Canadian Food Inspection Agency | Agence canadienne d'inspection des aliments

Canadian Food Inspection Agency




Our vision:
To excel as a science-based regulator, trusted and respected by Canadians and the international community.

Our mission:
Dedicated to safeguarding food, animals and plants, which enhances the health and well-being of Canada's people, environment and economy.

Evaluation of Cell Substrates and the Development of RT-PCR and Real-Time RT-PCR Assays for Peste des Petits Ruminants (PPR)

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Peste des petits ruminants (PPR) disease

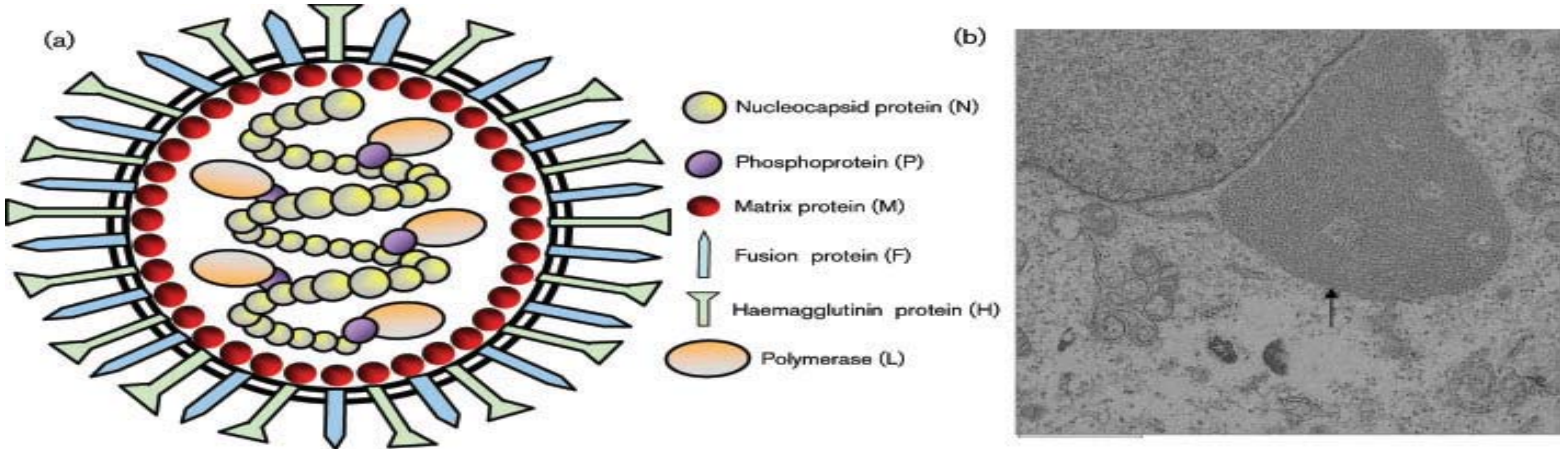
Acute viral disease which causes belongs to the *Morbillivirus* genus of the *Paramyxoviridae* family and characterized by fever, erosive stomatitis, enteritis, pneumonia and death.

The clinical disease resembles Rinderpest in ruminants, which is acute, and after an incubation period of 3-6 days, the clinical symptoms become apparent, which include high rise of temperature, oral and ocular discharges, severe pneumonia, dyspnoea, coughing, enteritis, severe diarrhoea followed by death.

The disease is highly contagious with morbidity and mortality rates reaching as high as 100 per cent and 90 per cent, respectively.

The disease is transmitted by aerosols between animals living in close contact and substantial amount of virus is known to be present in the ocular, nasal secretions and as well as faeces of the infected animals.

PPR virus



Virus	Genome	N	P	C	V	M	F	H	L
Peste des petits ruminants	66.98	79.66	58.65	54.02	54.61	89.82	82.74	59.14	83.44
Measles	70.50	82.70	65.35	65.34	64.55	92.26	85.98	67.33	87.73
Canine distemper	63.58	75.38	53.57	52.91	44.67	83.93	78.75	46.26	81.05
Dolphin morbillivirus	65.91	79.69	57.91	55.29	50.50	89.29	84.52	57.14	83.29

