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COMPARISON OF ANTIGEN DETECTION METHODS OF PESTE DES PETITS RUMINANTS VIRUS IN CLINICAL SAMPLES OF SMALL RUMINANTS

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Summary

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Peste des petits ruminants (PPR) is an acute and highly contagious viral disease of small ruminants such as goats and sheep. The diagnosis of PPR infection in sheep and goats populations can be strengthened with detection of antigen in clinical samples of susceptible populations. In present study, the PPR virus antigen detection was investigated and compared in clinical sheep and goat samples by agar gel immunodiffusion (AGID), haemagglutination (HA) tests and immuno-capture ELISA (IC-ELISA). The viral antigen was detected from 8.95% of the samples tested by AGID, 20.9% tested by HA test and 34.3% tested by IC-ELISA. Kappa statistics scores for HA versus AGID, IC-ELISA versus AGID and HA versus IC-ELISA were 0.45 (95% confidence interval CI 0.158–0.742), 0.359 (95% CI 0.01–0.69) and 0.035 (95% CI 0.309–0.694), respectively, and indicated a non-significant agreement between the tests. The major findings of this study were that the HA test was more sensitive than AGID for detection of PPRV antigen, but IC-ELISA was the most sensitive of all three. The rapid detection of viral antigen by appropriate methods will help in early diagnosis of infection and subsequent control of the PPR disease in Pakistan.

Key words: antigen detection, agar gel immunodiffusion test, ELISA, haemagglutination test, peste des petits ruminants virus

INTRODUCTION

Peste des petits ruminants (PPR) is an acute and highly contagious viral disease of small ruminants such as goats and sheep. PPR virus (PPRV) is a member of the genus *Morbillivirus,* family Paramyxoviridae. It has a single-strand negative sense RNA genome of ~16 kb (15,948 nucleotides) in length that encodes eight proteins including six structural proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), haemagglutinin protein (H) and large polymerase protein (L), and two nonstructural proteins V and C (Bailey *et al.*, 2005; Chard *et al.*, 2008).

Abubakar *et al.* (2008) have reported dramatic consequences with morbidity of 80–90% and mortality between 50 and 80% due to infection of PPRV in small ruminants. In Pakistan, it causes eco-

nomic losses of Rs 20.5 billion (USD 0.24 billion) annually. Clinical findings are fever, ocular and nasal discharges, sores in mouth, diarrhoea and pneumonia. The main routes of PPRV transmission are oral and aerosol; the oral, nasal and ocular excretions being the key sources of infection (Couacy-Hymann *et al.*, 2009).

The small ruminants infected with PPR are routinely diagnosed on the basis of clinical examination, gross pathology, histologic findings and laboratory confirmation. A number of diagnostic tests are used for the detection of PPRV, including isolation on cell culture, agar gel immunodiffusion (AGID), haemagglutination (HA) tests, immunocapture enzyme-linked immunosorbent assay (IC-ELISA) and reverse transcriptase polymerase chain reaction (RT-PCR) (Forsyth & Barrett, 1995; Nussieba *et al.*, 2008).

The present study aimed to detect the PPRV antigen in clinical samples of sheep and goats by agar gel immunodiffusion test, haemagglutination test and immuno-capture ELISA (IC-ELISA) and to compare their sensitivity.

MATERIALS AND METHODS

Experimental procedure

A total of sixty seven (67) different tissues samples (lung, liver, spleen, heart, kidney, intestine and lymph nodes), oral and nasal swabs were collected from PPR suspected sheep and goats from different locations of Pakistan and were used for antigen detection of PPRV using haemagglutination test (HA), agar gel immunodiffusion test (AGID) and immunocapture enzyme-linked immunosorbent assay (IC-ELISA).

