

# Application of countercurrent immuno-electro-osmophoresis to the serology of peste des petits ruminants

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## RÉSUMÉ

DUROJAIYE (O. A.), TAYLOR (W. P.). — Application de l'électrosynérèse à la sérologie de la peste des petits ruminants. *Rev. Elev. Méd. vét. Pays trop.*, 1984, 37 (3) : 272-276.

L'électrosynérèse a été utilisée pour la détection des anticorps de la peste des petits ruminants dans les sérums récoltés sur le terrain et dans ceux de moutons et de chèvres infectés expérimentalement. Les anticorps ont également été détectés dans les sérums de deux moutons sur trois, quatre ans après que ceux-ci aient été inoculés avec le virus PPR. Des réactions croisées sérologiques entre le virus PPR, celui de la peste bovine et celui de la rougeole ont aussi été démontrées par cette technique. L'électrosynérèse paraît convenir pour un diagnostic sérologique rapide de la peste des petits ruminants.

*Mots-clés* : Electrosynérèse - Sérums - Peste des petits ruminants.

## SUMMARY

DUROJAIYE (O. A.), TAYLOR (W. P.). — Application of countercurrent immuno-electro-osmophoresis to the serology of peste des petits ruminants. *Rev. Elev. Méd. vét. Pays trop.*, 1984, 37 (3) : 272-276.

Countercurrent immuno-electro-osmophoresis has been used in the detection of antibody to peste des petits ruminants in field sera and in the sera of experimentally infected sheep and goats. Antibody was also detected in the sera of 2 out of 3 sheep four years after the sheep were inoculated with PPR virus. Serological cross reactivity between PPR virus and rinderpest and measles sera was also demonstrable by this technique. Countercurrent-immuno-electro-osmophoresis is considered suitable for rapid serology of peste des petits ruminants.

*Key words* : C.I.E.O.P. - P.P.R. - Sera.

## INTRODUCTION

Peste des petits ruminants (PPR) is an acute contagious virus disease of goats and sheep characterised by fever, oculo-nasal discharge, stomatitis, diarrhoea and pneumonia. The disease is considered to be of economic importance in West Africa (2, 4). The serum neutralisation test (SNT) is the most widely used in the testing of PPR antibodies in sera of affected animals (7). Although the SNT is

most desirable, it is time consuming and many laboratories in the developing countries are unable to apply the test due to the cost of maintenance of tissue cultures.

It has been shown that PPR antibodies in sera of naturally and experimentally infected goats can be detected by the Ouchterlony agar gel precipitation technique (AGPT) (3, 4). In this study, countercurrent-immuno-electro-osmophoresis (CIEOP) has been applied to the serology of PPR and the results obtained form the basis of this report.

## MATERIALS AND METHODS

### Preparation of virus antigen

Virus antigen was prepared from post mortem tissues of PPR affected goats such as lymph nodes, lungs and ileo-cæcum. A 1 in 3 w/v dilution of organ was macerated in phosphate buffered saline. The homogenate was centrifuged at 1 000 r.p.m. for 10-20 minutes and the supernatant was used as the virus antigen.

### Preparation of serum

Blood was obtained by jugular venipuncture by means of vacutainers and allowed to clot at room temperature. It was then incubated at 37 °C for 1 hour after which it was kept at 4 °C overnight. The supernatant serum was separated from the blood, centrifuged for 30 min. at 1 000 r.p.m. and stored at - 20 °C until tested.

Sera were collected from 6 experimentally infected goats and sheep and from village goats in different locations of Maiduguri and Riyom in Northern Nigeria. Sera collected from normal goats previously tested were used as negative controls.

### Experimental infection in sheep and goats

3 Dorset Horn sheep and 3 goats of British breeds were inoculated with Nig 75/1 strain of

PPR virus previously passaged 6 times in primary lamb kidney tissue culture. Serum samples were collected daily from the infected sheep and goats as from day 1 of infection (Table I).