Percentage similarity of the RPV Kabete 'O' strain to other morbilliviruses

A. C. Banyard and others

Journal of General Virology (2010), **91**, 2885–2897

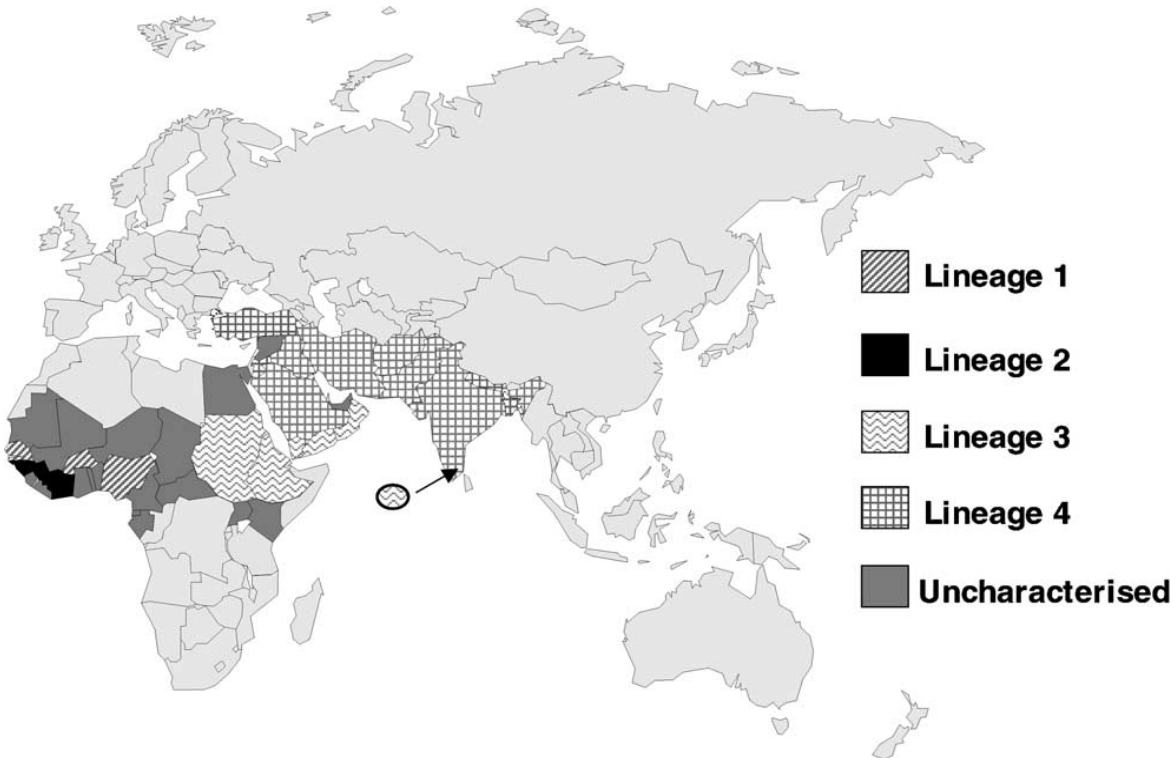
Geographical distribution of PPR

Table 2.1: First report of PPR from different countries

Sr. No.	Location / Country	Reference
1	Ivory coast	Garagadennec and Lalane (1942)
2	Senegal	Mornet *et al.* (1956)
3	Chad	Provost *et al.* (1972)
4	Togo	Benazet *et al.* (1973)
5	Benin	Bourdin (1973)
6	Ghana	Boniwell and Gibbs (1976)
7	Nigeria	Hamdy *et al.* (1976)
8	Oman	Hedger *et al.* (1980)
9	Sudan	Ali and Taylor (1984)
10	Saudi Arabia	Furley *et al.* (1987)
11	India	Shaila *et al.* (1989)
12	Jordan	Lefevre *et al.* (1991)
13	Israel	OIE (1993)
14	Ethiopia	Roeder *et al.* (1994)
15	Kenya and Uganda	Wamwayi *et al.* (1995)
16	Pakistan	Amjad *et al.* (1996)

Dr Ashish Master Thesis 2004

Figure 1: Geographic distribution of Peste des Petits Ruminants. I, II, III & IV correspond to different lineages of PPR distributed around the world.



