

Chromatographic Strip Technology: A Pen-side Test for the Rapid Diagnosis of Peste Des Petits Ruminants in Sheep and Goats

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Abstract: Outbreaks of peste des petits ruminants (PPR) in goats, reported in different parts of the country were investigated. The disease was confirmed at six places using immunocapture ELISA and a pen-side test (chromatographic strip), a recently developed technology for the diagnosis of PPR under field conditions. The results indicated that PPR was wide spread in the country causing high mortality in goats. Although sheep and goats were kept under the same roof, yet there was no evidence of PPR infection in sheep. The pen-side test proved to be a useful technology for the rapid diagnosis of PPR. It helped reduce losses by employing early control measures to contain the infection.

Key words: PPR, chromatographic strip, goats

Introduction

Peste des petits ruminants (PPR) is a highly contagious disease of sheep and goats, caused by a morbillivirus of the family Paramyxoviridae. It was first described in Cote d' Ivoire during 1942 (Diallo, 1997). The symptoms of the syndrome resembled " rinderpest", hence was named " peste des petits ruminants" (small ruminant plague). The disease has been reported in Africa, the Arabian Peninsula, most of the Middle East countries, India, Pakistan and Afghanistan (Hussain *et al.*, 1998). In severe disease, morbidity and mortality rates can reach upto 100 and 90%, respectively. Infected animals show clinical signs (fever, oculo-nasal discharges, erosive lesions in mouth, diarrhoea and pneumonia) similar to rinderpest (RP) in cattle and buffaloes from which it must be differentiated.

In Pakistan, PPR was first recognized during 1991 (Pervez *et al.*, 1993) and then in 1992 (Athar *et al.*, 1995). Both these reports were based upon clinical signs and post-mortem findings. The first laboratory confirmed outbreak of PPR occurred in Lahore during 1994 and the samples from infected animals were analyzed at the World Reference Laboratory for Rinderpest, UK (Hussain *et al.*, 1998). Since then, evidence of PPR outbreaks at several places in Pakistan has been

determined. This paper describes outbreaks of PPR during the year 2000 at various places in Pakistan.

Materials and Methods

Animals

The population used in the study was of goats suspected of PPR or animals within the herd that may have been in contact with infected goats. Samples were collected from animals during outbreaks in Islamabad Capital Territory (ICT), Livestock Experiment Station (LES) at Rakh Ghulaman district Mianwali and the northern areas of Pakistan.

Collection of samples

From sick animals, lachrymal fluids were collected as described previously (Bruning *et al.*, 1999). Briefly, sterile cotton swabs were inserted into and swirled around the conjunctival sac behind the eyelids (one swab for each eye). These specimens were tested at the spot using a chromatographic strip and the remaining portions were stored at 4°C before testing by IELISA in the laboratory.

From dead animals, mesenteric lymph nodes and spleen were collected and kept at -20°C until processed to detect PPR virus antigens.

Preparation of samples

The cotton material from each swab was cut-off and placed in the barrel of a 1.0 ml syringe. Sample was extracted by pressing cotton wool against the piston and saved in a tube. If the required quantity was not obtained, about 100 µl of PBS (pH 7.4) was added in the barrel and pressed again.

For mesenteric lymph nodes or spleen, about 100g of these samples were triturated and processed separately using a Pestle and mortar. The slurry was centrifuged at 2000 g for 10 min and supernatant used as test sample. In case the quantity of sample was not sufficient, PBS was mixed and centrifuged again.

Pen-side test; principle and operation

The assembled chromatographic plastic strips (Clearview™ prototype devices, Svanova Biotech, Sweden, Batch no. 5002) were used by loading 6 drops of sample onto a pad in sample window. On contact with the sample diluent, the freeze-dried Mab-labeled microspheres, already present on the pad, were re-hydrated and moved by capillary action along the nitrocellulose strip, placed below the pad, towards the immobilized band of trapping antibody. Any PPR virus antigen in the sample was bound to the antibody on the microspheres and the whole complex was then captured by the immobilized band of antibody (specific against PPR virus) on the nitrocellulose membrane. This resulted in accumulation of the dyed microspheres which gave rise

to a blue line in test window, indicating a positive result. Excess Mab-labeled microspheres continued migrating along the membrane until reached the band of immobilized rabbit anti-mouse antibody and resulted in second blue line in the control window Fig. 1. This internal control demonstrated that Mab-labeled microspheres had migrated along the length of the membrane.

Immunocapture ELISA

The immunocapture ELISA (IcELISA) for the detection of PPR virus antigens was carried out using IcELISA kit (CAMDA/OIE, France) as described by Libeau *et al.* (1994). Briefly, the ELISA plates were coated with a Mab against the N-protein of PPR virus. After incubation and washing, test antigen was added followed by the addition of a streptavidin horseradish peroxidase (HRPO) enzyme conjugate. The plates were incubated and washed, substrate was added and the reaction was stopped with sulfuric acid. Positive results were determined by comparison with the individual test control values.

Results

Six outbreaks at various places in the country were investigated (Table 1). Different clinical signs and post-mortem lesions observed are shown in Fig. 2 to 6. The disease affected goats of all ages, however, no clinical signs or mortality were recorded in sheep kept with sick animals at the same premises. The results of samples analyzed using Pen-side test and IcELISA are shown in Table 2. The strips were kept on an horizontal surface for up-to 10 min and in general, positive lines were observed in the test window within 5 min (Fig. 1).

