



Research Article

Evaluation of Haemagglutination Assay (HA) for the Detection of Peste des Petits Ruminants Virus (PPRV) in Faecal Samples of Recovered Goats

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ABSTRACT

This paper reports the findings of evaluation of Haemagglutination Assay (HA) for detection of Peste des Petits Ruminants (PPR) in faecal samples of sheep and goats persistently infected with PPR. Faecal samples (n=100) collected during an outbreak of PPR were subjected to HA and RT-PCR (gold standard). HA produced more positive results (77/100; 77%) as compared to RT-PCR (29/100; 29%). Kappa analysis indicated no agreement between HA and RT-PCR (kappa = -1.5159). In this study, we found that HA is a non-specific test for detection of PPR Virus (PPRV) in faecal samples of small ruminants, infected with PPRV. Therefore, other sensitive and specific laboratory test should be used for detection of PPRV in faecal samples of persistently infected animals.

Key Words: HA, sensitivity, specificity, PPR

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INTRODUCTION

Peste des Petits ruminants (PPR) or goat plague is a highly contagious and disastrous viral disease affecting small ruminants specially goats. The disease is caused by Peste des Petits Ruminants Virus (PPRV) that belongs to genus morbillivirus of family Paramyxoviridae (Gibbs et al., 1979). PPR is a Transboundary Animal Disease (TAD) of economic importance. The disease has detrimental effects to whole of the susceptible host population by provoking epidemics and pandemics (FAO, 1999).

Diagnosis of this disease may be carried out using different laboratory techniques like virus isolation, detection of PPRV antigen, detection of genome and nucleic acid sequencing and detection of specific antibodies in serum (Diallo, 2006). PPRV and Measles virus are unique among morbilliviruses as they possess haemagglutination activity. The agglutination of piglet red blood cells (RBCs) with tissue homogenate from PPR affected animals have been reported (Wosu et al., 1985). HA is a simple, quick and cheap diagnostic assay (Ezebie et al., 2004). A previous study used HA to determine the persistence of PPRV in faecal samples and reported persistence of PPRV in faecal samples of goats for 12 weeks (Ezebie et al., 2008). However, no information is available about the specificity and sensitivity of the test for detection of PPRV using faecal samples. Therefore, a study was conducted to evaluate the specificity and sensitivity of HA to detect PPRV in faecal samples.

MATERIALS AND METHODS

PPR Outbreak Profile

An outbreak was reported in an organized goat farm in sub urban area of Lahore, Punjab, Pakistan. The outbreak was confirmed by clinical signs, postmortem findings, RT-PCR and sero-conversion in affected animals using cELISA. The flock consisted of 140 goats with age ranging between 10–18 months. The flock had history of introduction of five new animals from a nearby livestock market. None of the animal had a history of vaccination against PPR. There was no outbreak of PPR in the nearby area/village. The morbidity rate was 100 %. However, 40 of 140 animals died during the outbreak with a mortality rate of 28.5 %.

Antigen

Faecal samples (n=100) were collected directly from rectum of goats twelve weeks post outbreak from recovered goats. The samples were transported to Animal Health Research Laboratories (AHRL) at National Agricultural Research Center (NARC), Islamabad in cold conditions. One gram of each faecal sample was thoroughly mixed with 3 mL of Phosphate Buffer Saline (PBS) having pH 6.8 and incubated at -20°C for 12 hours. After incubation homogenate was thawed and centrifuged at 15,000 rpm for 15 min at 4°C. Supernatant was collected in cryovials and kept at -20°C for further used as antigen.

Positive Control

A local PPRV isolate (PAK-KPI-06/NARC3) obtained on Vero cell culture was used as positive control for HA test. The isolate was serially passaged 12 times on Vero cells. The HA activity of the PPRV was monitored after each passage using 0.6% chicken RBCs. The highest HA titre (1: 1024) was obtained after 12th passage.

Red Blood Cells

Chicken blood collected in heparinized vacutaniner was washed three times using PBS (pH 6.8). Washed chicken RBCs at 1% concentration were used in HA (Wosu, 1985).

RNA Extraction

RNA for RT-PCR was extracted from homogenate of faecal material using RNeasy kit (Qiagen GmbH, Hilden, Germany) according to manufacturer's instructions. A negative control was also included during extraction to detect any possible contamination. Extracted RNA was stored at -80°C until further used. The quantity and purity of extracted RNA was determined using Nanodrop (Nano Drop 1000, Thermo scientific Wilmington, DE, USA). PPRV strain Nigeria 75/1 was used as control in RT-PCR to confirm the successful extraction of viral RNA (Balamurugan et al., 2012).

Haemagglutination Test

The HA test was performed as described by Wosu (1985) and Ezeibe et al., (2004). Briefly, two fold serial dilutions of each faecal sample suspension were made in 50 µL of sterile PBS (pH 7.2) in U bottom microtitration plate. Then 50 µL of chicken RBCs (1%) were added in each well. The positive and negative controls were included in each plate. The plate was incubated at 4°C for 45min and results were noted.

Table 1: A 2x2 table showing the results of faecal samples (n=100) analysed using Haemagglutination Assay (HA) and RT-PCR (Gold standard). The samples were obtained from goats recovered from PPR. Figures in parentheses are percentages.