P. Dhar et al. / Veterinary Microbiology 88 (2002) 153–159

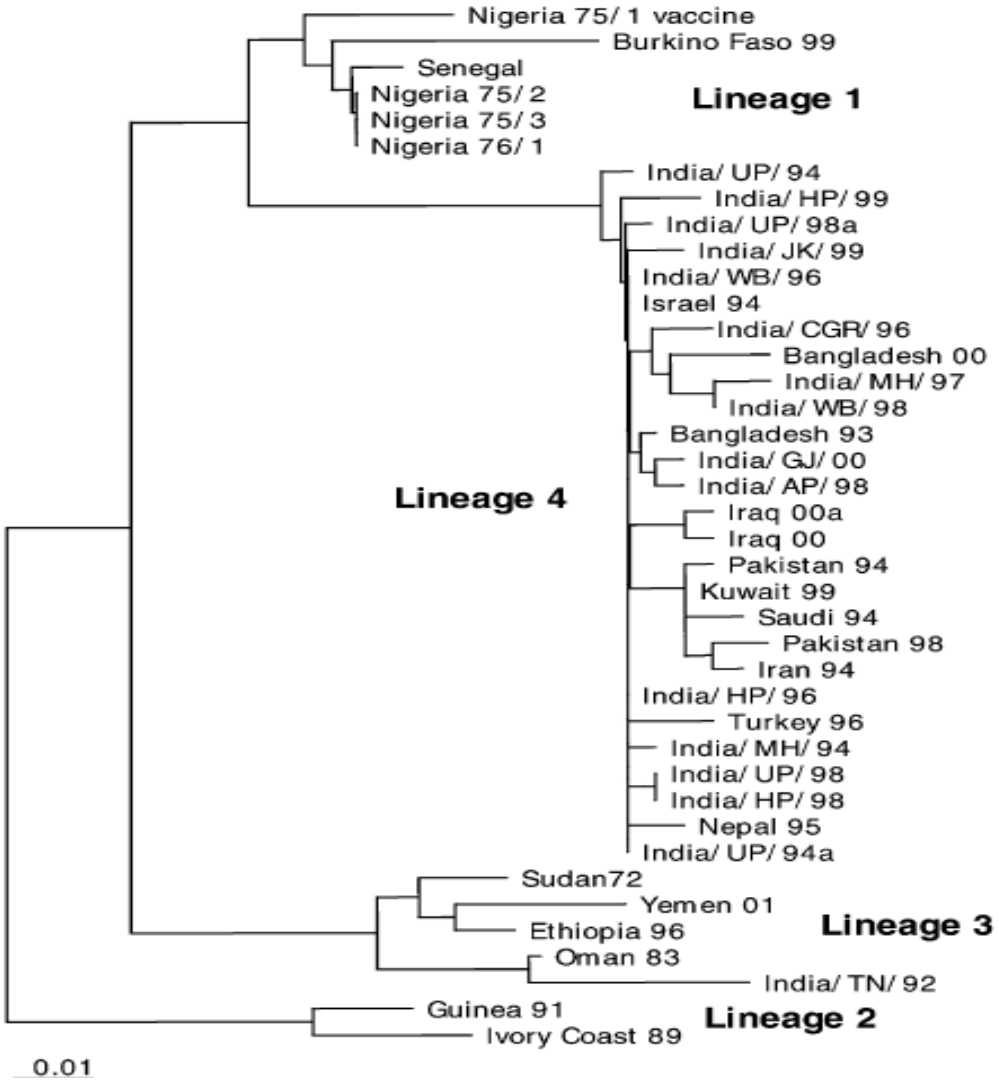


Figure 2: To shown the relationship between the different isolates of PPRV

Figure 3: Distribution of PPRV across Asia from 2000 to 2010

Laboratory tests

Serology to detection PPR both of antibodies and antigen with ELISA techniques; hemagglutination test; latex agglutination test; counter immunoelectrophoresis (CIEP) or agar gel immunodiffussion (AGID);

Using vero E6 and primary lamb kidney for attempted isolation PPR and can be confirmed by virus neutralization or molecular techniques.

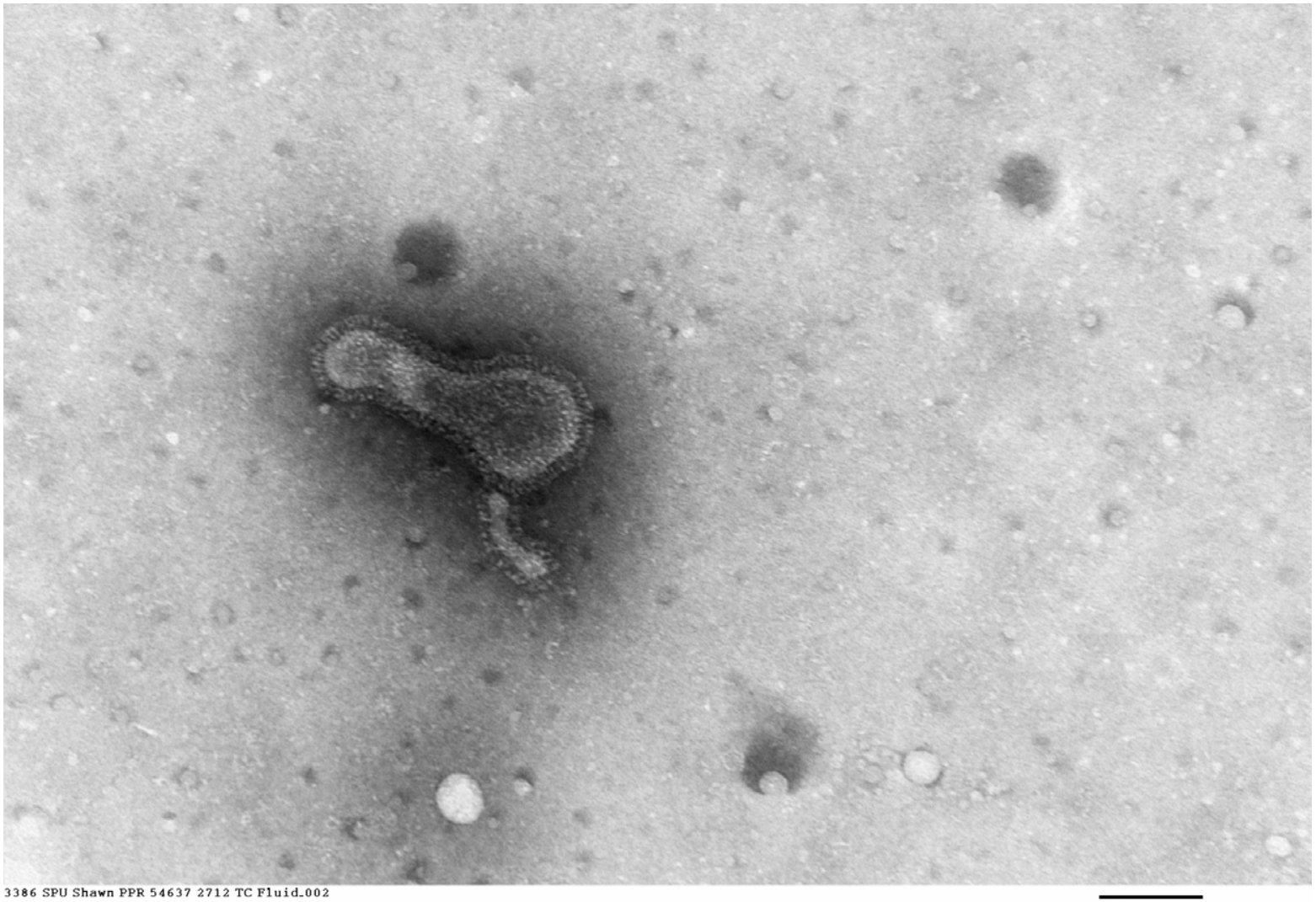
RT-PCR and Real-time RT-PCR.

