The detection of peste des petits ruminants (PPR) virus antigen by agar gel precipitation test and counter-immunoelectrophoresis

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(*Received* 31 *January* 1984; *accepted* 10 *May* 1984)

SUMMARY

The detectability of peste des petits ruminants (PPR) viral antigen in both ante-mortem secretions and necropsy samples from experimentally infected goats was investigated by both the agar gel precipitation test (AGPT) and counter-immunoelectrophoresis (CIE). Viral antigen was detected from 42·6% of the samples tested by the AGPT and 80·3% by CIE. The detection of viral antigen in a high proportion of the ocular and nasal secretions as well as the faeces and buccal scrapings, particularly from those collected within seven days of the onset of fever, by both techniques, would seem to obviate the need for lymph node biopsies or post-mortem samples in order to make a diagnosis of PPRV infection.

INTRODUCTION

The diagnosis of peste des petits ruminants virus (PPRV) and rinderpest virus (RV) infections may be achieved by a combination of clinical signs and epidemiology, gross and microscopic pathological lesions, virus isolation and characterization and serological tests designed to detect virus antigen and/or antibody.

Whereas clinical and epidemiological diagnosis are perhaps not conclusive, virus isolation may be definitive but expensive and time-consuming, and requires expertise and skill that are not readily available in many developing countries including Nigeria. Many of the serological tests have similar disadvantages to those associated with virus isolation. It is not surprising therefore that the agar gel precipitation test (AGPT) is the most frequently used technique for the detection of RV antigen and has been used for PPRV antigen detection also (Appel *et al.* 1981). Recently, Ali & Lees (1979) and Rossiter & Mushi (1980) reported on the use of counter-immunoelectrophoresis (CIE) for the detection of RV antigen and Majiyagbe, Nawathe & Abegunde (1980) for PPRV antigen and antibody detection.

For the detection of RV antigen by both techniques, lymph node samples are obtained from dead or slaughtered animals or from lymph node biopsies (Scott, 1967). Gum scrapings from infected animals have also been used for the detection of RV antigen (Scott & Brown, 1961; Kataria, Shrivastava & Majundar, 1977;

Thanappa Pillai & Abdul Khader, 1982). Recently, Forman, Rowe & Taylor (1983) also described the use of ante-mortem secretions for the rapid detection of RV antigen. Although PPRV antigen has been detected in the lymph nodes, the lungs and intestinal tissues of goats (Taylor & Abegunde, 1979; Gibbs *et al.* 1979; Hamdy *et al.* 1976) its detectability in other necropsy tissues as well as in ante-mortem secretions has not, to the best of our knowledge, been investigated. The present study was aimed at investigating the distribution of PPRV precipitinogen in various necropsy tissues as well as the usefulness of ante-mortem secretions in the diagnosis of PPR by both the AGPT and CIE.

MATERIALS AND METHODS

Experimental animals

Eleven West African dwarf goats under one year of age and which had been shown to be free of PPRV antibody were used for the study. The goats were de-wormed with Panacur (Hoechst, Frankfurt) at the rate of 7·5 mg/kg body weight on arrival, housed together in a loose pen and fed on maize-based concentrate and 'acha' hay at the rate of 0·5 kg per kg body weight, while water was offered *ad libitum*. Each animal was inoculated with 5 ml of PPRV-infected tissue homogenate subcutaneously twelve days after the goats were acquired. Thereafter the animals were examined at 08.30 h daily for clinical response.

Inoculum

The inoculum consisted of pooled 20 % (w/v) tissue homogenate prepared from a dwarf goat which was experimentally infected with 2 ml Vero cell-adapted PPRV (NIG.75/1, Taylor & Abegunde, 1979) containing $10^{5\cdot3}$ TCID50/ml. The goat was sacrificed six days after the onset of fever and tissues collected from the lymph nodes, spleen, lungs and the intestine and then tested for PPRV antigen by the AGPT. Positive tissues were then used for the preparation of the inoculum and penicillin (100 units/ml), streptomycin (100 mg/ml) and fungizone (20 units/ml) were added to the inoculum.

