

# Application of a precipitinogen inhibition test in the detection of antibody to peste des petits ruminants virus

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DUROJAIYE (O. A.). Application d'un test d'inhibition de la précipitation en gélose dans la détection de l'anticorps de la peste des petits ruminants. *Rev. Elev. Méd. vét. Pays trop.*, 1987, 40 (1): 17-20.

L'anticorps au virus de la peste des petits ruminants a été détecté dans les sérums d'une chèvre convalescente grâce au test d'inhibition de la précipitation en gélose. Une corrélation de 70 p. 100 est apparue entre les résultats obtenus par ce test et ceux obtenus avec le test de séroneutralisation. Le test d'inhibition de la précipitation en gélose peut donc être d'une aide précieuse dans le dépistage des anticorps peste des petits ruminants des sérums, pour les laboratoires ne pouvant pas appliquer le test de séroneutralisation. *Mots clés* : Chèvre - Peste des petits ruminants - Anticorps - Technique immunologique - Nigeria.

## INTRODUCTION

Peste des petits ruminants (PPR) is an acute, contagious virus disease of small ruminants characterised by stomatitis, enteritis and pneumonia. Laboratory confirmation of this disease has been based on virus isolation (4, 5) and the detection of diffusible precipitinogen in affected organs of sheep and goats (5, 2). In most cases, sera are tested for PPR antibodies by the serum neutralisation test (SNT). Unfortunately, many laboratories in developing countries do not have facilities required for the SNT (1). Subsequently, a test that can be performed under minimal laboratory conditions is desirable (1).

The principle of the precipitinogen inhibition test (P.I.T.) is based on the ability of antibody in serum to inhibit diffusible virus antigen (precipitinogen) from developing a precipitin line against hyperimmune serum in a normal Ouchterlony agar gel precipitation test (AGPT). YEDLOUTSCHNIG and STONE (8) applied this test for the detection of rinderpest antibody in bovine sera. The suitability of P.I.T. for the detection of antibody to *peste des petits ruminants* virus was investigated and result forms the basis of this report.

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## MATERIALS AND METHODS

### Precipitinogen Inhibition test

#### Precipitinogen

The precipitinogen (PPR virus antigen) was prepared as previously described by DUROJAIYE and TAYLOR (3).

#### Sera

Sera were collected randomly from abattoir goats in Plateau State, Nigeria. The sera were stored at -20 °C until tested.

20 µl of test serum was dispensed into each of six sterile Dram Bijou bottles lined on a tube rack. 20 µl of standard precipitinogen (virus antigen) was dispensed into the serum in the first bottle to make a 1 in 2 dilution of precipitinogen in serum. The above was thoroughly mixed and 20 µl was transferred into the serum in the next bottle to make a 1 in 4 dilution of precipitinogen in serum. This doubling dilution process was continued to the last bottle making a final 1 in 64 dilution of antigen in test serum.

Doubling dilutions of the precipitinogen in control normal sheep serum were carried out as described for the test serum above. The precipitinogen-serum mixtures were incubated at room temperature (25 °C) for 30-45 minutes. Each dilution of precipitinogen in serum was then tested against hyperimmune rinderpest serum in a normal AGPT.

### Agar gel precipitation test

Six ml of molten one per cent oxoid purified agar was dispensed into each 5 cm diameter petridish and allowed to set. Wells were punched in groups of seven in the agar (Fig. 1). Each well had a diameter of 5 mm, and a depth of 4 mm. In one group of wells, each peripheral well received 20 µl of one dilution of virus antigen in test serum. In the second group of wells,

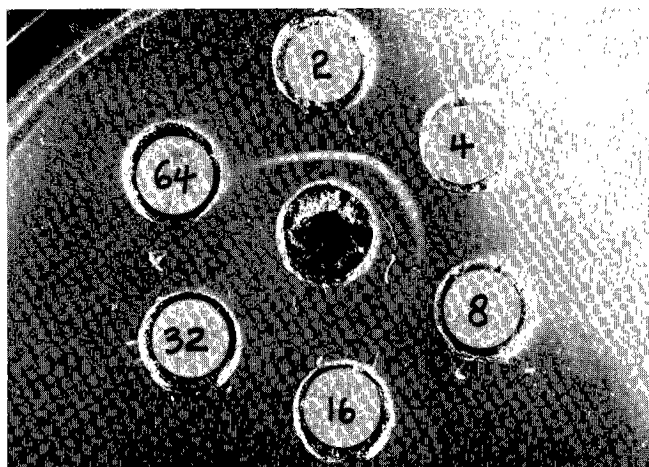


Fig. 1 : Titration of precipitinogen incubated in control normal sheep serum.