Objectives

Development of RT-PCR and Real-time RT-PCR assays for diagnostic Peste de Petits Ruminant virus.

To evaluate the utility of OA3Ts, OA4Ks1 and Vero E6 cells for PPR propagation, titration and serological detection in comparative manner using primary lamb kidney (LK) cells.

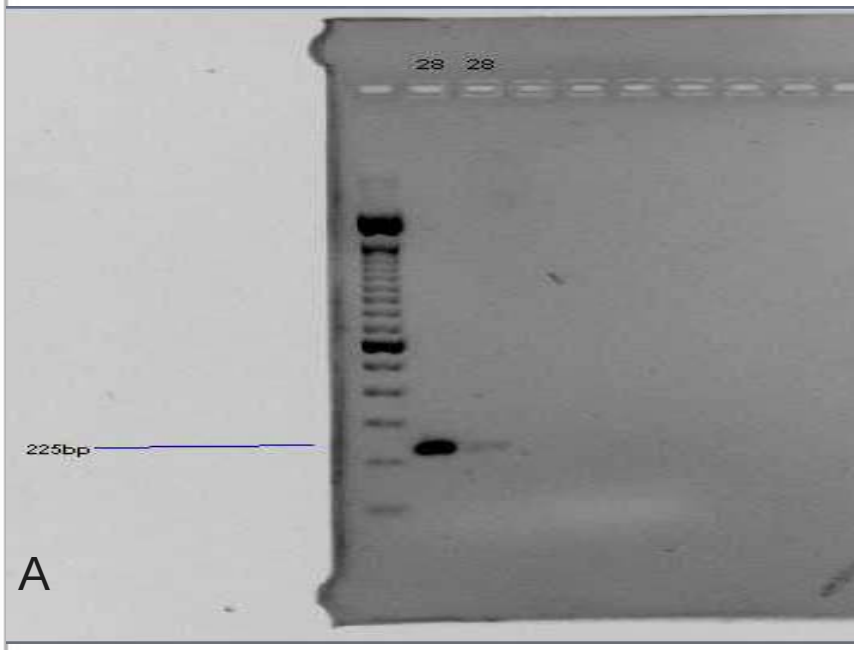
Virus detection in cell culture supernatants by electron microscopy

