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Haemagglutination as a confirmatory test for Peste des petits ruminants diagnosis☆

S. Manoharan[a,*], R. Jayakumar[b], R. Govindarajan[c], A. Koteeswaran[a]

[a] *Vaccine Research Centre-Viral Vaccines, Centre for Animal Health Studies, Tamil Nadu Veterinary and Animal Sciences University, Madhavaram Milk Colony, Chennai 600 051, Tamil Nadu, India*
[b] *Department of Animal Biotechnology, Madras Veterinary College, Chennai 7, Tamil Nadu, India*
[c] *Central University Laboratory, Centre for Animal Health Studies, Madhavaram Milk Colony, Chennai 600 051, Tamil Nadu, India*

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Abstract

Seven Peste des petits ruminants (PPR) viruses and a RPV (vaccine strain) and their respective antiserum raised in rabbits were used for reciprocal cross neutralization test and haemagglutination (HA) and haemagglutination inhibition (HI) test employing 0.5% chicken RBC. Although many differential diagnostic methods for PPR and RP (rinderpest) are available viz., immunocapture ELISA, 'N' gene radioactive cDNA probes, non radioactively labelled biotinylated cDNA probes, reverse transcription-polymerase chain reaction (RT-PCR) for 'F' gene along with reciprocal cross neutralization test, HA and HI tests could be used as a reliable alternative in field conditions particularly in places where RP and PPR are co-existing. The advantages of the tests have been discussed in comparison with reciprocal cross neutralization tests.

Keywords: Peste des petits ruminants virus; Rinderpest virus; Haemagglutination; Haemagglutination inhibition; Chicken red blood cell; Reciprocal cross neutralization test

1. Introduction

Peste des petits ruminants (PPR) is a highly contagious viral disease of small ruminants caused by a RNA virus of genus Morbillivirus in the family Paramyxoviridae. The etiological agent PPRV is serologically closely related to rinderpest virus (RPV), another member of the Morbillivirus causing clinically indistinguishable disease in large and small ruminants (Scott, 1990).

Methods to differentiate these two viruses are reciprocal cross neutralization test (Taylor, 1979; Taylor and Abegunde, 1979; Furley et al., 1987; Chandran et al., 1995), immunocapture ELISA (Libeau et al., 1994), mobility of 'N' protein in PAGE (Diallo et al., 1987), radiolabelled cDNA probe from 'N' gene (Diallo et al., 1989), non radioactively labelled bi-

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* Corresponding author. Fax: +91 044 2555 1573.
E-mail address: ulagaimano@yahoo.com (S. Manoharan).

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otinylated cDNA probes (Pandey et al., 1992) and reverse transcription-polymerase chain reaction (RT-PCR) for 'F' gene conserved region (Forsyth and Barrett, 1995). Among this, immunocapture ELISA and reverse transcription-polymerase chain reaction are routinely used for differentiation (OIE, 2000). A simple haemagglutination (HA) and its corresponding haemagglutination inhibition (HI) tests are accepted valid alternatives for differentiating PPRV and RPV (Wosu, 1985, 1991) since the other differentiating methods are cost prohibitive and time consuming for routine screening of large number of samples; a simple screening method to differentiate PPRV and RPV is the need of the hour in countries where rinderpest is reported along with Peste des petits ruminants. Hence, attempts were made to study the usefulness of the HA and HI tests, as a differentiating method in field conditions by comparing with the reciprocal cross neutralization test.

2. Material and methods

2.1. Viruses

The source of PPR viruses was from different outbreaks in Tamil Nadu and from other states of India (Table 1). Initially, the suspected tissues/infected tissue culture fluid of PPR viruses were confirmed for the presence of antigen and F gene by immunocapture ELISA and RT-PCR, respectively. The viruses were isolated (Libeau et al., 1994; Forsyth and Barrett, 1995) and adapted in Vero cells. The source of RPV was a vaccine strain in primary lamb kidney cells (RBOK strain) obtained from Institute of Veterinary Preventive Medicine, Ranipet, Tamil Nadu, India.

2.2. Antiserum production

Homologous antiserum for all the PPRV isolates and RPV was raised in rabbits as described by Obi et al. (1990). Briefly, Vero adapted PPRVs and RPV (2 ml) were homogenised with Freund's adjuvant and injected subcutaneously in the flank region. Subsequent three injections were given with incomplete adjuvant intramuscularly (2 ml) at 2 weeks interval. The blood was collected 7 days after last injection and the serum was separated and stored at −20 °C till use.

Table 1
Haemagglutination, haemagglutination inhibition and 50% serum neutralization titres of different PPRV isolates and RPV with their homologous and heterologous antiserum

Viruses	Species	Haemagglutination titre		Virus titre	Antiserum to different viral isolates																	
		Tissue suspension	Tissue culture fluid	$TCID_{50}$ log_{10}	Haemagglutination titre/50% neutralization end point in log10																	
					PPRV/IND/TN/97/1 (Coimbatore)		PPRV/IND/TN/96/1 (Dindugul)		PPRV/IND/TN/96/2 (Otteri)		PPRV/IND/TN/95/7 (Tirunelveli)		PPRV/IND/TN/87/1 (Arasur)		PPRV/IND/N AH/97/1 (Maharashtra)		PPRV/IND/ ORI/97/1 (Orissa)		Vaccine strain of rinderpest virus (RBOK)	
					HI	SNT	HI	SNT	HI	SNT	HI	SNT	HI	SNT	HI	SNT	HI	SNT	HI	SNT
PPRV/IND/TN/97/1 (Coimbatore)	Goat	32	64	6.0	1024	2.73	1024	2.57	1024	2.2	512	2.33	512	2.2	256	2.42	512	2.7	512	1.0
PPRV/IND/TN/96/1 (Dindugul)	Goat	32	128	6.5	1024	2.43	1024	2.43	512	2.2	512	2.33	256	2.43	256	2.3	512	2.43	256	0.8
PPRV/IND/TN/96/2 (Otteri)	Goat	64 oculonasal extract	64	7.0	1024	2.33	1024	2.43	1024	2.2	256	2.43	128	2.2	512	2.2	256	2.51	256	1.2
PPRV/IND/TN/95/7 (Tirunelveli)	Goat	32	128	6.25	1024	2.81	1024	2.73	512	2.43	512	2.73	512	2.51	512	2.57	512	2.33	256	0.7
PPRV/IND/TN/87/1 (Arasur)	Sheep	Not done	64	7.5	512	2.51	1024	2.63	1024	2.73	512	2.73	1024	2.51	256	2.57	256	2.33	256	0.7
PPRV/IND/NAH797/I (Maharashtra)	Goat	16	64	5.67	512	2.66	512	2.2	1024	2.33	256	2.51	1024	2.43	1024	2.63	512	2.66	128	0.8
PPRV/IND/ORI/97/ 1 (Orissa)	Goat	32	128	6.25	1024	2.33	512	2.3	512	2.2	256	2.43	512	2.42	512	2.5	512	2.73	256	1.0
Vaccine strain of rinderpest virus (RBOK)	Bovine	Nil	Nil	6.4	–	0.5	–	0.2	–	1.0	–	0.8	–	–	–	0.5	–	0.7	–	2.2