufacturers appear to influence the incidence of adverse reactions (10). Although the reported rates are much lower than those associated with the first available preparations of ERIG (46%) (11), further studies are required to improve the safety of this product.

**References**

Laboratory Techniques in Rabies


