Retour au menu

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Rapid diagnosis of *peste des petits ruminants* (PPR) infection, application of immunoelectroosmophoresis (IEOP) technique

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RÉSUMÉ

MAJIYAGBE (K. A.), NAWATHE (D. R.), ABEGUNDE (A.). — Diagnostic rapide de la peste des petits ruminants (PPR) par la technique d'électrosynérèse. *Rev. Elev. Méd. vét. Pays trop.*, 1984, **37** (1) : 11-15.

La technique d'électrosynérèse sur lame appliquée au diagnostic de la PPR est décrite. Elle se révèle plus sensible que l'immunodiffusion en gélose pour détecter les antigènes solubles ou la séroneutralisation pour la recherche des anticorps. Elle présente aussi l'avantage d'être rapide (2 à 3 heures) et de ne pas nécessiter de manipulations stériles. Comme pour les autres techniques, il existe une réaction croisée avec la peste bovine.

Mots clés : Peste des petits ruminants - Diagnostic - Electrosynérèse.

SUMMARY

MAJIYAGBE (K. A.), NAWATHE (D. R.), ABEGUNDE (A.). — Rapid diagnosis of *peste des petits ruminants* (PPR) infection, application of immunoelectro-osmophoresis (IEOP) technique. *Rev. Elev. Méd. vét. Pays trop.*, 1984, **34** (1) : 11-15.

A new diagnostic protocol for PPR infection using the IEOP technique is here described. The IEOP test has been compared with currently used routine diagnostic methods for PPR like the agar gel immunodiffusion, serum neutralization and virus isolation and identification. The IEOP test has proved to be a rapid and reliable tool for PPR diagnosis. Results can be obtained within 2-3 hours. Epidemiologic survey carried out using this new test confirmed PPR is wide spread in the country as well as a high survival rate amongst infected animals.

Key words : Peste des petits ruminants - Diagnosis - Immunoelectro-osmophoresis.

« *La Peste des petits ruminants* (PPR) » is a viral disease of sheep and goats resembling rinderpest clinically. The disease is generally more severe in goats in which it causes high mortalities especially in the humid south of West Africa.

The importance of PPR as a major economic disease in West African sheep and goats has been established (3). Whatever control measure is designed, the importance of accurate diagnosis of infection in new, as well as in already known endemic areas to the success of such a control measure cannot be taken for granted. This becomes more important as it has been reported that PPR might not be restricted to West Africa as previously thought. There is the strong possibility that PPR occurs in the Sultanate of Oman (4) and Sudan (W. P. Taylor-Personal communication). The diversity of symptoms and the epidemiologic picture of the disease was designated by its clinical observations in different geographical areas and might have contributed in no small measure to the confused nomenclature used in different countries.

Currently used diagnostic procedures for PPR include the agar gel precipitin test (AGPT) for detection of PPR antigen in lymph nodes and other tissues, complement fixation test (CFT), direct immunofluorescent test (DIFT), animal inoculation, virus isolation and identification, serum neutralization test (SNT) and the measle hemagglutination inhibition test (MHIT) (1, 6, 8). Of these, the AGPT, SNT and virus isolation and identification are the routinely used methods for

PPR diagnosis; with the exception of AGPT whose reliability is not 100 p. 100, the SNT and virus isolation are only meaningful when positive results are obtained in addition to their being laborious and time-consuming.

There is, therefore need for a more rapid, easy and reliable diagnostic protocol for PPR. This presentation deals with the application of the IEOP technique in PPR diagnosis and its comparison with other routinely used diagnostic schedules.

MATERIALS AND METHODS

Test Antigens

Caprine lymph node antigen was extracted from lymph node (preferably mesenteric) of suspected and experimental cases. Extracts were prepared by squeezing out fluid out of the lymph nodes or prepared as previously described for rinderpest fluid antigen (9). Tissue culture antigens were prepared in Vero or Fetal Lamb Kidney (FLK) cell cultures using appropriately adapted PPR virus strains (Vom Nig. 75/1 or Eruwa 79) as earlier reported (5). Control antigens were similarly prepared from normal caprine lymph nodes or uninfected tissue culture cells.

Test Sera

These consisted of samples obtained from abatoirs or during suspected field outbreaks. In addition, known positive and negative PPR sera from the Institute flock were included in the test. As further control, hyperimmune and convalescent rinderpest bovine sera were also used to monitor the tests.

