

## Identification of Peste-des-petits ruminants, Georgia

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### Background

The Peste-des-petits ruminants virus (PPRV) is the cause of a highly infectious transboundary animal disease that primarily affects sheep, goats and small wild ruminants. It is presently being targeted by international organizations for global eradication by 2030. Between January and March 2016, outbreaks of PPR were reported in three farms located near Tbilisi, the capital of Georgia. Of 3,740 susceptible sheep 415 (11%) showed symptoms of PPR.

### Methods

Organ and swab (nasal and ocular) samples were collected and tested in the Laboratory of the Ministry of Agriculture, Tbilisi using a PPR Antigen Capture ELISA (ID.Vet, France). Six positive samples were individually adsorbed onto the matrix of a ViveSTM transport tube (ViveBio, USA) and were shipped to the Institute for Veterinary Disease Control, Austria, for further characterization. Upon arrival in Austria, the samples were eluted from the ViveSTM with 1 ml of Dulbecco's Modified Eagle's Medium High Glucose medium and stored at -80°C. Total RNA was extracted from 200 µl aliquots using an RNeasy kit (Qiagen, Germany). The extracted RNA samples were analysed by RT-PCR to amplify a fragment of both the PPRV Nucleo-protein (N) and Fusion protein (F) genes. Three of the six samples tested were positive by RT-PCR. Amplicons were purified and sent for sequencing using standard Sanger methods at LGC genomics (Berlin, Germany). A phylogenetic tree of N and F gene segments from a representative selection of PPRV sequences available in GenBank was estimated using the maximum likelihood method available in MEGA6 employing the Kimura-2 parameter model of nucleotide substitution and 1000 bootstrap replications.

### Results

The phylogenetic analysis revealed that the PPRVs present in the three Georgian samples were identical and belonged to lineage IV. Notably, the N gene fragment sequences were more related to those of viruses from, Egypt, Eritrea, Ethiopia, and Sudan while the F gene fragment sequences clustered with viruses from Egypt, Ethiopia and Sudan. Unexpectedly, the N and F gene fragment sequences for viruses isolated from countries close to Georgia (e.g. Turkey, Iran and Iraq) were less similar to the Georgian viruses.

### Conclusion

This is the first report of PPR in Georgia. Since there is no obvious connection between Georgia and Egypt, Eritrea, Ethiopia or Sudan through the trade or import of small ruminants, further work is required to fully understand PPRV circulation at a regional level.

## Imaging of BVDV host cell entry

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### Background

Bovine viral diarrhoea virus is an economically important pathogen of cattle within the family Flaviviridae, genus Pestivirus. Its enveloped virus particles integrate three viral glycoproteins, Erns, E1 and E2. E2 is interacting with the cellular receptor CD46 to mediate virus entry via clathrin-coated pits. Fusion occurs in the endosome and results in the release of the viral nucleocapsid complex into the cytoplasm.

### Methods

E2 protein of the cytopathogenic BVDV strain C86 was tagged with a fluorophore and resulting virus particles were purified. Bovine CD46 was tagged with a fluorophore and inducibly expressed in porcine SK6 cells. The system was fixed at different time points after virus addition and imaged on a Zeiss AiryScan microscope.

### Results

Association of virus particles could readily be observed at 5, 8, 10 and 12 min after virus addition.

### Conclusion

We have established a system for the monitoring of BVDV host cell entry which will be employed to monitor virus entry using life cell imaging and to correlate these data with electron cryo microscopy to further elucidate morphological changes during virus entry.