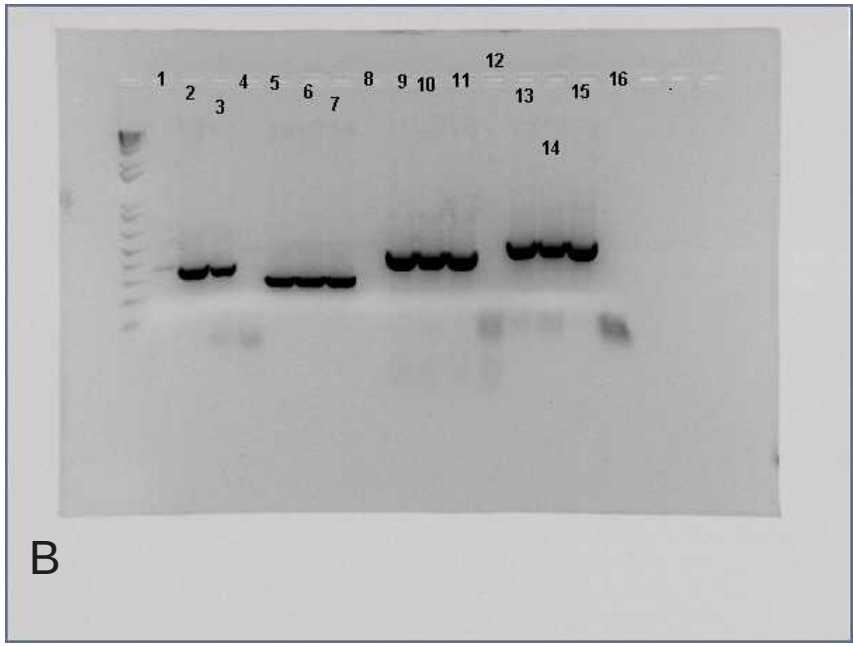


Primers designs for RT-PCR and Realtime PCR based on information lineage III from GenBank

Primers for RT-PCR	Amplicon size
NUF1 5'CCTTCCTCCAGCACAAAA 3'	225bp
NUR1 5' GCCGATCTTTGAGCCTCACGAG 3'	
NF1 5'TCTCGGAAATCGCCTCACAGACTG3'	350bp
NR1 5'CCTCCTCCTGGTCCTCCAGAATCT3'	
FNMY 5' CGCGCCTTCCTCCAGCATAAAA 3'	250bp
RNMY 5' TGGCCATGGCCTGTAGTCT 3'	
FPMY 5' ATGTTTATAGCCACGGGGGT 3'	500bp
RPMY 5' CTTGACTCTGGCACAGCTTGG 3'	
Primers for Realtime PCR	130bp
NrF1 5'TGACCAGGGAAGAAGTCACA 3'	
NrR1 5'TCGTCTTCAGGCATGATCTC 3'	
NrP 5'Fam TTGTCCTTCTCGTCGGGCCC 3'Tam	

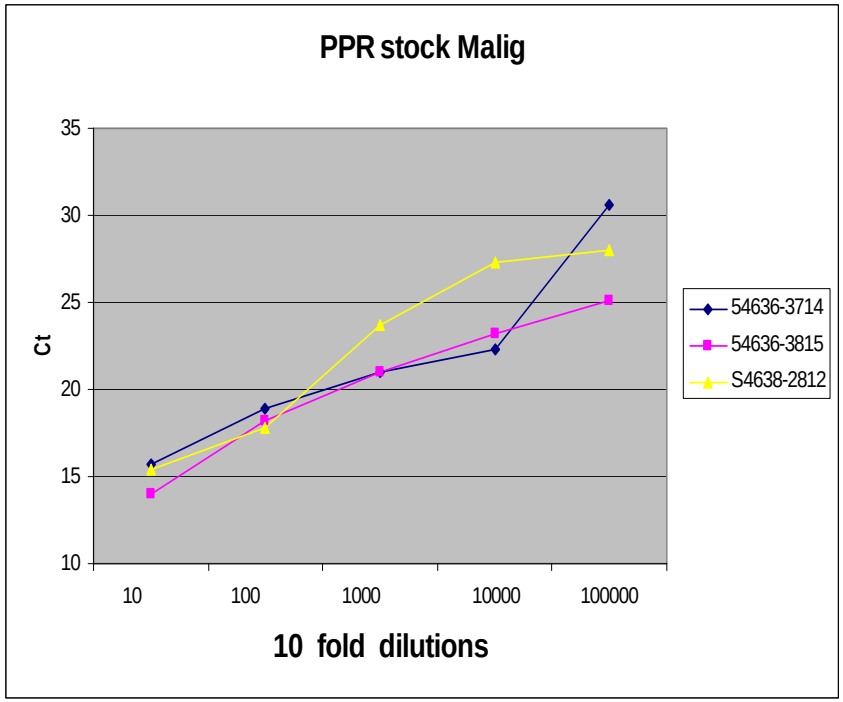
Primers Generate PCR products of the expected size

