Detection of antibodies to Borna disease virus (BDV) in Turkish horse sera using recombinant p40

Brief Report

H. Yilmaz¹, C. R. Helps², N. Turan¹, A. Uysal³, and D. A. Harbour²

¹University of Istanbul, Veterinary Faculty, Department of Microbiology, Avcilar, Istanbul, Turkey
²University of Bristol, Department of Clinical Veterinary Science, Bristol, U.K.
³University of Istanbul, Veterinary Faculty, Department of Internal Medicine, Avcilar, Istanbul, Turkey

Accepted August 8, 2001

Summary. The nucleoprotein of Borna disease virus (BDV-p40) was produced in a Baculovirus expression system using sf9 cells. The purity and specificity of the recombinant p40 was confirmed by SDS-PAGE and immunoblotting. The recombinant p40 was used in an ELISA to screen horse sera in Turkey. For this, 323 horses from selected cities in the Marmara region of Turkey were examined clinically and serum was collected from each. All horses were clinically healthy except for a few with wounds on the skin. Antibodies to BDV were detected in the sera of 82 (25%) of 323 horse sera. Six sera were selected that had low, medium or high OD values by ELISA and were analysed by Western blotting. All reacted specifically with p40 at a dilution of 1 in 1000. This is the first report of the detection of Borna disease in Turkey and needs further molecular biological investigations to compare the Turkish strains with those strains detected in Europe.

* Borna disease (BD) was first reported in the 18th century as a neurological disease of horses and was later characterised as a transmissible, progressive polioencephalomyelitis (see [13] for review). Borna disease virus (BDV) infection has since been reported in many warm blooded animals such as sheep, cattle, cat, dog, ostriches and people [3–5, 8, 10, 18, 23]. BDV infection has also been produced experimentally in many species [6, 9, 19, 21].

Borna disease virus (BDV) is a novel RNA virus that is the only member of the family Bornaviridae in the order Mononegavirales. The BDV genome has 6 open
reading frames, only two of which (p24 and p40) are expressed in the majority of infected cells [7]. Sequence analysis has shown a high level of nucleotide and amino acid conservation across isolates from many species, except for one isolate from an Austrian horse [20].

The virus is believed to be transmitted through nasal, conjunctival and salivary fluids and is highly neurotropic, localising in the central nervous system where it affects particularly the grey matter of the limbic system and brain stem [7]. Therefore the clinical signs (ataxia, depression, gait abnormalities) are mainly related to central nervous system abnormalities [5].

BD has been reported in many countries in the world including Central and Northern Europe, North America, Japan, Iran, Israel, Austria, United Kingdom and Sweden [1, 2, 4, 5, 12, 15, 17, 22]. However, there is no report on BD in Turkey. In this study, the presence of antibodies to BDV in Turkish horses was investigated by ELISA using a recombinant p40.

Horses included in the study were from the Marmara region of Turkey. This region is a predominantly agricultural region but there are many racing horses, army horses, private horses and working horses of gypsies. The racing horses are mainly located at the Turkish Jockey Club and Horse Sport Club in Istanbul, while most of the army horses are in Bursa. Gypsies generally have one or two horses as beasts of burden. Private horses generally belong to people who breed horses (racing and others) in a small stud either as a hobby or for commercial purposes.

The sera analysed in the study were collected from private, army and gypsies’ horses. Horse owners in selected cities were asked to participate in the study and consent was gained to bleed the horses. Clinical examinations were performed by checking the mouth, nares, eyes, lymph nodes (axillary, mandibular and prescapular) and skin. Horses were assessed for the presence of nervous disorders and the breed, sex and age of the animals was recorded. Blood was taken from the jugular vein using Vacutainers (Becton-Dickinson) without anticoagulant. A total of 323 horses were examined and sampled between June 1997 and June 1998.

Recombinant BDV p40 antigen was prepared by cloning the p40 gene of an equine isolate of BDV into the plasmid pFastbac/HTb (Fastbac Baculovirus expression system, Gibco BRL). This system fuses a hexa-histidine tag to the N-terminus of the expressed protein allowing purification by Ni-Agarose chromatography. Recombinant baculovirus was produced according to the manufacturer’s instructions and used to infect sf9 cells at an moi of between 5 and 10. Seventy-two hours after infection the cells were harvested and recombinant hexa-histidine-tagged p40 was purified under native conditions using Ni-Agarose according to the manufacturer’s instructions. Purity of the protein was assessed by SDS-PAGE and Coomassie Blue staining. Figure 1 shows a Coomassie Blue stained SDS-PAGE gel of purified recombinant BDV p40. Estimated purity of the protein was greater than 99%. A band slightly smaller than p40 can also be seen on the gel, which probably represents the p38 isoform, produced from use of the second in-frame initiation codon present in the p40 open reading frame [7], since it is also detected by horse sera positive for BDV by ELISA. Serial