Standardization of the Peste des Petits Ruminants (PPR) Haemagglutinin antigen for Haemagglutination–Inhibition Test

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Abstract


Samples of tissue culture Peste des Petits Ruminants (PPR) monospecific vaccine were incubated with equal volume of aluminium-magnesium silicate. The cultured PPR virus which were haemagglutination negative became haemagglutination positive following the incubation with AMS. The haemagglutination was in each case inhibited by a known PPR antiserum. It has been concluded that incubation with AMS can be adopted to convert cultured PPR virus to standard PPR haemagglutinin antigen for use in haemagglutination inhibition test to confirm diagnosis of PPR.

Key words: PPR virus, HA antigen, HI test, aluminium magnesium silicate.

Introduction

Peste des Petits Ruminants (PPR) is considered to be the most important cause of morbidity and mortality among sheep and goats in West Africa, Asia and in the Middle East (Ozkul et al., 2002; Abdollahpour et al., 2006). Ramachadran et al. (1993) reported that PPR could produce agglutination of RBC of some species but observed that the test has a major limitation because cultured PPR virus could not produce agglutination of any RBC making it unreliable to adopt the simple haemagglutination inhibition (HI) test for confirmation of PPR diagnosis in the field.

HI test is simple, cheap and yet reliable. Need exists, therefore, to standardize the commonly available PPR antigen for easy evaluation of efficacy of PPR vaccination efforts.

Aluminium-magnesium silicate (AMS) is a natural ore made of particles composed of thousands of submicroscopic platelets stacked in sandwich fashion. The faces of the platelets carry negative electrical charges while the edges have positive charges (VenkataKrishnan and Chenicoff, 1995). This property makes it...
possible for AMS to stick to cells. Since AMS can adsorb to cells, it may be able to remove the cells and so free the viral haemagglutinins to attach to specific red blood cells to produce haemagglutination. This study was conducted to test this hypothesis.

**Materials and Methods**

Tissue cultured monospecific PPR Vaccines (Nigeria 75/1) from the National Veterinary Research Institute, Vom Nigeria were reconstituted as recommended for use in vaccinating sheep and goats. One milliliter of each reconstituted vaccine was mixed with 1 g of AMS (12%). The vaccine-chemical mixtures were incubated at room temperature for one hour before they were centrifuged at 3000 rpm for 10 min. The supernatants were used alongside portions of each of the vaccines which were not incubated with the chemical for haemagglutination test. Chicken red blood cells were used as indicator (Ramachandran et al., 1993).

A 0.6% chicken red blood cell concentration was prepared (Wosu, 1984). Briefly the HA test was done by dispensing 30 µl of phosphate buffered saline (PBS) of pH 6.8 into wells in rows of a microtitre plate (Coastex, USA). Then 30 µl of the two supernatants and of portion of the vaccines not incubated with any chemical were dispensed into different rows on the same plate and serially double diluted two fold. The 0.6% chicken RBC was added to each well in the three rows at the rate of 30 µl per well. RBC control and viral control were included in the protocol on the same plate.

The RBC control consisted of wells which contained only 30 µl PBS to which 30 µl of 0.6% chicken RBC was added. The virus controls consisted of the portions incubated with the chemical which were serially double diluted in different rows and equal volume of a known PPR anti-serum added to each well. The antigen-antiserum mixture were rocked to mix well and allowed to stand at room temperature for 45 min before 30 µl of the 0.6% chicken RBC was added to each well.

The whole set up was incubated at 4°C overnight. HA titres were read only if the known PPR anti-serum inhibited the haemagglutination by the antigens and if the RBCs in the RBC control wells settled to discrete bottoms.

Reciprocals of the highest dilution of each antigen which gave complete haemagglutination was recorded as it's HA titre.

**Results and Discussion**

All the PPR vaccines (Cultured virus) were haemagglutination negative. However portions of the same vaccines incubated with AMS became HA positive. When the incubation with AMS was repeated on each vaccine portion, the HA titres increased until it got to a maximum and then remained constant.

Wosu (1985) reported that cultured PPR virus could not produce haemagglutination of any species RBC. Ramachandran et al. (1993) confirmed failure of cultured PPR virus to agglutinate RBCs and observed that this was a major limitation to use of HA test for confirmation of diagnosis of PPR.

Incubating the cultured PPR viral samples with AMS to activate its haemagglutinin appears a simpler and cheaper process than the method used by

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0 = When not incubated with AMS; 1 = When incubated with AMS once; 2 = When incubated with AMS twice; 3 = When incubated with AMS thrice; 4 = When incubated with AMS four times
Ramachandran et al. (1993). The method may be easily applicable in less sophisticated laboratories as found in most parts of Africa, Asia and Middle East where PPR is presently a big problem.

The result of the present study suggests that PPR tissue cultured monospecific vaccine (Nigeria 75/1) which is confirmed PPR virus can be converted to a standard PPR haemagglutinin by incubating it with AMS. So HI test can be adopted for sero monitoring and surveillance of PPR.

References