### Preparation of agar

A 1.2 p. 100 (w/v) agar was prepared by boiling 1.2 g of oxoid agar in 98.8 ml 0.05 M barbitone acetate buffer (pH 8.6). Three ml of molten agar was layered on each microscopic slide and allowed to set. Six pairs of wells were punched in the agar on each slide and the distance between two wells of a pair was 6 mm while the distance between adjacent pairs of wells was 8 mm. The diameter of each well was 3.5 mm.

### Filling of wells

For each pair of wells, the well on the anode side was filled with 10 µl of serum while the well on the cathode side was filled with 10 µl of antigen.

### Procedure of electrophoresis

The electrophoresis bath (Shandon) was filled with 0.05 m barbitone acetate buffer and the agar slide containing the reactants was placed on a bridge in the apparatus. Both edges of the slide were connected to the buffer

TABLE N° 1 - Testing of PPR experimental transmission sera by C.I.E.O.P.

| Days post infection | A n i m a l n° : |        |        |         |         |         |
|---------------------|------------------|--------|--------|---------|---------|---------|
|                     | Goat 1           | Goat 2 | Goat 3 | Sheep 1 | Sheep 2 | Sheep 3 |
| 0                   | -                | -      | -      | -       | -       | -       |
| 1                   | -                | -      | -      | -       | -       | -       |
| 2                   | -                | -      | -      | -       | -       | -       |
| 3                   | -                | -      | +      | -       | -       | +       |
| 4                   | -                | -      | +      | -       | -       | +       |
| 5                   | -                | -      | +      | -       | -       | +       |
| 6                   | -                | -      | +      | -       | -       | +       |
| 7                   | +                | +      | +      | -       | -       | +       |
| 8                   | +                | +      | +      | +       | +       | +       |
| 9                   | +                | +      | +      | +       | +       | +       |
| 10                  | +                | +      | +      | +       | +       | +       |
| 28                  | +                | +      | +      | +       | +       | +       |

Key : - = Negative ; + = Positive.

in the trough by means of porous paper such that current was able to flow freely through the agar on the slide. The trough was covered with its lid and the power pack was switched on.

Tests were run at 10 milliamps per slide at room temperature for 30-60 minutes after which the agar was examined for precipitin lines by means of a viewer.

### Serum neutralisation test (SNT)

SNT was carried out as described by TAYLOR (7).

## RESULTS

### Antibody development in experimentally infected sheep and goats

It was possible to monitor the development of PPR antibody in experimentally infected sheep and goats by the use of C.I.E.O.P. Antibody was detected as early as day 3-post infection in two of them and consistently from day 8 in all the rest (Table I).

Also, antibody was detected in the sera of 2 out of 3 sheep four years after they were experimentally infected with PPR virus.

### Detection of antibody in field sera

C.I.E.O.P. detected antibody in convalescent field sera obtained from Northern Nigeria. 82 (59.8 p. 100) of the 137 sera tested were found to be positive. The results obtained with the serum neutralisation test (SNT) is similar to those of C.I.E.O.P. to

some extent. Of the 80 sera that were positive by the C.I.E.O.P., 71 (88.8 p. 100) were positive by the SNT (Table II).

### Cross reactions of PPR virus antigen with heterologous sera

Precipitin lines were formed between PPR virus antigen and measles and rinderpest sera.

Results obtained with C.I.E.O.P. were proved to be specific in that there was no precipitation with standard normal sheep serum used as control. Also, precipitin lines withstood washing in phosphate buffered saline (PBS) for 3 days with daily changes of PBS and the precipitin lines were stained with coomassie Blue (Fig. 1).