each peripheral well received 20  $\mu$ l of one dilution of virus antigen in control normal sheep serum. In both groups, the central well was filled with 20  $\mu$ l of hyperimmune rinderpest serum. The agar plate was incubated at room temperature in a humidified chamber and examined for precipitin lines after 16-24 h.

The final dilution of precipitinogen which reacted with the hyperimmune serum to form a precipitin line was regarded as the titre of the precipitinogen. The titre of precipitinogen incubated in normal serum (TC) was compared to the titre of that incubated in test serum (TT) and the ratio of the reciprocal of both titres TC/TT was regarded as the inhibition index. An inhibition index of 2 may suggest the presence of antibodies in a test serum but in order to reduce the risk of non-specific reactions, test sera with inhibition indices  $\geq 4$  were scored positive and those with inhibition indices  $< 4$  were scored as negative.

### Serum Neutralisation Test (SNT)

Procedure adopted for SNT was as described by

TABLE I Survey of PPR antibody in sera of goats by the use of the precipitinogen Inhibition test.

Serum Batch	No. of Sera Tested	No. of sera positive		
		P.I.T.	S.N.T.	Both P.I.T. and S.N.T.
1	15	12	12	10
2	24	17	8	7
3	21	5	8	3
4	27	2	2	1
5	31	3	3	2
Total	118	39	33	23

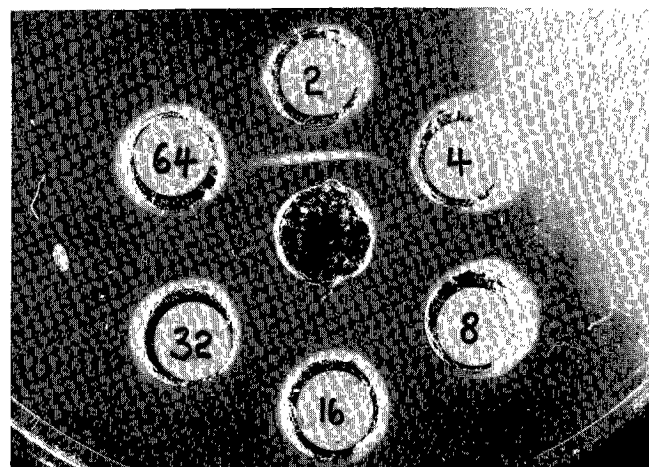


Fig. 2 : Titration of precipitinogen incubated in immune test serum.

TAYLOR (6).

## RESULTS

When the precipitinogen was incubated in some of the test sera, there was four-fold or eight-fold reduction in its titre compared to the titre obtained when the precipitinogen was incubated in normal serum. In Fig. 1, for example, the titre of precipitinogen incubated in normal serum was estimated to be 1:32. When the precipitinogen was incubated in an immune test serum, the titre was 1:8. This showed a four-fold reduction in the titre of the precipitinogen. Thirty-nine (33 p. 100) of the 118 sera tested by PIT were positive (Table I). The maximum inhibition index obtained was 8 (Table II). In comparison, 33 (28 p. 100) of the serum samples were positive by the serum neutralisation test. 23 (19.5 p. 100) were positive by both the PIT and the SNT.

TABLE II Inhibition Indices of positive sera in the precipitinogen Inhibition test.

