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Letter to the Editor

# Failure to detect Borna disease virus antigen and RNA in human blood

**Abstract**

*Background:* Borna disease virus (BDV) is the etiological agent of a rare progressive meningoencephalitis that affects mostly horses and sheep. There is an unresolved debate whether also humans are susceptible to infection with BDV and if so, whether this might be associated with neuropsychiatric diseases. One recent key publication employing an ELISA-based sandwich assay reported prevalences of BDV-specific circulating immune complexes in human blood as high as 30% in the normal population and up to 100% in psychiatric patients [Bode L, Reckwald P, Severus WE, Stoyloff R, Ferszt R, Dietrich DE, et al. Borna disease virus-specific circulating immune complexes, antigenemia, and free antibodies—the key marker triplet determining infection and prevailing in severe mood disorders. Mol Psychiatry 2001;6(4):481–91]. However, this report did not examine for the physical presence of BDV antigens in human blood, and therefore, these seemingly high prevalences may not reflect Borna virus-specific signals.

*Objectives:* We attempted to correlate string plasma signals in the particular sandwich ELISA system with the presence of BDV antigens.

*Study design:* Four preselected plasma samples with high reactivity in the described assay were analysed by immunoaffinity purification and highly sensitive real-time RT–PCR.

*Results:* Neither method did provide any evidence for the presence of viral proteins or nucleic acids.

*Conclusions:* Our findings argue against the concept that the described sandwich ELISA reliably detects BDV-specific antigens in human blood, therefore do not support the hypothesis that BDV is a pathogen of humans.



*Keywords:* Borna disease virus; Mental disorders; Viral diagnostics

*Sir,*

Borna disease virus (BDV) causes a non-purulent meningoencephalitis in horses and other animals (Rott and Becht, 1995). Previous sero-epidemiological studies have suggested that BDV infections are associated with neuropsychiatric diseases in humans, but conclusive evidence for this hypothesis is lacking, which is in part due to the lack of generally accepted diagnostic markers to verify a BDV infection *intra vitam* (Lipkin et al., 2001). By employing an ELISA-based diagnostic sandwich assay with the two monoclonal antibodies (mAb) W1 and Kfu2 it was recently reported that BDV-specific circulating immune complexes (CIC) are detectable in about 30% of plasma samples from healthy human blood donors and in up to 100% of psychiatric patients (Bode et al., 2001). However, neither this report nor any other study in the literature did provide compelling evidence for the physical presence of viral antigens in ELISA-reactive blood samples. This raised the possibility that the reported high prevalence of individuals with a BDV infection was at least in part due to cross-reactivity of the mAbs with plasma components and emphasized the need for thorough validation of diagnostic assays for BDV.

We attempted to detect BDV antigen in human plasma samples exhibiting high reactivity in the described sandwich ELISA (Bode et al., 2001) by immunoaffinity purification. A proteinG agarose-based affinity-matrix with immobilized W1/Kfu2 was prepared and shown to specifically retain the viral N and P antigens from lysates of persistently BDV-infected culture cells in a low pH-reversible manner (Fig. 1A). We then fractionated on a W1/Kfu2 affinity-column 5 ml aliquots of four different human apherese plasma with strong reactivities (rating: +++) in the CIC-ELISA described by Bode et al. (Fig. 1B). We easily detected by immunoblotting the viral N and P proteins in BDV-infected cell extract, but failed in parallel to obtain specific signals for viral proteins in any of the eluates (Fig. 1B). Immunoglobulin heavy and

