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Detection of antibodies against infectious Borna disease virus
— a comparison of three serological methods —

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보르나병 바이러스 항체검출을 위한 연구
—세 가지 혈청진단법의 비교—

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초록 : Borna disease(BD) virus 특이항체검출에 대한 세 가지 혈청진단법(간접형광항체법, 세포효소면역반응법, 혈청중화시험)의 정확도를 비교하기 위해 BD virus를 실험적으로 감염시킨 273수의 토끼의 혈청으로 시험하였다.

혈청중 간접형광항체법에 의하여 판정된 123혈청들은 모두 세포효소면역반응법에 의해서 양성으로 판정되었으나 혈청중화시험법에 의해서는 단지 27혈청에서만 양성으로 판정되었다. 혈청중화시험법은 간접형광항체법과 세포효소면역반응법에 비하여 민감도가 훨씬 낮게 나타나 BD virus의 혈청학적 연구에는 간접형광항체법 및 세포효소면역반응법의 활용도가 높을 것으로 생각된다.

Key words : Borna disease virus, Indirect immunofluorescence antibody test, Cell enzyme-linked immunosorbent assay, Serum neutralization test.

Introduction

Borna disease(BD) virus infections in horses and sheep are of economic importance. They cause an encephalomyelitis which is named after the city Borna in Sexony, were severe enzootics of the horse population occurred.[1~3] BD virus shows exclusive affinity to the central nervous system(CNS). Besides natural infections a broad variety of animal species can be infected experimentally, leading either to disease and death or to a lifelong virus persistence without clinical symptoms. The veterinary importance of this infection at the beginning of this century stimulated a variety of clinical, pathological and virological studies, and soon BD become a most interesting research topic. Several publications have given surveys on different aspects of the disease.[4]

The virus has not yet been characterized. Its outstanding ability to induce a persistent infection of neurons has attracted the attention of clinicians, pathologists, virologises and immunologisets and recently also the interest of psychologists, since leaning deficiencies due to persistent BD virus infection have been discovered.

An antibody response in the naturally infected and artificially infected or vaccinated horse and experimentally infected rabbit could only be shown sporadically in numerous animals with symptoms of BD[5], although the brains of infected horses carry significant amounts of specific antigen.[6]

A variety of tests have been reported for the detection and measurement of antibodies to infectious BD virus. These include indirect immunofluorescence antibody test(IFA), complement fixation(CF), immuno- diffusion(ID) and serum neut-

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ralization(NT) test.[7~10] Recently the cell enzyme-linked immunosorbent assay(CELISA) method has been used for detection of BD virus antibody by Pauli et al.[11] However, a detailed comparison of the CELISA method with other standard serological procedures has not yet neen reported. In this study, the CELISA and NT are compared with other standard thchniques IFA presently used for the serological confirmation of BD infection.

Materials and Methods

Viruses : The rabbit-adapted BD virus, strain V[12,13], was used. Ten percent suspensions(w/v) in Eagle's medium, Dulbecco's modification, were prepared by sonication(Branson sonifier, 20 cycles/min, 40mA at 4℃ 1min) of brain pieces, followed by centrifugation at 1000 g for 10min

Sera : Serum samples were obtaind from prof. Ludwig in Virologie, Freie Universitaet Berlin, im Robert Koch Institut, Germany, which were collected from the experimentally, strain V infected rabbits with rabbit-abapted BD virus.

Cell cultures : Embryonic brain cells from rabbits were obtained by mancing the brain and aspirating the tissue once through an infection needle(Luer Lock 18G1 1/2, 4012). This material was suspended in minimal essential medium-(MEM) containing 10% fetal calf serum and seeded into plastic tissue-culture plates. Cells which still remained attached to the plates 24h later were passaged again and used for the experiments when the monolayer become confluent.