## DISCUSSION

Counter-current — immuno-electro-osmophoresis has been shown to be suitable for the serology of peste des petits ruminants. One of the main advantages of the C.I.E.O.P. is its rapidity in producing results as precipitin lines were often visible after the test was run for thirty minutes. The C.I.E.O.P. is simple to perform, requiring very small quantities of reagents. Results obtained suggest that C.I.E.O.P. can be used for seroepidemiological studies as well as experimental studies on PPR.

PAN, DE BOER and HESS (6) found C.I.E.O.P. to be rapid and accurate in the detection of antibody in sera of pigs infected with african swine fever (ASF) virus. They also reckoned C.I.E.O.P. to be superior to complement fixation test and the AGPT in the

TABLE N° 2 - Survey of field sera for PPR antibody by C.I.E.O.P.

| Bath of Sera | N° Tested | Number Positive |        |                            |
|--------------|-----------|-----------------|--------|----------------------------|
|              |           | C.I.E.O.P.      | S.N.T. | Both C.I.E.O.P. and S.N.T. |
| 1            | 49        | 17              | 13     | 13                         |
| 2            | 52        | 31              | 29     | 26                         |
| 3            | 15        | 14              | 15     | 14                         |
| 4            | 21        | 18              | 21     | 18                         |
| Total        | 137       | 80              | 78     | 71                         |

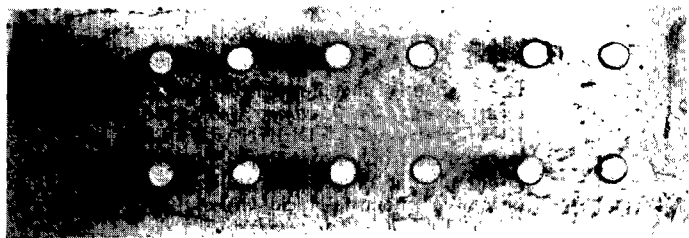


Fig. 1a. — C.I.E.O.P. slide stained with Coomassie blue showing precipitin lines in positive samples.



Fig. 1b. — Close up of an unstained precipitin line between a pair of wells.

detection of ASF antibodies. HOEKSTRA and DEINHARDT (5) applied C.I.E.O.P. in the quantitation of group specific antigen and antibodies of C. Type ribonucleic acid leukemia and sarcoma viruses. Similarly, BERLIN and PIROJBOOT (1) found C.I.E.O.P. to be useful in the rapid detection of antibody to influenza virus. The rapidity, simplicity and sensitivity of the C.I.E.O.P. make it a suitable technique in serological studies of many virus diseases including PPR.

## CONCLUSIONS

1. Counter current immuno-electro-osmophoresis has been used in the detection of

antibody to peste des petits ruminants in the sera of naturally affected and experimentally infected sheep and goats.

2. C.I.E.O.P. has also been used to demonstrate cross reactivity between measles and rinderpest sera and PPR virus.

3. Results obtained with C.I.E.O.P. were similar to those obtained with the serum neutralisation test to some extent.

4. C.I.E.O.P. is considered suitable for serological studies on PPR.

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## RESUMEN

DUROJAIYE (O. A.), TAYLOR (W. P.). — Aplicación de la inmuno-electro-osmoforesis en la serología de la peste de los pequeños rumiantes. *Rev. Elev. Méd. vét. Pays trop.*, 1984, 37 (4) : 272-276.

Se utilizó la inmuno-electro-osmoforesis para descubrir anticuerpos de la peste de los pequeños rumiantes en los sueros recogidos sobre terreno y en los de ovejas y cabras experimentalmente infectadas.

Se observaron también anticuerpos en los sueros de dos ovejas de tres, cuatro años después de la inoculación con el virus PPR. Se demostraron también por dicha técnica reacciones cruzadas serológicas entre el virus PPR, el de la peste bovina y el del sarampión. Parece que se puede utilizar la inmuno-electro-osmoforesis para un diagnóstico rápido de la peste de los pequeños rumiantes.

*Palabras claves* : Electrosinerosis - Sueros - Peste de los pequeños rumiantes.

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