A bovine viral diarrhea virus (BVDV) amplification method combined with an enzyme immunoassay was developed to detect BVDV antigens in seropositive cattle. Reconstitution assays conducted by adding decreasing amounts of BVDV (Singer strain) to Madin-Darby bovine kidney (MDBK) cells showed that the sensitivity threshold of the combined assay was 10^-7 TCID50. BVDV amplification was carried out in polycation (DEAE-Dextran and polybrene)-treated MDBK cells. Treated cells were able to replicate both ether-treated virus and neutralizing antibody-coated virus. Ammonium chloride decreased virus replication in polycation-treated cells, suggesting viral penetration by endocytosis. BVDV detection was tested in leukocytes from 104 seropositive cattle from 2 unvaccinated commercial closed dairy herds with high seroprevalence. Lysates and co-cultures of peripheral blood leukocytes (PBL) were tested, directly or after up to 6 blind passages in normal or polycation-treated cells. BVDV was detected in 10/104 cattle after only one co-culture of PBL in treated cells. No virus was detected in whole blood or plasma samples. BVDV positive and negative cattle were retested three times, achieving consistent results. The finding of immune carriers supports the possibility that these animals may constitute an epidemiological risk.

Keywords
Bovine viral diarrhea virus (BVDV), epidemiology, detection, amplification, polycations, cELISA.