Haemagglutination test

The HA test was performed as described by Wosu (1985) using 0.5 % chicken and sheep red blood cells (RBC) with each of the tissue extracts (antigen) of 67 samples. In each of the 12 wells of a U-bottomed micro titre plate, 50 µL of PBS was added. In the first well of each series, 50 µL of tissue extract (sample) was added and mixed well giving 2-fold dilutions (1:2). Fifty µL of the suspension was transferred from the first to the second well and mixed thoroughly. Process was repeated up to the 11^{th} well. Then 50 µL of the material was discarded from the 11^{th} well. To each well, 50 µL of the 0.5% RBC were added to each well from the 1^{st} to the 12^{th}. Well no. 12 in each series acted as control (no antigen was added). The plates were incubated 20–30 min at room temperature. The presence of haemagglutinin was indicated by mat formation and its absence by button formation.

Agar gel immunodiffusion test

Noble agar 1% (Oxoid, UK) was prepared by adding 1 g Noble agar to 100 mL buffer solution. About 15 mL of the agar was put in each of the Petri dishes to achieve a 4 mm thickness of the agar gel in the plate. On solidification, wells in the agar were made using a gel punch (5 mm) following a hexagonal pattern with a central well. The wells were then sealed with molten agar. The central well was filled with 10 µL of hyperimmune serum (obtained from Veterinary Research Institute, Lahore), one peripheral well was filled with 10 µL control PPR antigen and the remaining peripheral wells – with 10 µL of test samples (67 samples). Petri dishes were incubated at 37 °C in humid chamber for 24 h. A white precipitation line between the test sample and the anti PPR serum was recorded as positive result.

Immunocapture enzyme-linked immunosorbent assay

All the samples were tested by immunocapture-ELISA kit as described by Libeau *et al.* (1994). PPR IC-ELISA Kit was manufactured by Biological Diagnostic Supplies Ltd., Flow Laboratories and Institute for Animal Health Pirbright, Surrey, England for PPRV antigen detection. Ortho-phenylenediamine (OPD) was used as chromogen and the absorbance was measured at a wavelength of 492 nm. Percent positivity (PP) values were calculated by ELISA Data Interchange (EDI) software (FAO/IAEA, Vienna, Austria). The following formula was used to convert the optical density values to percentage positivity:

$$PP = \frac{100 - (ODc/ODt)}{ODref} \times 100$$

where: ODc – optical density of control sample; ODt – optical density of test sample; ODref – median optical density of PPR reference antigen.

The samples with PP > 18% were considered as positive.

Statistical Analysis

The concordance between the three tests (HA, AGID and IC-ELISA) was determined using kappa statistics.

RESULTS

All 67 samples were tested for antigen detection by HA, AGID and IC-ELISA. Out of them, 14 (21%) samples were found positive for PPRV antigen by HA, a detail of which is given in Table 1. Out of 67 samples 8.95% (6) samples were found positive for PPRV antigen by AGID, as shown in Table 2. The results of IC-ELISA showed that 34.3% samples were found positive for PPRV (Table 3).

The results of chi-square test for HA versus location (P=0.006) and AGID versus location (P=0.049) were significant which indicated that locations and test results were associated. The result for IC-ELISA versus location (P=0.006) indicated a much higher association with locations.

Kappa statistics were applied to evaluate the concordance between the three tests (HA, AGID and IC-ELISA). Kappa

Table 1. Results from PPRV antigen detection in tissues samples of small ruminants by the haemagglutination test

Area	Number (percentage)		Total
	Negative	Positive	
Faisalabad	8 (61.53)	5 (38.46)	13 (19.40)
Sargodha	7 (87.50)	1 (12.50)	8 (11.94)
Islamabad	19 (95.00)	1 (5.00)	20 (29.85)
Mardan	10 (100.0)	–	10 (14.92)
Rawalpindi	–	2 (100.0)	2 (2.99)
Attock	5 (62.50)	3 (37.50)	8 (11.90)
Peshawar	1 (100.0)	–	1 (1.50)
Northern Areas	2 (50.00)	2 (50.00)	4 (5.97)
Quetta	1 (100.0)	–	1 (1.50)
Total	53 (79.10)	14 (20.90)	67 (100.0)

Table 2. Results from PPRV antigen detection in tissues samples of small ruminants by agar gel immunodiffusion test

