

Response to the Letter to the Editor by Wolff et al. (2006)

Presence of Borna disease virus (BDV)-specific structural components in human blood plasma

Sir,

Investigations in using ELISA to detect antigen and CIC (Bode et al., 2001) have provided preliminary evidence that among healthy donors up to 30% may have been exposed to BDV and for detection of antigen in plasma (N/P proteins) in up to 5%. Detection of BDV antigen and CIC has also been reported in patients with acute depression (Bode et al., 2001; Kamhieh, 2005). Further evaluation of the specificity of these assays – based on two monoclonal antibodies (mAbs; W1/Kfu2) – is welcome, as is development of procedures for confirmatory testing of ELISA-positive sera.

In our view, there are some methodological issues with the experiments reported by Wolff et al. (2006) concerning testing of plasma samples through immune affinity purification.

1. There appears to be no dispute (Wolff et al., 2006) that the antibodies W1 and Kfu2 (Bode et al., 2001) react strongly with BDV proteins in lysates of infected cells. However, lysates are not appropriate to validate the sensitivity of the system. Instead, defined amounts of recombinant proteins, comparable to those reported for reactive samples, should be used to “spike” non-reactive sera and thus validate the experimental system. No such data are presented.
2. Of the sera analysed, neither the provenance nor antigen-ELISA reactivity (in addition to CIC results) at the time of testing are reported. Likewise, Wolff et al. (2006) fail to comment on the efficiency of a low-pH elution for dissociation of bound antigen-antibody complexes. For example, in many immune affinity purification protocols, low pH elution is followed by high pH elution to ensure dissociation of all immuno-affinity bound molecules.
3. The finding of immunoglobulin heavy and light chains in the eluates is expected as a result of purification of BDV-specific CIC. Furthermore, in both reactive and non-reactive plasma, the explanation that presence of

human anti-mouse antibodies (HAMA) could account for purification of immunoglobulins, is not considered (Wolff et al., 2006). Using a commercially available kit (Roche) HAMA (unrelated to BDV-ELISA reactivity) were detected in up to 10% of Australian sera (psychiatric patients, multi-transfused patients, blood donors, pregnant women) with levels up to 79.3 ng/ml (Kamhieh, 2005).

The letter did not report whether BDV genomic RNA was present prior to analysis. In order to optimise detection, particularly with a low-titre labile RNA virus such as BDV fresh plasma samples should be used. It is unfortunate that the authors did not include analysis of a human serum known to be BDV RNA positive at the time of testing.

For the reasons outlined above, we suggest that the data presented by Wolff et al. (2006) do not support a conclusion that the sandwich ELISA fails to detect BDV-specific antigens in human blood. A simple analysis for antigen and CIC with the anti-BDV-N-specific mAb Bo 18 used and mAb W1, used in the original tests (Fig. 1), would provide a comparison of the sensitivity of the system applied (Wolff et al., 2006) with ELISA (Bode et al., 2001).

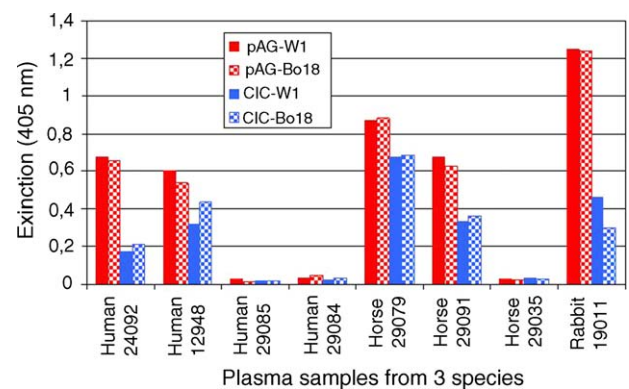


Fig. 1. Detection of BDV-specific native antigens (pAG) and immune complexes (CIC) in randomly selected plasma samples from human subjects, horses and an experimentally infected rabbit by ELISA. Comparison of anti-BDV N/p40 monoclonal antibodies W1 and Bo 18, used in parallel as catching antibodies (both hybridoma supernatants diluted 1:500). Bo 18 supernatant was kindly provided by Wolff. Extinction values refer to 1:2 dilution in the pAG-ELISA and 1:20 dilution in the CIC-ELISA; cut off value \leq Ext. 0.1 (for detailed methods, see Ref. (Bode et al., 2001)).

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References

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