RAISING OF RABBIT HYPERIMMUNE SERUM FOR LABORATORY DIAGNOSIS OF PESTE DES PETITS RUMINANTS (PPR) VIRUS

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ABSTRACT

Agar gel immunodiffusion (AGID) test is a simple and rapid method for the diagnosis of Peste des petits ruminants (PPR) which requires the preparation of potent specific hyperimmune serum. This study deals with the raising of hyperimmune serum against PPR virus in rabbit. Four different inocula included PPR vaccine reconstituted in phosphate buffer saline, PPR antigen suspended in phosphate buffer saline, PPR vaccine in oil adjuvant and PPR antigen in oil adjuvant were used. All inocula were inoculated at different intervals and concluded that inoculum contained PPR antigen in oil adjuvant provoked better immune response against PPR virus in rabbits.

Key Words: Hyperimmune serum; PPR, Rabbits.

INTRODUCTION

Peste des petits ruminants (PPR) is an acute and highly contagious viral disease of small ruminants, which is characterized by high fever, anorexia, necrotic stomatitis, oculonasal discharge, diarrhoea and respiratory distress. Disease is of high economical important because of high mortality rate, especially among young animals and restriction on livestock tradings. This disease was firstly reported by Gargadennec and Lalanne (1942) in Ivory Coast. Morbidity and mortality rates in small ruminants vary but can be high as 100% (Lefevre *et al*., 1991; Anderson and Mckay, 1994). PPR is prevalent in most of Africa, the Middle East countries and in recent years, in India (Shaila *et al*., 1996) and Pakistan (Amjad *et al*., 1996).

Diagnosis of PPR is mainly based on routinely tests such as agar gel immunodiffusion (AGID), counter immunoelectrophoresis (Obi and Patrick, 1984), haemagglutination test (Wosu, 1991), immunocapture enzyme linked immunosorbent assay (Singh *et al*., 2004). Polymerase chain reaction (Forsyth and Barrett, 1995) or virus isolation (Brindha *et al*., 2001). Among these, agar gel immunodiffusion (AGID) is a simple, cheap and rapid test (White, 1958) that can be performed in any laboratory and even in field (OIE, 2004). The present study was aimed to raise the hyperimmune serum against PPR in rabbits which will be used as diagnostic tool in AGID.

MATERIALS AND METHODS

Virus: PPR virus Nigeria 75/I (PPR 75-1 LK 6 Vero 75) obtained from Centre de cooperation Internationale en recherche agronomique pour le developpement (CIRAD) France, was used as seed virus and from this, live attenuated PPR cell culture vaccine was prepared at Veterinary Research Institute, Lahore.

Cell Line: African green monkey kidney (Vero) cells were maintained in minimal essential medium supplemented with nystatin, penicillin and streptomycin sulphate and 10 % foetal calf serum.

Rabbits: Four Chinchilla rabbits over four month of age were used. The rabbits were dewormed, checked for faecal ova after 5 days deworming and found negative.

Preparation of PPR antigen: PPR antigen was prepared in laboratory as described in Manual on the diagunosis of rider pest (FAO, 1996). Attenuated PPR virus Nigeria 75/I was grown on African green monkey kidney (Vero) cells until cytopathic effects were observed through the cell sheet. The monolayer was then washed with phosphate buffer saline (PBS) and cell was scraped from the glass. The cells were disrupted to release antigen by suspending them in PBS and freeze and thawn several time at -20^{o}C.

Experimental Design: The experiment was conducted in two phases; in the first phase of the experiment two different inoculums were prepared as follow.

***Inoculum A*:** This was prepared by reconstituting the PPR vaccine (100 doses) in 10ml phosphate buffer saline (PBS).

***Inoculum B*:** Inoculum B was prepared by suspending PPR antigen (which was prepared by infecting two 162 cm^2 tissue culture with PPR virus Nigeria 75/I) in 10ml PBS. The rabbits were randomly divided into two groups i.e. group A and B (each group comprising of two rabbits). Inoculum A and B were given to group A and B respectively according to schedule shown in Table-1.

Table-1 Inoculation schedule of inoculum A and B

Day of Injection	Quantity Of Inoculum	Rout of Administration
Zero day	1 ml	I/V
7 day	1ml	Intraperitoneally
11 day	2ml	Intraperitoneally
15 day	4ml	Intraperitoneally

The rabbits were bled after one and two weeks of the last injection and serum was collected for AGID test. In the second phase of the experiment two other inoculums were prepared;

***Inoculum C*:** Inuculum C was the PPR vaccine in oil adjuvant. This was prepared by reconstituting the one vial of PPR vaccine (100 doses) in 2.4ml of PBS, and mixed with 0.1 ml of Tween 80. This mixture was added to 2.5ml mixture of liquid Paraffin and Arlacel, which was prepared by mixing of nine parts of Liquid Paraffin with one part of Arlacel while stirring.

Inoculum D: This inoculum was the PPR antigen in oil adjuvant: It was prepared as inoculum C. The rabbits were randomly divided into two groups i.e. group C and D (each group comprising of two rabbits). Inoculum C and D were given to group C and D respectively according to schedule shown in Table-2.

Table-2 Inoculation schedule of inoculum C and D

Day of Injection	Quantity Of Inoculum	Rout Of Administration
Zero day	1 ml	Subcutaneous
21 day	1ml	Subcutaneous
35 day	2ml	Subcutaneous

The rabbits were bled after one two, three and four weeks of the last injection and serum was collected. The sera obtained from all the four groups were subjected to agar gel immunodiffusion test (AGID) to determine their antibody titre against PPR virus.

RESULTS AND DISCUSSION

Antibody production is a complex biological process and it is not always possible to follow any recommendations and guidelines outlined in the literature as procedure and protocols have to be modified depending on the antigen. For some purpose a single injection may be sufficient but in general higher antibody yield are obtained by administering a series of injections (Cruickshank *et al*. 1968).

In present study, sera obtained from all the groups were subjected to AGID test.

The sera collected from group A showed no precipitation line, the sera collected from group B showed very weak precipitation line, the sera collected from group C showed no precipitation line but the sera collected from group D (in which inoculum D was injected) showed strong precipitation line. This inoculum D which showed the strong precipitation line was diluted to different dilutions and subjected to AGID and showed the precipitation line up to 1:8 dilution. The sera were divided into aliquots and store in the freezer (-20^{o}C).

In group A and C, sera did not show precipitation line, which might be due to too much or too little antigen (Hanley *et al*. 1995) which may have induce tolerance rather than an active immune response for the given antigen. In group B and D weak and strong precipitation lines were observed respectively, this finding is in accordance with observation of Jenning (1995) who studied that antibody formation is enhanced by the use of certain adjuvant substances. The prolonged exposure of the antigen to the immune system enhanced the immune response by attracting and stimulating immune cells. Kaeberle (1986) has also observed that antigen plus an adjuvant greatly enhances the antibody titre compared with the antigen without adjuvant.

The present study has suggested that antigen containing adjuvant provided better immune response against PPR virus in rabbits.

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