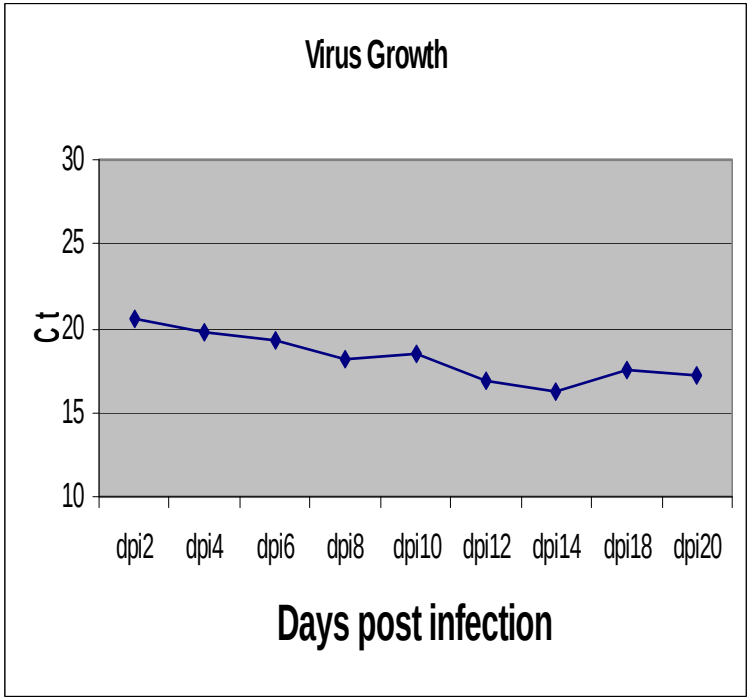




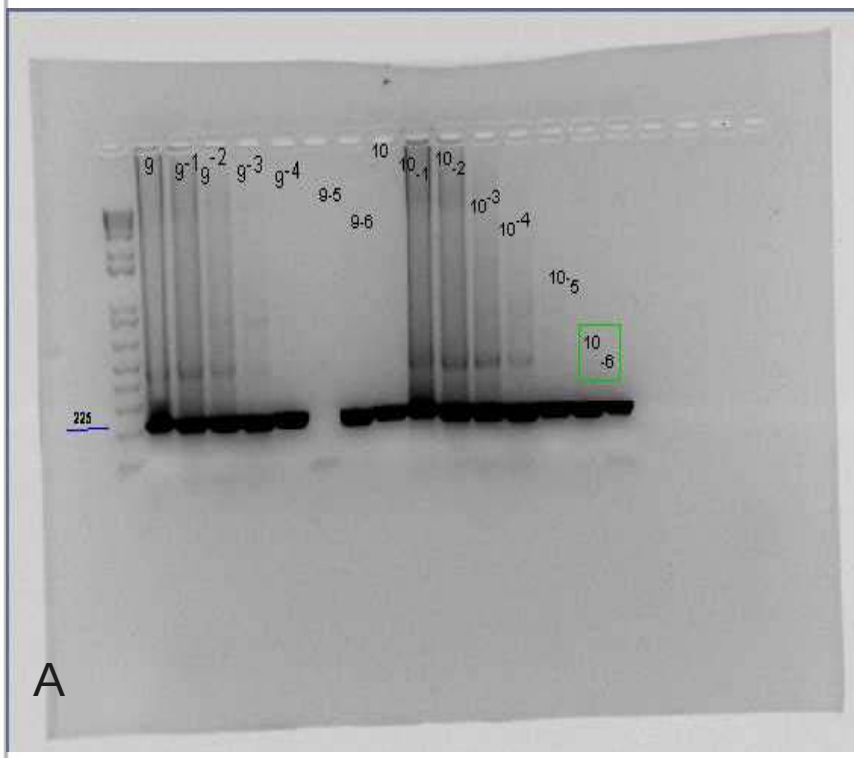
A. # 28 to shown 225 bp with primers NUF1 and NUR1
B. Lane 1, 2 and 3 are showing 350 bp with primers NF1 and NR1; Lane 5,6,7 are showing 225 bp with primers NUF1 and NR1; Lane 9,10,11 and lane 13,14,15 are showing around 500 bp with primers PNMY and RNMY1 or RNMY2

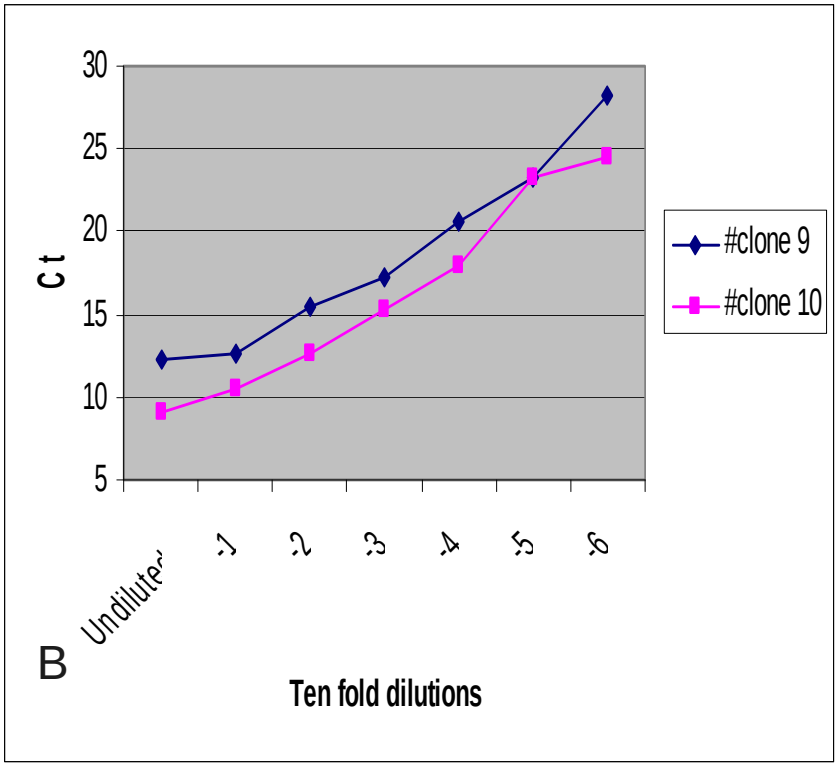
Semi-quantitative detection of PPR by Realtime RT-PCR