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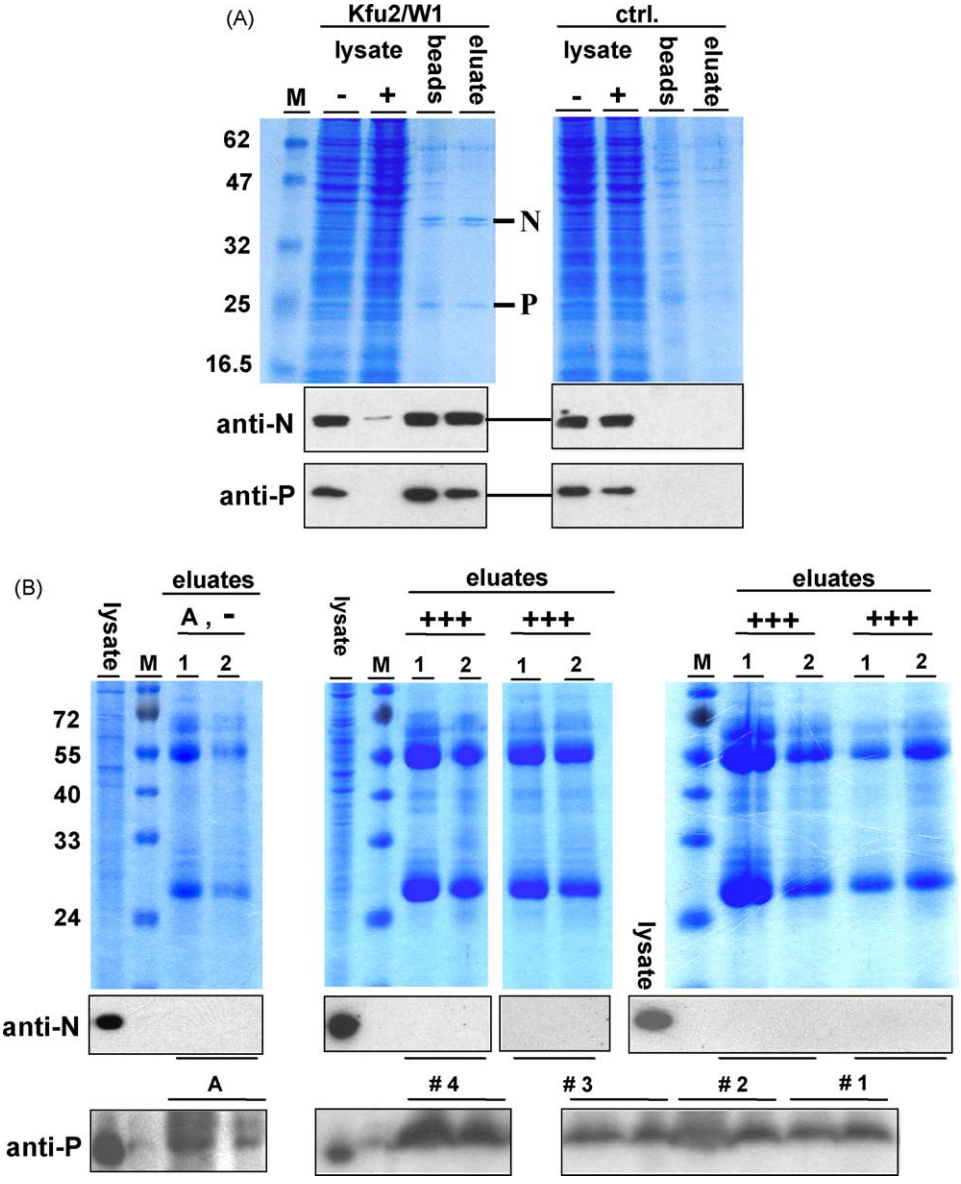


Fig. 1. (A) Characterisation of an immunoaffinity matrix with specificity for the BDV N and P proteins. The 20 μl virus-specific (lanes, Kfu2/W1) and control (lanes, ctrl.) affinity matrix was incubated with extract from $2.5 \times 10^6$ persistently BDV/He80-infected 293 cells. The beads were washed and viral antigens were eluted in two volumes of 0.1 M glycine–HCl, pH 2.8. Aliquots of the fractions were separated on SDS gels and analyzed by Coomassie staining (upper panel) and immunoblottings (lower panels) using the BDV N-specific mAb Bo18 and P-specific rabbit antiserum. The 5 μl aliquots of the lysate before and after incubation with the affinity beads (lanes, lysate − and +) were separated in parallel with a 2.5 μl aliquot of the bead fractions before elution and 5 μl of neutralized pH 2.8-eluate (lanes,"beads" and "eluate"). M, molecular mass markers. (B) Fractionation of human plasma by Kfu2/W1 affinity chromatography. About 5 ml of four plasma samples each with strong reactivities in the CIC-ELISA (lanes, +++) and of an unreactive control plasma (lanes, A, −) were diluted 1:10 and passaged over the Kfu2/W1 affinity-column (volume: 1 ml). Proteins retained after washing were eluted with two 1 ml fractions of 0.1 M glycine–HCl and further concentrated 25-fold by TCA precipitation. Aliquots corresponding to 125 μl of the original eluate were run on SDS gels (lanes, 1 and 2). The separated proteins were analysed by Coomassie staining (upper panels) and by immunoblotting for the viral N and P proteins (lower panels). The positions and numbers of corresponding plasma samples in the N and P blots are indicated by horizontal lines. Note that the band with a slightly slower migration than the viral P protein was also stained when pre-immune instead of P-specific serum was used, indicating the detection of cross-reactive IgG light chain by the secondary antibody.

light chains were found in the eluate fractions, which were presumably retained by the backbone of the column.

About 5 ml of the reactive plasma samples should contain at least 500 ng of BDV antigen (Bode et al., 2001). Using purified recombinant N protein the limit of detection in our immunoblot system was approximately 5 ng per protein band. However, in the concentrated eluate fractions that each represented at least 1/8 of the input volume, no virus-specific signals were observed. Thus, our study failed to detect BDV antigens in four plasma donations with high reactivity in the described diagnostic ELISA test (Bode et al., 2001). This rather suggests that the signals in these samples were

due to other reasons such as cross-recognition of plasma proteins by the mAbs W1 and/or Kfu2. All reactive samples also failed to give a signal when analyzed in a highly sensitive real-time RT–PCR that detected 150 copies/ml of a synthetic BDV RNA transcript, further arguing for the absence of virus or viral RNP. Detailed informations on the methods used are available from the authors on request. In conclusion, our findings do not support the hypothesis that the sandwich ELISA as described by Bode et al. reliably and selectively detects BDV-specific antigens in human blood. They strongly argue against the appropriateness of this test as tool for the diagnostics of BDV infections in humans. Further investigations are necessary to establish whether or not BDV is a pathogen of humans and to determine its prevalence.

## References

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