Cell enzyme-linked immunosorbent assay : For antibody titrations the second passage of newborn rabbit brain cells were seeded in 96-well microtitre plates(NUNC) and one day later with supernatant virus.[14] In the case of antibody titration, 20-50 focus forming units(ffu) per well were inoculated. The investigations of a variety of fixation and staining procedures resulted in the following scheme, which gave optimal results : Medium poured off, cells in the plates fixed with 3% formaldehyde in PBS(pH:7.5) at 4℃. When required for use, poured off the fixative, washed once and incubated with Triton ×-100 in PBS(TX-PBS) for ½ h at foom temperature, then twice washed with PBS plus 1% foetal calf serum(fcs) and incubated with geometrically diluted serum samples for 1h at room temperature. Thereafter thrice wasched with PBS/fcs and incubated with appropriately diluted anti-immunoglobulin antibodis coupled to horseradish peroxidase for 1h at room temperature. Again three washes with PBS/fcs followed by incubation with bydrogen peroxide(H_2D_2) activated 30amino-9-ethyl-carbazol till foci turned reddish brown, and the reaction stopped by wasching with tap water. Cell foci could easily be evaluated(Fig 1).

Indirect immunofluorescence antibody test : The indirect fluorescense antibody test was performed as described earlier.[15] A first step of IFA was similar to CELISA. As second antibody was used fluorescein-isothiocyanat-(FITC-)coupled goat anti-rabbit-IgG(Nordic, Bochum). The titer is given as the dilution of antibody still showing a clear positive antigen accumulation in the nucleus and cytoplasm of the cells forming a focus(Fig 2).

Serum neutralization test : Equal volumes of stock virus containing 25ffu/well and twofold dilutions of post-infectious sera were incubated for 1h at 37℃. A similar preparation of virus mixed with normal rabbit serum was used as a control. The incubation maxture was then inoculated for virus assay. The test was performed as a plaque reduction test. The test was evaluated, 5 to 6 days post-infection, with the help of the CELISA. The esrum dilution leading to 50% focus reduction is given in all neutralization experiments.

Statistical terminology : Sensitivity, specificity and positive predictive valus(PPV) were to assess the relative merit of the serological tests.[16] Sensitivity is the number positive detected by both tests divided by the total number positive in the "standard" test(IFA). Specificity is the number negative detected by both tests divided by the total number negative in the standard test. PPV is the number of positives by both tests divided by the total number positive in the trial test. PPV is a measurement of true-positives in the trial test.

Results

The sample population in this study was composed of serum samples from 237 individual rabbits. One hundred twenty three of 273 serum samples tested were positive by IFA and twenty seven of 123 serum samples in rabbit and measured by all three serological tests to BD virus(Table 1).

IFA antibody titers ranged from 640 to 163840 in rabbit-serum samples, which were positive by three serological tests. CELISA antibody titers as well as ranged from 640 to 163840 by three serological tests, while NT antibody titers ranged from 11 to 213 in rabbit serum samples. 96 negative serum samples by NT ranged antibody titers by IFA and CELISA also from 40 to 163840.

CELISA antibodies to BD virus were present solely in the 123 serum samples positive by IFA. Therefore the specificity and PPV were 100% for those two tests in rabbit-serum samples(Table 2). NT antibodies to BD virus were present in 27

of the in rabbit serum samples which were positive by IFA(senitivity, 22%). The specificity of NT was 100%, while the PPV was 60%.

Table 1. Antibody titers to BD virus measured by three serological method in 27 rabbit serum samples found positive

Serum samples	BD viral antibodies measured by		
	IFA	CELISA	NT
1	640*	640	27
2	2560	1280	213
3	10240	10240	27
4	10240	10240	12
5	20480	40960	80
6	20480	40960	12
7	20480	20480	18
8	20480	20480	64
9	20480	20480	29
10	20480	40960	80
11	20480	20480	80
12	40960	40960	27
13	40960	81920	46
14	40960	81920	46
15	40960	81920	25
16	40960	81920	11
17	81920	163850	18
18	81920	40960	11
19	81920	163850	40
20	81920	163850	16
21	81920	163850	16
22	81920	163850	16
23	81920	163850	20
24	81920	81920	15
25	163840	81920	32
26	163840	163840	14
27	163840	81920	213

* : Titers are expressed as the reciprocal of the dilution.