Test samples

Conjunctival, nasal and faecal swabs and buccal scrapings were obtained from the goats on days 4, 5, 7 and 10 after the onset of fever using sterile cotton-wool swabs (Medical Wire and Equipment Co. Ltd, Wilts, U.K.) or a spatula in the case of buccal scrapings. The samples were suspended in 150 μl of phosphate-buffered saline, pH 7·2 (PBS) and then stored at −20 °C until examined. During post-mortem examination of goats that either died or were slaughtered, tissues were collected from trachea, lungs, mediastinal, pre-scapular and mesenteric lymph nodes, tonsils, liver, kidney, spleen, testis, urinary bladder, small intestine, colon, caecum and rectum. The tissues were extracted as 20 % (w/v) homogenates in PBS and then stored at −20 °C until they were examined.

Antigen

For the preparation of the PPRV positive control antigen, the PPRV NIG.75/1 (Taylor & Abegunde, 1979) was grown in Vero cells. At maximum CPE, the cells

and the supernatant medium were harvested and clarified at 2000 rev./min for 10 min (Mistral 4L, MSE, U.K.). The cell deposit was then washed with cold PBS, centrifuged at the above speed and the supernatant discarded. The cell deposit was resuspended in $\frac{1}{250}$ original volume of cold PBS, then subjected to three alternate cycles of freezing and thawing followed by centrifugation at 2000 rev./min for 10 min. The supernatant was then collected and stored at −20°C for use as the positive PPRV control antigen. The negative control antigen consisted of normal goat lung and lymph node homogenates.

Antiserum

The antisera used for the AGPT consisted of rinderpest hyper-immune rabbit and PPRV hyper-immune goat sera, while for the CIE goat PPRV hyper-immune serum was used. The rinderpest hyper-immune rabbit serum was obtained from the Animal Virus Research Institute, Pirbright, U.K., while the PPRV hyper-immune serum was prepared by inoculating two PPR-immune goats with pooled tissue homogenate from a goat that was experimentally infected with Vero cell-adapted PPRV (NIG. 75/1) as described under the section on inoculum. The homogenate was administered subcutaneously at the rate of 5 ml/kg body weight twice at weekly intervals. The animals were bled seven days after the last injection and sera extracted from clotted blood were stored at −20°C until used.

Agar gel precipitation test (AGPT)

The AGP tests were performed according to the methods described by Scott & Brown (1961) with some modifications as described by Forman, Rowe & Taylor (1983). The tests were carried out using 4 ml 1 % Ion agar no. 2 (Oxoid) in distilled water with 0·1 % sodium azide, in 5 cm petri dishes. Wells (six peripheral and one central), 3 mm in diameter and 2 mm apart, were cut out of the agar plates and 10 μl reagent volumes were used throughout the tests. Rinderpest hyper-immune or PPR hyper-immune serum was put into the central well, positive and negative PPR antigens into two diametrically opposed peripheral wells and the test samples into the remaining wells in the rosette. The plates were put under a humidified chamber and examined 2, 4, 6 and 24 h later.

Counter-immunoelectrophoresis (CIE)

The CIE assays were carried out as described previously (Majiyagbe, Nawathe & Abegunde, 1980; Makinde & Majiyagbe, 1982). The gel used was agarose (Sigma, medium EEo, type 2) and was prepared as 0·8 % (w/v) in 0·025 M barbital sodium acetate buffer, pH 8·6, containing 1 g sodium azide per litre. A standard commercial electrophoresis tank with a power supply (Elepos, Toyo, Japan) was used. Clean, greaseless 76 × 26 mm microscope slides were pre-coated with agar film, and after these had dried, 8 ml of the gel were layered on to each of the slides. Then six parallel rows of wells each 3 mm in diameter were cut along the major axis of each slide, pairs of wells being 5 mm apart. PPR hyper-immune serum was put in the anodal wells and test samples in the cathodal wells. Each set of tests included appropriate negative and positive controls. The assays were run for 45 min, using 0·1 M barbital sodium acetate buffer, pH 8·6, containing 1 g sodium azide per litre at a constant current of 3 mA per slide or 15–20 mA per tray of five slides, after

