A SOLID PHASE AGGREGATION OF COATED ERYTHOCYTES (SPACE) TEST FOR THE DETECTION OF PESTE DES PETITS RUMINANTS (PPR) VIRUS ANTIGEN

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Abstract

A solid phase aggregation of coated erythrocytes (SPACE) test was developed for the diagnosis of peste des petits ruminants (PPR). A dilution of 1:2000 of PPR antiserum raised in rabbits was used in the test to detect antigen in clinical and postmortem samples obtained from goats experimentally infected with homogenates from field cases. PPRV antigen was demonstrated in 165 of 342 (48.2%) clinical samples and in 89 of 120 (74.2%) postmortem caprine tissues. Agar-gel precipitation test (AGPT) detected PPRV antigen in 40 of 61 (65.6%) clinical samples and in 22 of 45 (48.8%) postmortem tissues. The utility of the SPACE test as a diagnostic tool on the field is being investigated.

Key Word: SPACE Test, PRPV, Antigen, Nigeria

Introduction

Red blood cells have been coated with antigens or antibodies for use in immunological tests (Coombs, 1981) or for the study of immune-complex mediated diseases (Meryhew *et al*, 1990). The solid phase aggregation of coated erythrocytes (SPACE) test was evolved for use in microtitre plates to quantify antigens or antibodies by means of agglutination. It has been applied for the detection of rotavirus and rinderpest virus antigens (Bradburne *et al*, 1979; Bansal *et at*, 1987). It is necessary for the test that the coated erythrocytes are agglutinable with specific antibolies and do not spontaneously aggregate or lyse (Steinitz and Tamir, 1985).

We hereby report the application of this test for the detection of pests des petits ruminants (PPR) viral antigen in experimentally-infected goats.

Materials and methods

PPRV Anti-serum:

Hyperimmune serum against PPRV was raised in rabbits following serial inoculations as per the method of Scott and Brown (1961). This serum is monospecific to PPRV and was produced using vero cell-adapted, sucrose-gradient purified Nigerian PPRV isolate - NIG 75/1 (Obi and Ojeh, 1989).

Test samples:

342 clinical samples and 120 postmortem samples from twelve male kids experimentally infected with a proven PPR field virus, were used for the test. Some of the samples, especially the ante-mortem ones, were kept at 4°C for up to two months before being tested. Uninfected goat fetus tissues and a PPR positive lung homogenate were used as negative and positive controls respectively.

Purification of immnoglobulins:

The caprylic acid method of IgG isolation and purification was carried out on the rabbit anti-PPR hyperimmune serum as per Russ *et al* (1983).

Coating of erythrocytes:

This was as per the method of Bansal *et al* (1987). Sheep blood was collected in Alsever's solution and kept at 4°C for two days before washing three times with phosphate buffered saline (PBS, pH 7.4) at room temperature. 0.9ml of purified immunoglobulin (corre-

Coating of erythrocytes:

This was as per the method of Bansal *et al* (1987). Sheep blood was collected in Alsever's solution and kept at 4°C for two days before washing three times with phosphate buffered saline (PBS, pH 7.4) at room temperature. 0.9ml of purified immunoglobulin (corresponding to 5 mg/ml of IgG) was mixed with 0.5 ml washed erythrocytes (rbc). 1.0ml of 0.4% glutaraldehyde and 2.0ml PBS were added to the mixture, mixed gently but thoroughly, and incubated at room temperature for two hours. After this, 1.0ml of 0.2M L-lysine monohydrochloride in physiological saline solution (PSS) was added, and kept at 4°C overnight.

The erythrocyte suspension was then washed three times, with five minute intervals between each washing, resuspended at 1.0% concentration in PBS and kept at 4°C prior to use.

The test:

A 1:2000 dilution of the PPRV hyperimmune serum was prepared in carbonate-bicarbonate buffer (pH 9.6) and used to sensitise the round bottom haemagglutination plates, and left for two hours at room temperature, followed by four washings in PBS-Tween. 100μl of serial 2-fold dilution of samples in PBS were added to the wells plus 100μl of 0.5% suspension of sensitised RBC. The plates were incubated at room temeterature for two hours and thereafter read.

Some of the samples that were available were tested in a standard agar-gel preciptation test (AGPT) (Scott and Brown, 1961).