Immunoelectroosmophoresis (IEOP) Test

The gel used was agarose (sigma, medium EEO, type II). The tank buffer was a barbital sodium-sodium acetate buffer, pH 8.6, 0.1 M containing 2 gm sodium azide per liter. The gels where made into 0.8 p. 100 (W/V) solutions using as solvent 0.025 M tank buffer. The IEOP protocol was essentially as described previously with some modifications (5, 7). A standard horizontal commercial electrophoresis tank with power supply capable of delivering constant voltage or current was used. Greaseless, pre-cleaned and labelled 76 × 26 mm microscope slides were precoated with agar film. Eight mls of gel was layered on to the precoated slide supported on a levelling table capable of holding from one to 16 slides. Six parallel rows of wells were cut along the major axis of the slide, pairs of wells along this axis were 5 mm apart. Each well was 3 mm in diameter while the wells in each pair were 5 mm apart.

Test sera were placed in wells at the anode side while the antigen was at the cathode side. Each set of tests included appropriate negative and positive controls. The filled slides were placed on a support inside the tank containing cooled buffer and were connected with the buffer by means of a strip of filter paper. The electrophoresis was performed at room temperature for 45 to 60 minutes at a constant voltage of 10 volts per cm of gel. Current reading was usually stable at 3-10 milliamps (ma) when a single slide was being run or 15 to 40 ma when the full complement of 5 slides were run at once. The slides were examined for precipitation lines and results recorded immediately after the run. They were then placed in trays or petri dishes containing phosphate buffered saline (PBS, calcium and magnesium free) pH 7.6 for about 2 hours, read again and results compared with previous one. If staining was required, the slides were left overnight in PBS, then in distilled water for 6 hours before drying. This was achieved by covering the slide with a lint-free filter paper soaked with distilled water, and placing on a rack to allow to dry overnight at room temperature. The thin sheet of dried gel was stained for 5 minutes in 0.5 p. 100 Naphthalene Black 12 B or Coomassic Brilliant Blue (R250) followed by clearing in a mixture of 7.5 p. 100 glacial acetic acid and 50 p. 100 ethanol destainer. The slides were allowed to air dry at room temperature and stored as a permanent record.

PPR serum Neutralisation Test (SNT)

Some of the serum samples found positive by the IEOP test for PPR antibody were checked by SNT using 100 $TCID_{50}$ virus in FLK cell cultures.

Agar gel immunodiffusion (AGID) Test

Antigen preparations from experimental, suspected field samples or tissue culture were tested in the AGID test as previously described (9) using rabbit hyperimmune rinderpest serum.

Retour au menu

RESULTS

All the antigens prepared from experimental PPR and tissue cultures were positive in the AGID test. The same is true of samples examined from field cases except that in some cases, final confirmation was made after staining the washed and dried slides.

Both lymph node and tissue culture antigen preparations were used in evaluation of the IEOP test and the results correlated well with the AGID tests or virus isolation when attempted from known samples.

For detection of antibody in serum samples, standardised tissue culture antigen preparations were used. The antigen titres were 1 : 16 (Vero) and 1 : 32 (FLK), respectively in a IEOP checkerboard titration (Table I).

Serum samples from the Institute's flocks, from field outbreaks, and abbatoirs in various parts of the country were screened by the IEOP test. The results are summarised in table II.

The results of SNT on some IEOP positive sera are shown in table III. Out of a total of 42 IEOP positive sera, 35 (83 p. 100) were positive in the SNT, thus indicating a good measure of correlation between the two tests.

DISCUSSION

We have compared the IEOP test with present routine diagnostic methods for PPR i.e. AGID, SNT and virus isolation. Our results show that the IEOP is a useful and reliable tool for PPR diagnosis. It is 8 to 16 times more sensitive than the AGID, with the result that it can be employed for the detection of antigen as well as antibody. It is a fast method which gives results within 2 to 3 hours and does not require sterile conditions for manipulations.

The IEOP test will be highly adaptable for use in titration of PPR serum antibody, epidemiologic work as well as in monitoring the efficacy of control programmes.

As mentioned earlier, the IEOP results correspond with those of AGID and SNT results. However, the correlation with SNT is not 100 p. 100. The seven samples which were negative on SNT were weakly positive in the IEOP test.