Table 1. *Results of examination of ante-mortem and post-mortem samples for PPR virus antigen*

Samples	Number positive/number tested (%) AGPT	CIE
Ante-mortem		
Ocular swab	6/11 (54·5)	7/11 (63·6)
Buccal scrapings	6/11 (54·5)	7/11 (63·6)
Nasal swab	7/11 (63·6)	7/11 (63·6)
Faecal swab	7/11 (63·6)	7/11 (63·6)
Post-mortem		
Tracheal mucosa	0/11 (0)	10/11 (90·9)
Lungs	5/11 (45·5)	10/11 (90·9)
Mediastinal lymph node	5/11 (45·5)	10/11 (90·9)
Pre-scapular lymph node	5/11 (45·5)	11/11 (100)
Mesenteric lymph node	6/11 (54·5)	11/11 (100)
Tonsil	5/11 (45·5)	10/11 (90·9)
Liver	0/11 (0)	5/11 (45·5)
Kidney	0/11 (0)	6/11 (54·5)
Spleen	6/11 (54·5)	11/11 (100)
Testis	2/11 (18·2)	8/11 (72·7)
Urinary bladder	0/11 (0)	7/11 (63·6)
Small intestine	6/11 (54·5)	10/11 (90·9)
Colon	9/11 (81·8)	10/11 (90·9)
Caecum	7/11 (63·6)	11/11 (100)
Rectum	7/11 (63·6)	10/11 (90·9)
Total	89/209 (42·6)	168/209 (80·3)

which the slides were examined for visible precipitation lines. The slides were then placed in trays containing calcium, magnesium-free phosphate-buffered saline, pH 7·6 (Ca^{2+}/Mg^{2+} – free PBS) and left overnight at room temperature, after which the results were read again. The slides were subsequently placed in distilled water for 6 h, dried and then stained for 5 min in 0·5 % naphthalene black 12B and then cleared in 7·5 % glacial acetic acid – 50 % ethanol destainer. The slides were then dried at room temperature and stored as permanent records after the final readings.

RESULTS

Agar gel precipitation test (AGPT)

Out of the 209 ante-mortem and necropsy samples which were examined, 89 (42·6 %) were positive for PPRV antigen. Precipitinogens were detected in ocular and nasal secretions, buccal scrapings, faeces, lungs, mediastinal, pre-scapular and mesenteric lymph nodes, tonsils, spleen, testis, small intestine, colon, caecum and rectum, but not in trachea, liver, kidney and urinary bladder mucosa (Table 1). In general, all the samples which gave positive reactions against PPR hyper-immune

Table 2. *Summary of the results of PPRV antigen detection in ante-mortem samples*

Animal number	Day of sampling after onset of fever	Number positive/number tested (%) for PPRV antigen	
		AGPT	CIE
421	4	4/4 (100)	4/4 (100)
435	4	4/4 (100)	4/4 (100)
423	4	4/4 (100)	1/4 (25)
432	4	3/4 (75)	3/4 (75)
425	4	3/4 (75)	1/4 (25)
429	5	4/4 (100)	4/4 (100)
431	7	2/4 (50)	0/4 (0)
424	7	2/4 (50)	1/4 (25)
437	10	0/4 (0)	4/4 (100)
428	10	0/4 (0)	3/4 (75)
434	10	0/4 (0)	3/4 (75)

goat serum also gave positive reactions against rinderpest hyper-immune rabbit serum. However, while lines of precipitation were seen in four to six hours with the PPR hyper-immune serum, the rabbit rinderpest serum gave positive results in two to four hours in many cases.

