Identification of immunodiagnostic antigens for cerebrospinal filariasis in horses by western blot analysis

Masataka TAKESUE1,Yuki OSAKA1, Masanori MURANAKA2, Yoshinari KATAYAMA2 and Hiromi IKADAI1*

1Laboratory of Veterinary Parasitology, School of Veterinary Medicine, Kitasato University, Aomori 034-8628, Japan
2Microbiology Division, Epizootic Research Center, Equine Research Institute, Japan Racing Association, Tochigi 329-0412, Japan

In the present study, the serum and cerebrospinal fluid of horses diagnosed with Setaria digitata cerebrospinal filariasis were analyzed by western blot. The results revealed S. digitata protein bands measuring 65, 34, 22, and 18 kDa in molecular weight. In particular, the 18 kDa band is a possible candidate for clinical immunodiagnosis on the basis of western blot findings.

Key words: cerebrospinal filariasis, Setaria digitata, western blot

Nematodes of the genus Setaria are filarial parasites commonly found in the peritoneal cavity of cattle and other ungulates. Three species, Setaria digitata, S. marshalli, and S. labiatopapillosa, are reportedly found throughout Japan [1, 2].

Generally, Setaria nematodes do not induce disease in their hosts. However, when the parasite infects uncommon hosts such as horses, sheep, and goats, it frequently migrates into the central nervous system, causing tissue damage or potentially acute or lethal upper cerebrospinal filariasis (CF). CF causes severe neurological symptoms in horses. There are some infectious diseases in horses that resemble CF such as West Nile virus infection, Japanese encephalitis, and protozoal illnesses [1, 3–5]; therefore, it is important to avoid misdiagnosis. Unfortunately, there are currently no established immunodiagnostic methods for CF in spite of the fact that other similar diseases can be detected through these methods. Currently, diagnosis of CF relies solely on histopathological analysis.

The present study aimed to establish a method to differentiate between equine CF and other infectious diseases of the equine central nervous system by using a Setaria antigen. Western blot analysis was performed on the serum and cerebrospinal fluid (CSF) of horses inoculated with Setaria digitata antigen via the medullary cavity and of horses carrying a variety of infectious diseases in addition to CF. Furthermore, we investigated the Setaria proteins that could be used as indicators for immunodiagnosis.

Serum and CSF were prepared from horses inoculated with S. digitata antigen. Male and female S. digitata specimens were frozen, pulverized, and dissolved in 2 m of 30 µg/ml gentamicin saline solution. This solution was then injected into the medullary cavity of a horse. After approximately 1 month, the procedure was repeated, and 2 weeks later, serum and CSF samples were collected.

Serum and CSF samples were also collected from 3 horses diagnosed with CF, 3 horses diagnosed with protozoal encephalitis (Sarcocystis neurona infection), and 1 horse diagnosed with spinal stenosis. Western blot analysis was performed to detect the specific proteins for S. digitata. After electrophoresis in 12% polyacrylamide gel and transfer to a membrane, prepared S. digitata antibody solutions (1:10) collected from the inoculated horses were incubated with each sample. After washing with PBS five times, the membranes were incubated with alkaline phosphatase conjugated anti-horse IgG isolated from goat serum (Cappel, U.S.A.). Signals were detected with nitro blue tetrazolium (Wako) combined with 5-bromo-4-chloro-3-indolyl phosphate (Wako).

The results showed a significant amount of variably sized proteins of both low and high molecular weights for both the serum and CSF samples. It was apparent that inoculation triggered S. digitata antibody production. There were weak nonspecific responses in the 80–94 kDa and 45 kDa
regions in both the serum and CSF samples taken before inoculation (Fig. 1). Western blot results were obtained for the inoculated serum/CSF, and the CF serum/CSF showed positive bands at 65, 34, 22, and 18 kDa (Fig. 1). Bands from the protozoal encephalitis samples closely resembled the 65 and 34 kDa bands from the inoculated samples, but the 22 and 18 kDa bands that were seen in the specifically positive CF samples were not observed in the spinal stenosis samples. All of the CF samples showed 22 and 18 kDa bands, and no 18 kDa band was present in any of the serum/CSF samples from the horses diagnosed with other cerebrospinal diseases. These results suggest that the 18 kDa protein signal in the serum and CSF samples could serve as a specific indicator for equine CF. However, it is unknown whether these signals are detected in other CF cases such as those caused by *S. marshalli* or *S. labiato-papillosa*, although these parasites have not been reported to cause equine CF in Japan.

In conclusion, the 65, 34, 22, and 18 kDa signals, especially the 18 kDa band, appear to contain important proteins useful for immunodiagnosis of *S. digitata* CF. By evaluating a larger number of CF cases and identifying the proteins involved, we may eventually be able to use western blot analysis of serum and CSF samples to diagnose equine CF.

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