HA	RT-PCR		Total
	Negative	Positive	
Negative	16 (22.5)	7 (24.1)	23 (23.0)
Positive	55 (77.4)	22 (75.8)	77 (77.0)
Total	71 (71.0)	29 (29.0)	100 (100.0)

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

The amplification of extracted RNA was done using PPRV specific primers based on Nucleoprotein (N) gene (Forsyth and Barrett, 1995). Extracted RNA (2.5 µL) was used for amplification by one step RT-PCR. Each PCR reaction mix (25 µL) tube contained QIAGEN one step 5X RT-PCR buffer with 12.5 mM of MgCl₂, 2 µL of enzyme mix, 10mM of each dNTP's, 5 unit of RNAs inhibitor and 25 pm of forward and reverse primers. The amplification was performed in a 9902 thermal cycler (Applied Biosystems, Courtaboeuf, France). Thermocycler was programmed for an initial reverse transcription for 30 min at 50°C, a PCR activation for 5 min at 95°C, 30 cycles of amplification (1 min at 95°C, 1 min at 50°C, and 2 min at 72°C), and a final extension step at 72°C for 10 min. The amplified PCR products were

electrophoresed in 1% agarose gel at 120 V for 15–20 min. The expected band size of the PCR product was 351bp.

Data Analysis

The amount of agreement between the results of HA and RT-PCR was determined using Kappa statistics (Dohoo, 2009). Receiver operating characteristic (ROC) curve of sensitivity was plotted against 1-specificity and area under curve was calculated (Thrusfield, 2005) using STATA 11.2 software.

RESULTS

Of 100 samples tested, both tests agreed on the status of 38 samples; 22 were positive by both tests and 16 were negative by both tests. However, both tests showed a greater disagreement; of 77 samples that were positive by HA, only 22 were positive by RT-PCR. Similarly, HA declared 23 samples as negative; of these 23, only 16 were negative by RT-PCR (Table 1). These findings suggest a poor sensitivity and specificity of HA for detection of PPR antigen in faecal samples as is evident by the area (0.4711) under ROC curve (Figure 1).

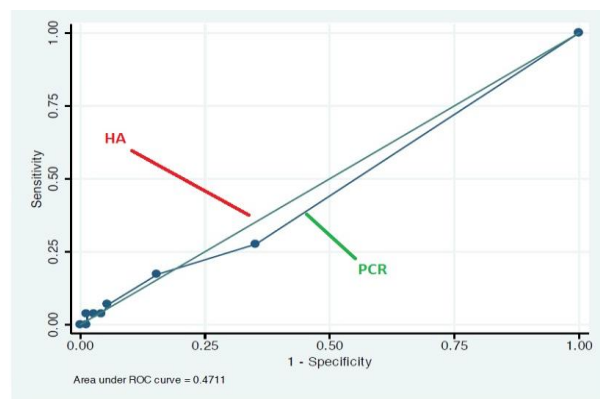


Figure 1: ROC curve showing sensitivity and specificity of Haemagglutination Assay (HA) and RT-PCR for detection of PPRV antigen in faecal samples from PPR recovered goats

DISCUSSION

Peste des Petits Ruminants (PPR) is one of the most devastating diseases of small ruminants. It is a highly transmissible and frequently fatal disease of sheep and goats (Gibbs et al., 1979). Various diagnostic tests such as HA, virus isolation, ELISA and RT-PCR are being used for the diagnosis of PPR. Some of these test (ELISA, RT-PCR and isolation) are expensive, require skilled personals and sophisticated laboratory. However, HA is a simple and cheap test and has been used for the diagnosis of PPR (Wosu, 1985; Ezeibe et al., 2004). For example, a study reported HA activity of porcine RBCs with extract from PPR infected mesenteric lymph nodes (Wosu, 1985). However, HA activity with chicken and human 'O' RBCs have also been observed (Ezeibe et al., 2004). The chicken RBCs were used for HA in this study as they are economical and easily available.

HA test can be performed on samples obtained from live and dead animals. Ocular, nasal, oral discharges and faecal samples obtained from PPR affected live animals have

been used for detection of PPR antigen using HA (Manoharan et al., 2005; Abubakar et al., 2012). Therefore, in case of an outbreak samples can be taken from live and dead animals to detect PPR antigen using HA.

To study the persistence of PPR in animals recovered from PPR various studies have used different samples. For example, a recent study where nasal, ocular and oral swabs were analysed using real time PCR, from goats challenged with PPR virus indicated persistence of PPRV for 40 days (Liu et al., 2012). However, a previous study has indicated detection of PPRV in faecal samples of PPR recovered goats for 12 weeks using HA which clearly indicates the persistence of PPRV for longer duration in faecal samples (Ezeibe et al., 2008). Therefore, faecal samples appear to be the sample of choice for persistence studies. However, Ezeibe et al., (2008) did not evaluate the specificity and sensitivity of HA when faecal samples were used to detect presence of PPR antigen. The results of our study ($\kappa = 1.5159$) indicated no agreement between HA and RT-PCR which suggests that HA is a non-specific test when faecal samples are used for detection of PPR antigen. Therefore, HA is not a good choice for detection of PPR antigen in persistence studies and more specific tests such as RT-PCR should be used for this purpose.

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