Counter-immunoelectrophoresis

PPRV antigen was detected in 168 (80·3 %) of the 209 samples which were examined by the CIE. Antigen was detected in the above-named samples which were positive by the AGPT as well as trachea, liver, kidney and urinary bladder (Table 1). In general, most test samples gave a single line of precipitation in the CIE assays except nine samples which gave two, and one sample which produced three precipitation lines. Some samples gave positive lines after 30 min in the CIE assays, while some non-specific lines which usually disappeared after washing the slides in Ca^{2+}/Mg^{2+}-free PBS were also recorded.

Ante-mortem samples

Out of the 44 ante-mortem samples, i.e. ocular and nasal secretions, buccal scrapings and faeces, which were tested, 26 (59·1 %) were positive for PPRV antigen by the AGPT. On the other hand, 28 (63·8 %) of the samples were positive by the CIE.

While the AGPT detected PPRV antigen more frequently in ante-mortem samples collected within seven days of the onset of fever, this technique did not detect antigen in ante-mortem samples collected 10 days after the onset of fever. Conversely, the CIE detected PPRV antigen in samples collected up to 10 days after the onset of fever. However, this technique detected viral antigen in only 25 %, i.e. the buccal scrapings only, which were collected from three goats on days 4 and 7 after the start of fever. The above results are summarized in Table 2.

Table 3. *Summary of detection of PPRV antigen in post-mortem samples*

Animal number	Outcome	Day of sampling after onset of fever	Number positive/number tested (%) for PPRV antigen	
			AGPT	CIE
429	Died	5	5/15 (33·3)	12/15 (80·0)
421	Slaughtered	5	7/15 (46·7)	13/15 (86·7)
435	Died	7	5/15 (33·3)	14/15 (93·3)
423	Died	7	9/15 (60·0)	12/15 (80·0)
432	Slaughtered	9	4/15 (26·7)	12/15 (80·0)
431	Slaughtered	9	7/15 (46·7)	12/15 (80·0)
425	Died	9	10/15 (66·7)	14/15 (93·3)
424	Died	9	6/15 (40)	14/15 (93·3)
437	Slaughtered	14	4/15 (26·7)	12/15 (80·0)
428	Slaughtered	14	1/15 (6·7)	13/15 (86·7)
434	Died	14	5/15 (33·3)	12/15 (80·0)

Post-mortem samples

The AGPT detected PPRV antigen in 63 out of 165 (38·2 %) of the post-mortem samples while the CIE detected the same antigen in 190 (84·8 %) of the samples. It was observed that PPRV antigen was detectable more often in the colon (81·8 %), the caecum and the rectum (63·6 %) and the spleen, the small intestine and the mesenteric lymph nodes (54·5 %) by the AGPT. Precipitinogens were detected in about 45·5 % of the samples collected from lungs, mediastinal and pre-scapular lymph nodes, as well as tonsils and lungs by this technique (Table 1). Unlike the AGPT, the CIE detected PPRV antigen in a very high proportion of all the tissues which were examined.

In addition, while PPRV antigen was detected more often in samples collected from goats which died or were slaughtered within nine days of the onset of fever by the AGPT (Table 3), the CIE detected viral antigen in many samples which were collected up to 14 days after the onset of fever.

DISCUSSION

The present investigation showed that PPRV antigen could be detected in ante-mortem secretions and necropsy samples by both the AGPT and CIE, and thus confirmed the observation of Majiyagbe, Nawathe & Abegunde (1980) and Appel *et al.* (1981) that the CIE and AGPT respectively could be useful tools for the diagnosis of PPRV infection. It was interesting to note that precipitinogens were detectable in tissues other than lymph nodes, intestines and lungs, which are the most commonly used test samples for the detection of PPRV antigen. This observation provides the diagnostician with a wider choice of test materials, although it would seem that the above-mentioned tissues still remain the test materials of choice.