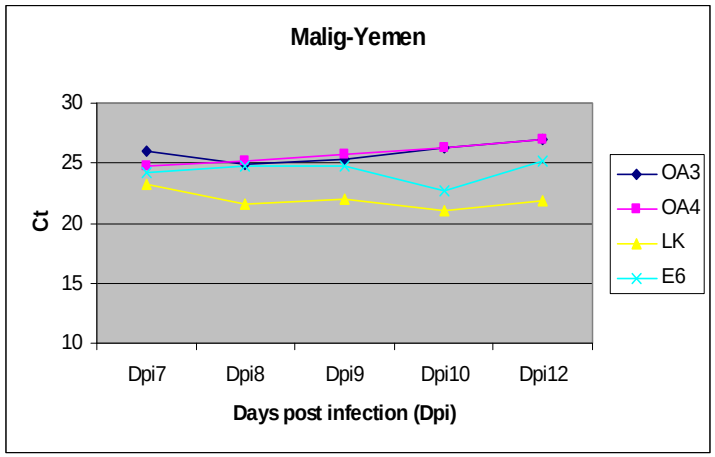
Design of plasmid for standard curve to use in quantitative Realtime RT PCR

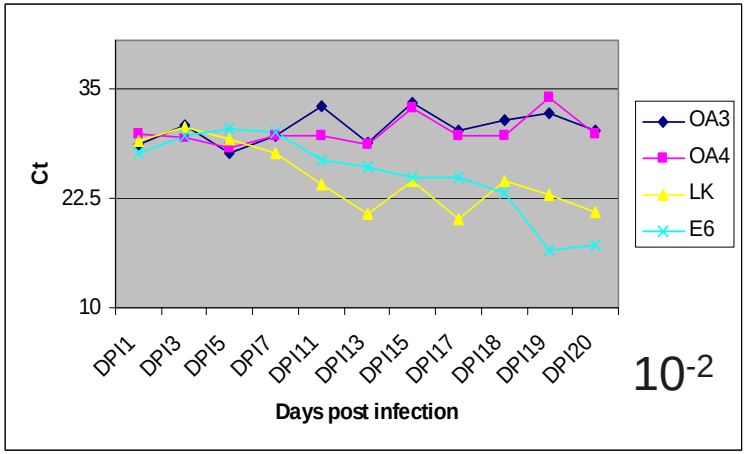


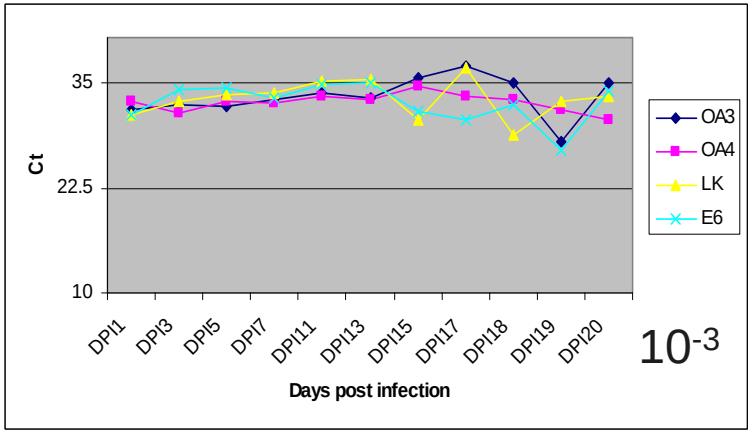


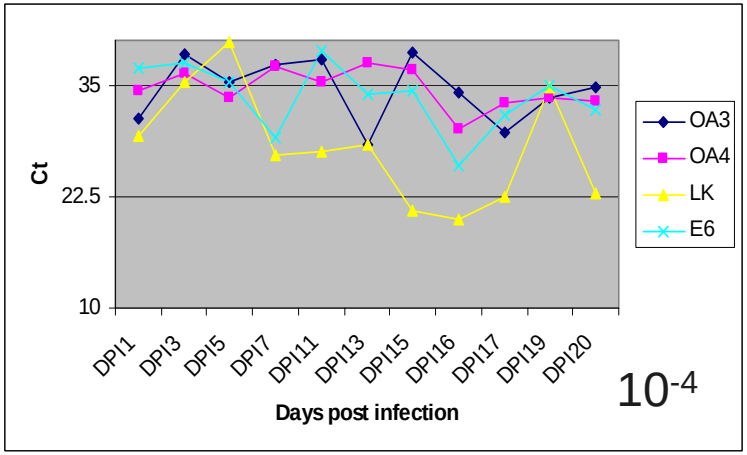
A. Ten fold dilution clone 9 and 10 with primer NUF1 and NUR1
B. To shown Ct value of clone 9 and clone 10