Serum Batch	No. Tested	No. Positive	No. with Inhibition Indices of	
			4	8
1	15	12	4	8
2	24	17	8	9
3	21	5	5	0
4	27	2	2	0
5	31	3	3	0
Total	118	39	22	17

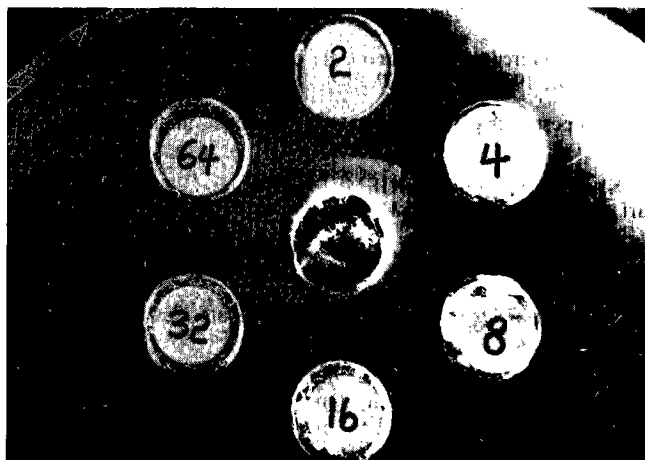


Fig. 3 : Titration of precipitinogen incubated in normal sheep serum. Titre estimated to be 1:8.

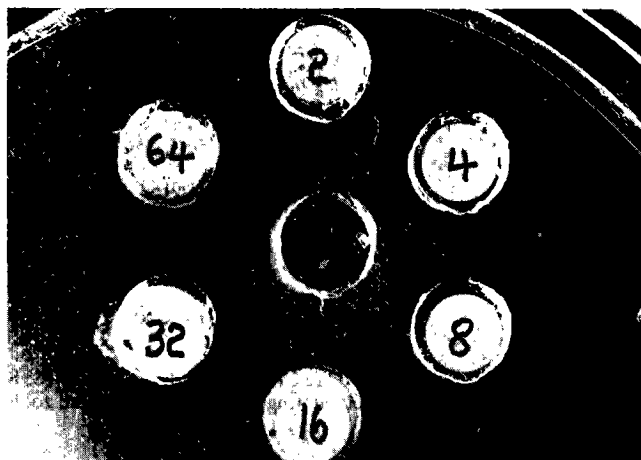


Fig. 4 : Titration of precipitinogen incubated in immune test serum showing complete inhibition of precipitinogen evidenced by absence of precipitin lines.

## DISCUSSION

The precipitinogen Inhibition test was found useful in the detection of PPR antibodies in convalescent sera. PIT is therefore potentially useful in screening field sera for PPR antibodies. This is of practical importance in laboratories ill-equipped for the performance of the SNT. It was observed that more positives were

found with this test (33 p. 100) than with the neutralisation test (28 p. 100). Similar observations were made by YEDLOUTSCHNIG and STONE (8) in convalescent rinderpest sera. This observation may be due to the fact that the PIT and SNT measure different types of antibodies and therefore may have different types of sensitivities in the detection of these antibodies. It is recommended that in critical cases, sera screened by PIT should also be examined by the SNT at the earliest opportunity.

**DUROJAIYE (O. A.).** Application of a precipitinogen inhibition test in the detection of antibody to peste des petits ruminants virus. *Rev. Elev. Méd. vét. Pays trop.*, 1987, **40** (1): 17-20.

Antibody to the virus of peste des petits ruminants was detected in convalescent goat sera by means of a precipitinogen Inhibition Test. There was a 70 p. 100 correlation between results obtained with this test and those obtained with the serum neutralisation test. The precipitinogen inhibition test can be valuable in the screening of sera for antibodies to peste des petits ruminants in laboratories lacking facilities for performing serum neutralisation test. *Key words* : Goat - Peste des petits ruminants - Antibody - Immunological technique - Nigeria.

**DUROJAIYE (O. A.).** Aplicación de una prueba de inhibición de la precipitación en gelosa para la detección del anticuerpo de la peste de los pequeños rumiantes. *Rev. Elev. Méd. vét. Pays trop.*, 1987, **40** (1): 17-20.

Se evidenció el anticuerpo al virus de la peste de los pequeños rumiantes en los sueros de una cabra convaleciente por medio de la prueba de inhibición de la precipitación en gelosa. Existe una correlación de 70 p. 100 entre los resultados obtenidos por esta prueba y los obtenidos con la prueba de seroneutralización. Puede ser muy útil la prueba de inhibición de la precipitación en gelosa para la puesta en evidencia de los anticuerpos peste de los pequeños rumiantes en los sueros por los laboratorios que no pueden utilizar la prueba de seroneutralización. *Palabras claves* : Cabra - Peste de los pequeños rumiantes - Anticuerpo - Técnica inmunológica - Nigeria.

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O. A. Durojaiye

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