The observation that the AGPT detected PPRV antigen in about 42·6 % and the CIE in 80·3 % of the samples examined seems to indicate that the latter technique is more sensitive than the former. This is in agreement with the

observation by Ali & Lees (1979) and Rossiter & Mushi (1980) that the CIE is a more rapid and sensitive procedure for detecting RV antigen. In this study, although lines of precipitation could sometimes be seen 30 min after connecting the CIE test samples to a power source, and were complete after 45 min, it was necessary to soak the slides in PBS for two to three hours followed by another two hours in distilled water to eliminate non-specific lines or confirm weak positives before a final reading was made. In contrast, many of the positive samples were already so by two to four hours after setting up the AGPT using rinderpest rabbit hyper-immune serum. Thus, while the CIE may be more sensitive, it may not necessarily be more rapid than the AGPT using the modifications of Forman, Rowe & Taylor (1983). It is therefore suggested that under field conditions or in situations where laboratory facilities are poor, the AGPT would be a more feasible and also a reliable test for detecting PPRV antigen.

Some points of interest seem to have emerged from the present study. While the CIE detected antigens in a high proportion of samples from goats that died or were slaughtered up to 14 days after the onset of fever, antigen was detected in only a small percentage of samples collected about 14 days after the onset of fever by the AGPT. Brown & Scott (1960) reported that precipitinogen was detected in the lymph nodes of rinderpest-infected cattle on the first day of fever and last detected on the eighth day, with the highest percentage positives occurring on days 3 to 5 of fever. Thus it would seem as if the optimal time for sampling for the AGPT for PPRV diagnosis would be within the first seven days of fever, while for the CIE samples could be collected up to 14 days after the onset of pyrexia. Secondly, it was interesting to note the rather high percentage of ante-mortem secretions from which PPRV antigen could be detected. About 54·5 % of the ocular and buccal and 63·6 % of the nasal and faecal swabs were positive by the AGPT. It was also noted that about 75–100 % of the ante-mortem samples collected four to five days after and about 50 % of those collected seven days after the onset of fever contained PPRV antigen detectable by the AGPT. The use of the above ante-mortem secretions for PPRV antigen diagnosis has not been previously reported. In Nigeria most livestock owners would resist the slaughter of goats or lymph node biopsies in order to confirm a diagnosis of PPRV infection. It would seem from the above results that ante-mortem secretions as shown for RV by Forman, Rowe & Taylor (1983) would be suitable test samples for the detection of PPRV antigen. In any case it is suggested that such samples be obtained within the first seven days of the onset of pyrexia.

The observation that the CIE detected PPRV antigen in such organs as kidneys, liver, tracheal mucosa and urinary bladder, which were negative by the AGPT, may lead one to speculate that the goat hyper-immune serum may pick up non-specific reactions. Alternatively, this observation may be due to the higher sensitivity of the CIE over the AGPT. Although there is no report on the distribution of PPR virus or viral antigen in the various tissues of PPRV-infected goats, it has been reported that in rinderpest virus (RV) infection in cattle the RV could be isolated from the liver (Todd & White, 1914); the kidney (Daubney, 1928); the trachea (Jacotot, 1931); the testis (Van Saceghem, 1933) and the urine (Nicolle & Adil Bey, 1899). In the AGPT no false positive reaction was observed with the goat hyper-immune serum, since all the samples which gave positive lines of

precipitation with the PPR goat hyper-immune serum were also positive with the rabbit rinderpest hyper-immune serum.

Finally, it is important to note that both the AGPT and the CIE are group-specific and may not distinguish between PPRV and RV infections. With the recrudescence of bovine rinderpest in Nigeria, it is important that PPRV diagnosis using both the AGPT and the CIE be supported by other laboratory tests as well as clinical and epidemiological results.

The senior author gratefully acknowledges the help of Dr A. Lamorde, the Director of the National Veterinary Research Institute, Vom, Nigeria for facilities to carry out this work in his Institute and Dr K. A. Majiyagbe of the same institute for the use of his facilities. We also wish to acknowledge the secretarial assistance of Miss M. A. Isoh who typed the manuscript.

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