The virus antigen was detected in lachrymal fluids from all the sick animals showing body temperature >103 °F. Lachrymal fluids from 12 animals showing temperature <103 °F were negative by both the assays. However, 4 died later-on, were found positive for PPR when lymph nodes and spleen were tested by both the assays.

Table 1: Prevalence of PPR in goats at various places in Pakistan

Place	Animals at risk	No. of animals died	Mortality(%)
LES, Rakh Ghulaman	515	260	50.48
Islamabad Capital Territory (ICT)			
Private Farm1	55	13	23.63
Private Farm 2	83	07	8.43
Rawalpindi district			
Private Farm	77	23	29.87
The Northern Areas (District Diامر)			
Khinar-Nala	540	116	21.48
Chilas town	12	05	41.66

Table 2: Results of Pen-side tests and ICeLISA used for the diagnosis of PPR in goats

Place	No. of samples collected			No. of samples positive By Pen-side Test (%)			No. of samples positive By IcELISA (%)		
	A	B	C	A	B	C	A	B	C
LES, Rakh Ghulaman	14	07	07	09(64)	05(71)	05(71)	09(64)	07(100)	07(100)
Islamabad Capital Territory (ICT)									
Private Farm 1	04	03	03	02(50)	02(67)	02(67)	02(50)	03(100)	03(100)
Private Farm 2	03	03	03	02(67)	02(67)	02(67)	02(67)	03(100)	03(100)
District Rawalpindi									
Private Farm	05	02	02	04(80)	01(50)	01(5)	04(80)	02(100)	02(100)
The Northern Areas (District Diamer)									
Khinar Nala	07	03	03	04(57)	03(100)	03(100)	04(57)	03(100)	03(100)
Chilas town	02	NA	NA	02(100)	NA	NA	02(100)	NA	NA
A = Lachrymal fluids B= Mesenteric lymph nodes C = Spleen NA = Not Available									

Lymph nodes and spleens collected from dead animals were also analyzed for the presence of PPR virus antigens. Samples collected from all eighteen dead animals were found positive by IcELISA. However, five of these samples gave negative signal when processed by Pen-side test.

Almost all sick animals at the private goat farm 1 (ICT) developed mouth lesions similar to those seen in contagious pustular dermatitis (Fig. 6). However, it did not cause any mortality in the herd.

Discussion

Laboratory analysis of samples collected from all six outbreaks confirmed the presence of PPR in goats. Significance of PPR has recently been realized in Pakistan and outbreaks of disease similar to PPR have been reported from various parts of the country based upon clinical signs and post-mortem lesions. These observations and previous reports (Pervez *et al.*, 1993; Athar *et al.*, 1995; Hussain, 1996; Ayaz *et al.*, 1997; Tahir *et al.*, 1998) indicate that the disease is wide spread in Pakistan. However, true picture about the prevalence of this disease is not clear mainly due to lack of proper diagnostic facilities and inefficient reporting system. Most field veterinarians confuse PPR with pleuropneumonia which is another limiting factor to control PPR in the country.

A test that can be performed easily under field conditions with high enough specificity can play a significant role to contain infection at the face of an outbreak. The Pen-side test, described above, has proved to fulfil this criteria. These prototypes of the chromatographic strip

for the diagnosis of PPR and rinderpest has recently been supplied for field trials in Pakistan (FAO-UN TCP/PAK/8923). Similarly, the strip test for PPR was capable of identifying this disease at all the premises included in present studies. The discrepant results obtained for some of the samples (Table 2) by strip test and IcELISA may be due to the reason that IcELISA is based upon the Mab against the N portion, whereas the Mab used in strip test recognize an epitope on the H protein of PPR virus. The exposure of such epitopes may differ from sample to sample and the tissue materials other than virus may interfere between antigen antibody interaction.

PPR virus is present in lachrymal fluid at high concentrations shortly before or at the onset of pyrexia. Therefore, various authors have recommended lachrymal fluid as an ideal sample for viral antigen detection (Libeau *et al.*, 1994; Anderson *et al.*, 1996). Results of our studies also support previous findings and there was also complete homology between the Pen-side test and IcELISA. Lachrymal fluids found negative for both the tests, may have been collected from animals during early or late stage of infection when antigen levels were outside their detection limit. However, both tests when used on a herd basis were capable of identifying positive herds or premises. Therefore, it is important to collect samples from several animals which may be at different stages of the disease.

During an outbreak of PPR in goats in ICT, the infection was further complicated due to a disease similar to contagious ecthyma (Fig. 6). Almost 35 of 55 animals showed severe mouth lesions (papules, pustules, ulceration covered with thick tenacious scabs). Such Secondary infection due to orf virus, a type species of the genus Parapox virus, has also been reported following PPR outbreaks in goats (Radostits *et al.*, 1994). Our experience in the field has proved that strip test is rapid, easy to perform and does not require sensitive skills and equipment. In a country like Pakistan where insufficient diagnostic facilities and trained man-power are a constraint in the reporting of any economically important livestock disease, this Clearview™ technology is highly valuable to minimize the losses caused by various infections.

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