Comparing virus replication in different cell lines by Realtime RT-PCR

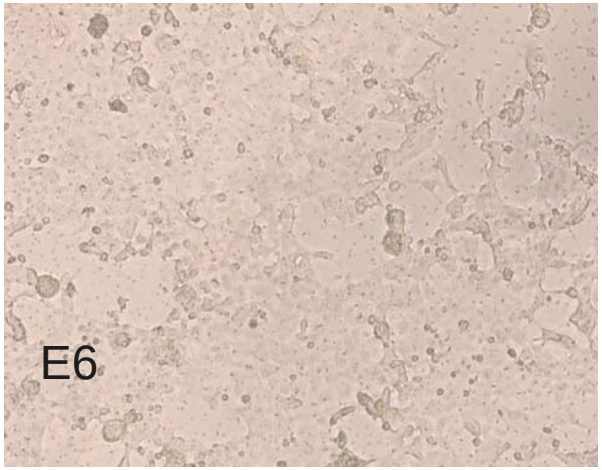


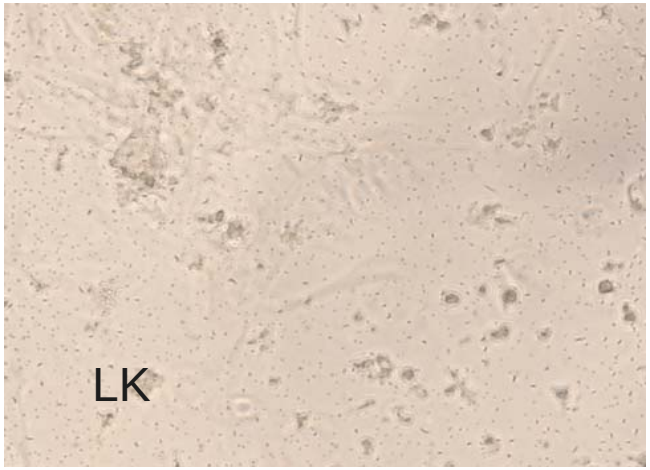


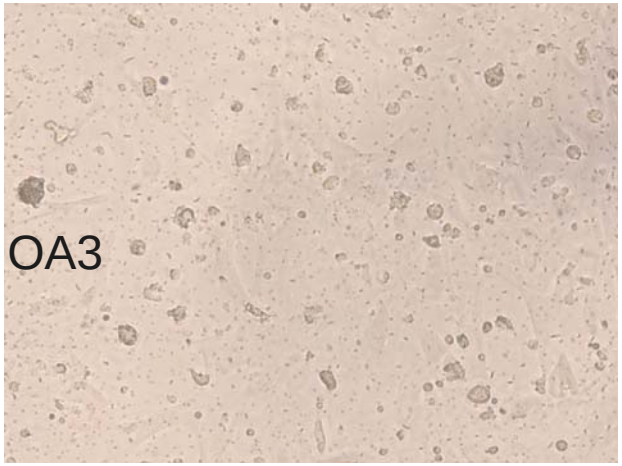


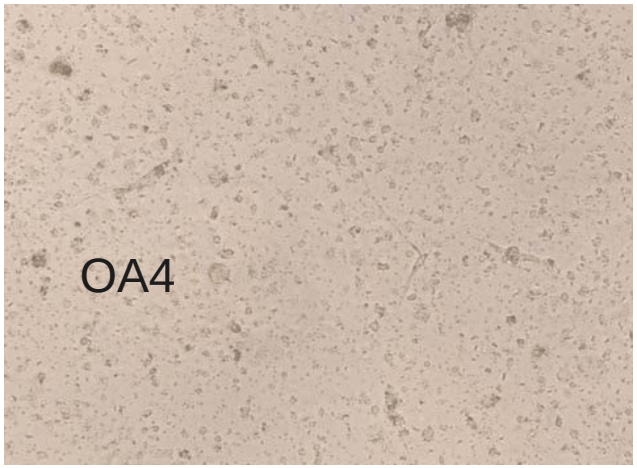


CPE in PPR virus infected cells









Conclusions:

We have established primers both of RT-PCR and Real Time RT-PCR to carry out diagnostics for Peste de Petits Ruminant virus

Currently evaluating PPR viral growth using TCID50 to confirm RT-PCR results

Acknowledgements

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