Area	Number (percentage)		Total
	Negative	Positive	
Faisalabad	9 (69.23)	4 (30.76)	13 (19.40)
Sargodha	8 (100.0)	_	8 (11.94)
Islamabad	20 (100.0)	_	20 (29.85)
Mardan	10 (100.0)	–	10 (14.92)
Rawalpindi	1 (50.00)	1 (50.00)	2 (2.99)
Attock	7 (87.50)	1 (12.50)	8 (11.90)
Peshawar	1 (100.0)	_	1 (1.50)
Northern Areas	4 (100.0)	_	4 (5.97)
Quetta	1 (100.0)	–	1 (1.50)
Total	61 (91.04)	6 (8.95)	67 (100.0)

Table 3. Results from PPRV antigen detection in tissues samples of small ruminants by immunocapture enzyme-linked immunosorbent assay

Area	Number (percentage)		Total
	Negative	Positive	
Faisalabad	5 (38.46)	8 (61.53)	13 (19.40)
Sargodha	5 (62.50)	3 (37.50)	8 (11.94)
Islamabad	15 (75.00)	5 (25.00)	20 (29.85)
Mardan	8 (80.00)	2 (20.00)	10 (14.92)
Rawalpindi	1 (50.00)	1 (50.00)	2 (2.99)
Attock	5 (62.50)	3 (37.50)	8 (11.90)
Peshawar	1 (100.0)	_	1 (1.50)
Northern Areas	3 (75.00)	1 (25.00)	4 (5.97)
Quetta	1 (100.0)	–	1 (1.50)
Total	44 (65.67)	23 (34.30)	67 (100.0)

statistics scores for HA vs AGID, IC-ELISA vs AGID and HA vs IC-ELISA were 0.45 (95% confidence interval 0.158–0.742), 0.359 (95% CI 0.01–0.69) and 0.035 (95% CI 0.309–0.694) respectively, which indicate a non-significant agreement between the tests.

DISCUSSION

PPRV antigen detection was investigated and compared in clinical samples of small ruminants by HA, AGID and IC-ELISA. Chicken RBCs used in the test showed better results as compared to sheep RBCs. In Sudan, Nussieba *et al.* (2008) also reported PPRV antigen detection in samples by HA. Similarly in India, Manoharan *et al.* (2005) used haemagglutination as a confirmatory test for Peste des petits ruminants diagnosis.

The results of AGID showed 8.95% positive samples in the present study. In Sudan, the PPRV antigen was detected in 77.5% of the samples tested by AGID (Nussieba *et al.*, 2008). Recently, Munir

et al. (2009) also detected PPRV antigen in 21.4% of samples of small ruminants in Pakistan. These rates were higher that the results from the present study. Results of both tests revealed HA test was more sensitive than AGID for detection of PPRV antigen. This result was in concordance with Nussieba *et al.* (2008). Moreover, the HA test is quick, simple, cheap and reliable confirmatory test for the diagnosis of PPRV.

The results of IC-ELISA showed that 34.3% of samples were positive for PPRV antigen. Abubakar *et al.* (2008) utilized the same IC-ELISA technique for detection of PPRV in goats in Pakistan and found out a prevalence of 40.98%. However, seropositivity for PPRV in ruminants in Pakistan was reported to be 33.33% by competitive ELISA (Khan *et al.*, 2007). All studies in Pakistan showed variation in the prevalence of PPRV and this was probably due to the intensity of movements of the small ruminants in different provinces or geographic regions.

Munir *et al.* (2009) investigated the comparative efficiency of competitive ELISA (c-ELISA), standard agar gel immunodiffusion (AGID) and precipitinogen inhibition test (PIT) for the diagnosis of Peste des Petits Ruminants (PPR) in Pakistan and concluded that c-ELISA is used as standard since it has the best sensitivity and specificity and can be utilized for samples which are not kept under ideal conditions.

In conclusion, the rapid detection by suitable and appropriate methods of antigen and nucleic acid detection of PPRV in infected animals will help in early diagnosis of infection and subsequently control of the PPR disease in Pakistan.

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