Supplementary information

Materials and methods

Virus isolation

The inocula were prepared as described by Rubbenstroth *et al.* [2] with slight modifications. The brains were homogenized in DMEM containing 2% fetal calf serum (FCS), and then sonicated on ice. The homogenates were centrifuged at 10,000 × g for 10 min at 4°C. The supernatants were filtrated with 0.45 μm filter, which was used as the inocula. The inocula were added to semi-confluent QT6 cells, and the media were changed within 12 hours after the inoculation. The cells were first passaged after 24 hours, and then the cells were maintained in DMEM containing 10% FCS. The cells were passaged every three or four days.

RT-PCR and sequencing analysis

The RNAs were isolated from the brain samples and the inoculated cells using QIAamp Viral RNA Mini Kit (QIAGEN), High pure viral nucleic acid kit (Roche), or RNeasy Plus Mini Kit according to the manufacturer's instructions. The extracted RNA samples were reverse-transcribed with random hexamer using Verso cDNA Synthesis Kit (Life Technologies) or PrimeScript RTase (TaKaRa) in accordance with the manufacturer's protocol, which was used as the templates for the PCR analyses.

PCR was carried out using Ex Taq Hot Start Version (TaKaRa) and primers MH175 (5'-AARGARTAYYTIAAYGARTGYATGGAYGC-3') and MH170 (5'-GGRTTYTCYTTYTTICTCCARTAAAANGC-3') [1] with the following cycles: initial denaturation at 94°C for 2 min, 40 cycles of 94°C for 30 sec, 55°C for 30 sec and 72°C for 20 sec, and final extension at 72°C for 3 min. The PCR products were analyzed by

agarose gel electrophoresis. For sequencing, the PCR products were purified with NucleoSpin Gel and PCR Clean-up (MACHEREY-NAGEL) or by ethanol precipitation. The purified PCR product of sample 1 (Table 1) was cloned with TOPO TA Cloning Kit for sequencing (Life Technologies) and sequenced with the M13 forward primer (5'-GTAAA ACGACGGCCAGT-3'). The other PCR products were directly sequenced with primers MH175 or MH170.

Immunofluorescence assay (IFA)

For IFA, the inoculated cells were fixed with 4% paraformaldehyde for 10 min at room temperature (RT). The reaction was quenched with 0.1M glycine in PBS for 10 min at RT. The cells were permeabilized with 0.1% saponin in PBS for 10 min, and then blocked with 5% FCS in PBS for 30 min. The cells were incubated with rabbit anti-Borna disease virus 1 (BoDV-1) P antibodies diluted at 1:500 with 1% FCS in PBS for 1 h. After four times washing with PBS, the cells were reacted with anti-rabbit IgG-Alexa Fluor 488 (Life technologies) diluted at 1:1000 with 1% FCS in PBS for 1 h. The cells were washed four times with PBS, and stained with DAPI for 10 min. After five times washing, the images were obtained using LSM-710 confocal microscope (Zeiss).

Western blotting

The inoculated cells were once washed with PBS, and directly lysed with SDS sample buffer. The cell lysates were electrophoresed with 12% acrylamide gel, and then transferred to a PVDF membrane. The membrane was blocked with 3% skim-milk in Tris-buffered saline containing 0.1% Tween-20 (TBS-T). The blot was incubated with

rabbit anti-BoDV-1 P antibodies (HB03) diluted at 1:1000 with 3% skim-milk in TBS-T for 1 h at RT. After three times washing with TBS-T, the membrane was incubated with anti-rabbit-Ig conjugated with horseradish peroxidase (Dako) for 1 h at RT. After four times washing with TBS-T, the membrane was incubated with ECL Prime Western Blotting Detection System (GE healthcare), and analyzed by Fusion Solo S (Vilber Lourmat).

References

1. M. Horie, K. Ueda, A. Ueda, T. Honda and K. Tomonaga 2012. Detection of Avian bornavirus 5 RNA in Eclectus roratus with feather picking disorder. *Microbiol Immunol* **56**: 346-349.

2. D. Rubbenstroth, M. Rinder, B. Kaspers and P. Staeheli 2012. Efficient isolation of avian bornaviruses (ABV) from naturally infected psittacine birds and identification of a new ABV genotype from a salmon-crested cockatoo (Cacatua moluccensis). *Vet Microbiol* **161**: 36-42.