There is therefore a strong possibility of incomplete neutralization of PPR virus during SNT and thus would be read as negative.

The IEOP test when used for PPR has one draw-back — the test is not specific for PPR alone as it cross-reacts with rinderpest virus (RV) (2, 9). It will therefore detect antigen and antibody due to RV. However, based on clinical and pathologic findings, a diagnosis of PPR can be made in conjunction with the result of the IEOP test without recourse to the laborious reciprocal cross neutralization test. Moreover, it has been observed that the number and intensity of the precipitin arcs are more for the homologous than the heterologous system (MAJIYAGBE, unpublished observation). This can also be used to distinguish between PPR and RV reaction in the IEOP test. The result of our limited country-wide survey confirms that PPR is wide-spread. The pattern of observation is similar to an earlier one reported (11). However, the percentages suggest an increase in the survival rate of infected animals

TABLE N°I-Result of IEOP checkboard titration of PPRV tissue culture antigen preparations

Serum dilution	Vero-PPRV antigen					FLK - PPRV antigen					
	1:2	1:4	1:8	1:16	1:32	1:2	1:4	1:8	1:16	1:32	1:64
Neat	+	+	±	+	-	+	+	+	+	+	-
1 : 2	+	+	+	+	-	+	+	+	+	+	-
1 : 4	+	+	+	+	-	+	+	+	+	+	-
1 : 8	±	±	+	+*	-	+	+	+	+	+*	-
1 : 16	-	-	±	-	-	±	±	+	+	+	+

+ = Positive (precipitin line formed) ; - = Negative (no precipitin line formed) ;
± = Partial positive ; * Antigen titres. Vero antigen = 16 units.
FLK antigen = 32 units. 4 units of antigen used in test.

Retour au menu

TABLE N°II-Serum samples screened by IEOP for PPRV antibody

Sera Source	Total N°	N° Positive	Percentage Positive	1977 Survey *
Mangu Mkt.	35	26	74.3	-
Vom	43	33	76.7	-
ILCA, Ibadan	14	8	57.1	-
Eruwa	45	17	37.7	-
Makurdi	35	24	68.6	-
Sokoto				
Goat	15	15	100	-
Sheep	24	24	100	-
Kaduna				
Goat	70	54	77.1	50.0
Sheep	52	32	61.5	66.7
Gumel	21	21	100	-
Katsina	16	16	100	-
Kano				
Goat	106	50	47.2	37.8
Sheep	101	48	47.5	47.2
Jos				
Goat	93	60	64.5	45.5
Sheep	21	16	76.2	62.5
Lake Chad Basin				
Village	14	14	100	-
Total	833	458	54.98	

* Data extracted from Taylor (1979).

TABLE N°III-Correlation between IEOP and SN tests for PPRV antibody

N° Sera tested	IEOP positive[a]	SNT positive[b]
10	10 (100.00)[d]	10 (100)
12	12 (100.00)	10 (83.3)
20	20 (100.00)	15 (75.0)
42	42[c] (100.00)	35 (83.3)

a = 4 units of PPRV - FLK antigen extract.
b = SNT done in Fetal Lamb Kidney cells culture using 10^{-2} virus dilution
c = 7 out of the 42 samples were positive only after staining IEOP slides
d = Numbers in brackets represent percent positives

especially in the face of repeated challenges in the field. The current use of TCRV in the field to protect young stock will further help in the control of PPR. The IEOP technique will be very suitable in monitoring the response of these vaccinated animals.

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RESUMEN

MAJIYAGBE (K. A.), NAWATHE (D. R.), ABEGUNDE (A.). — Diagnóstico rápido de la peste de los pequeños rumiantes por la técnica de electrosineresis. *Rev. Elev. Méd. vét. Pays trop.*, 1984, **37** (1) : 11-15.

Se describe la técnica de electrosineresis sobre lámina aplicada al diagóstico de la peste de los pequeños rumiantes. Es más sensible que la inmunodifusión en gelosa para descubrir los antigenos solubles o la seroneutralización para la búsqueda de los anticuerpos.

Tiene también la ventaja de ser rápida (2 a 3 horas) y de no necesitar manipulaciones esteriles.

Como con demás técnicas, existe una reacción cruzada con la peste bovina.

Palabras claves : Peste de los pequeños rumiantes - Diagnóstico - Electrosineresis.

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