Results

The SPACE test detected PPRV antigen in 165 out of 342 clinical samples (48.2%) and in 89 out of 120 post-mortem samples (74.2%). The highest dilution factor picked in the test run to indicate a positive sample was 1:4 using a control positive. This was due to the prozone effect in the negative control in which [illegible] dilution was shown to be positive.

The AGPT detected antigen in 40 out of 61 (65.6%) clinical samples and in 22 out of 45 (48.8%) postmortem samples. Of these same samples used for the AGPT, SPACE detected 38 (62.3%) and 41 (91.1%) respectivitely.

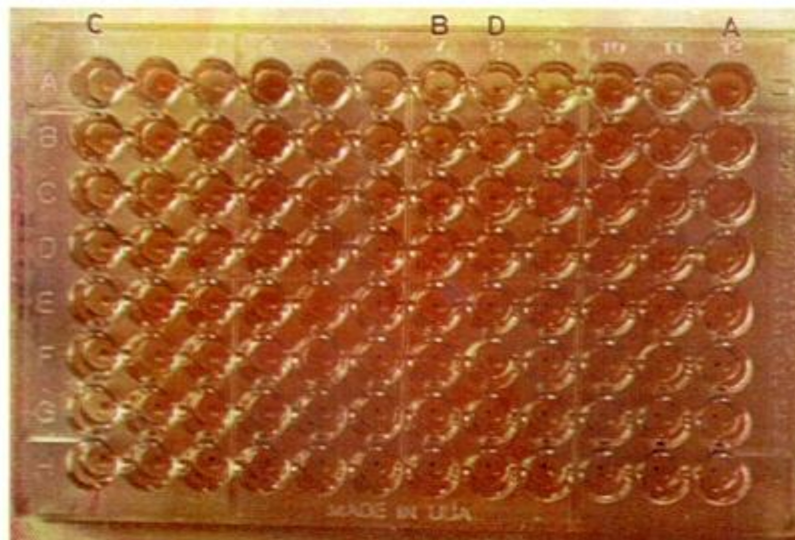

Plate 1:A SPACE Test in Round-bottom Microtire Plate

A - Positive Control

B - Negative Control

C - Positive Sample

D - Negative Sample

Table I: Results of examination of samples from PPR infected goats by AGPT and space tests.

Samples	AGPT NO. Positive/No. tested	SPACE No. Positive/ No. Tested
Antemortem		
Ocular swabs	11/15	32/81
Buccal Scrapings	6/11	41/67
Nasal swabs	13/18	30/73
Rectal swabs	6/9	42/77
Lymph node biopsies	4/8	20/44
	40/61 (65.6%)	165/342 (48.2%)
Postmortem		
Tonsils	1/3	6/12
Trachea	2/6	11/12
Lymph nodes	4/7	9/12
Lungs	2/3	11/12
Liver	2/5	7/12
Kidney	1/3	7/12
Spleen	3/5	9/12
Abomasum	3/3	10/12
Intestines	2/4	9/12
Rectum	2/6	10/12
	22/45 (48.9%)	89/120 (74.2%)

Discussion

The SPACE test has been adapted for the detection of PPRV antigens in experimentally infected goats. All twelve goats proved positive to PPRV as examined by the AGPT and SPACE tests.

The fact that SPACE could detect antigens in PPRV-infected tissues after eight weeks of storage of such tissues in PBS at 4°C and the fact that it could detect positive in 74.2% of the tissue samples and 48.2% of the clinical samples might earn it a place in the array of diagnostic tests for both antemortem and postmortem detection of PPR. The failure of the test to detect infection in 51.8% of the clinical samples may suggest that some of the samples were obtained prior to the establishment of the virus; that there are fewer antigens in some of the samples and that samples were still obtained after the shedding of the virus has stopped. Failure to detect infection in 25.8% of the tissues also suggests fewer antigens in the tissues or their inaccessibility or non-recognition by the detecting immunoglobulin-coated erythrocytes.

The test was simple to perform, for large sample sizes could be tested in a day; no bulky equipment or hazardous materials are used; colour visualisation is simple and recognisable and the plates are easy to handle and store.

It now remains to evaluate the true value of a diagnostic SPACE test for PPR in appropriate control trials such as in a comparison of different diagnostic methods for PPRV antigens in cell cultures; in differentiating between PPRV and rinderpest virus infections, and in detecting antigens after prolonged periods of storage of samples after natural and experimental infections.

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