Table 2. Comparison and results obtained by indirect immunofluorescence antibody test(IFA) and serum neutralization test(NT) with those obtained with the cell enzyme-linked immunosorbent assey(CELISA) in sera from 273 rabbits

Trial test	IFA +	IFA −	Sensitivity (%)	Specificity (%)	PPV (%)
CELISA					
+	123	0	100	100	100
−	0	150			
NT					
+	27	0	22	100	100
−	96	150			

Discussion

The results reported here that in comparison with IFA and CELISA was both sensitive and specific in detecting BD viral antibodies in rabbit sera(Table 2).

BD viral antibodies can be detected by CFT, ID, IFA and NT. Danner et al[17] campared IFA with CFT and ID tests. They had found that the IFA was more sensitive than another tests. CFT found irregularly the BD viral antibodies in infected horses. Immunodiffusion test was less sensitive to BD viral antibodies. In the raesent studies was observed also that the IFA was sensitive. Pauli et al[11] found the CELISA for BD viral antibodies to be a more sensitive test than IFA. They also found that CELISA titers were ten to 100 times higher than IFA titer values. However, in the present studies was observed that the IFA titer values almost correspond to CELISA titer values. The titer is very subjective, the results may vary between observers.

The results reported here suggest that the CELISA offers an easy, rapid and less expensive laternative to the IFA and a more sensitive to the NT for detecting BD viral antibodies. Furthermore the CELISA no need fluorescent microscope and time consuming purification of antigens is thus avoid, which need in other CELISA techniques.

NT is the low sensitivitiy of this method in dectecting BD viral antibodies. NT is not suitable for diagnosis of BD viral antibodies.

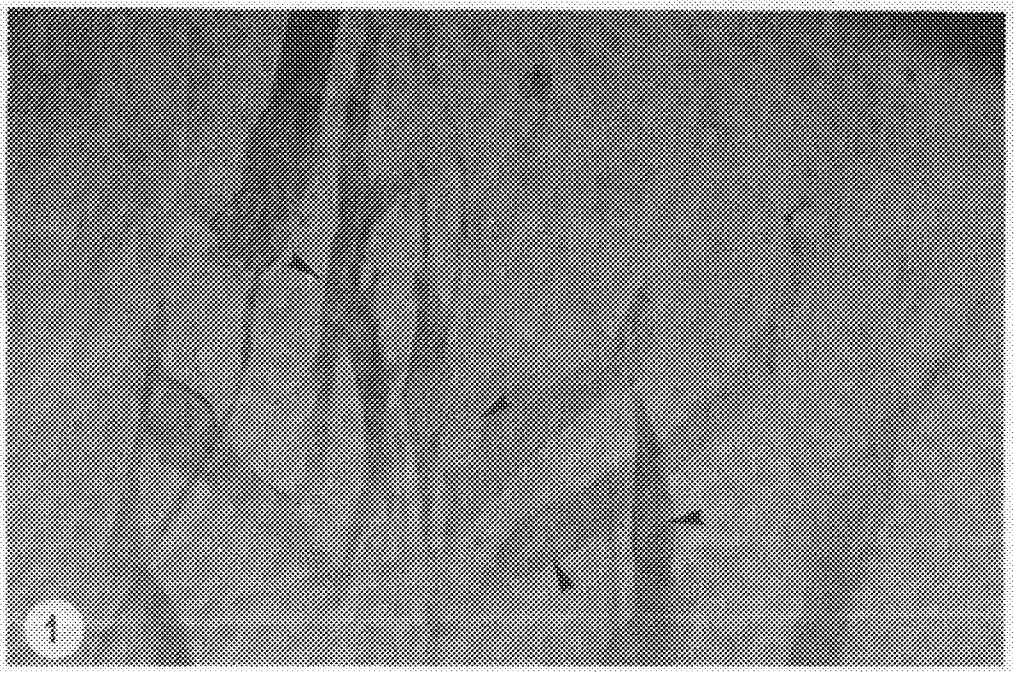
Summary

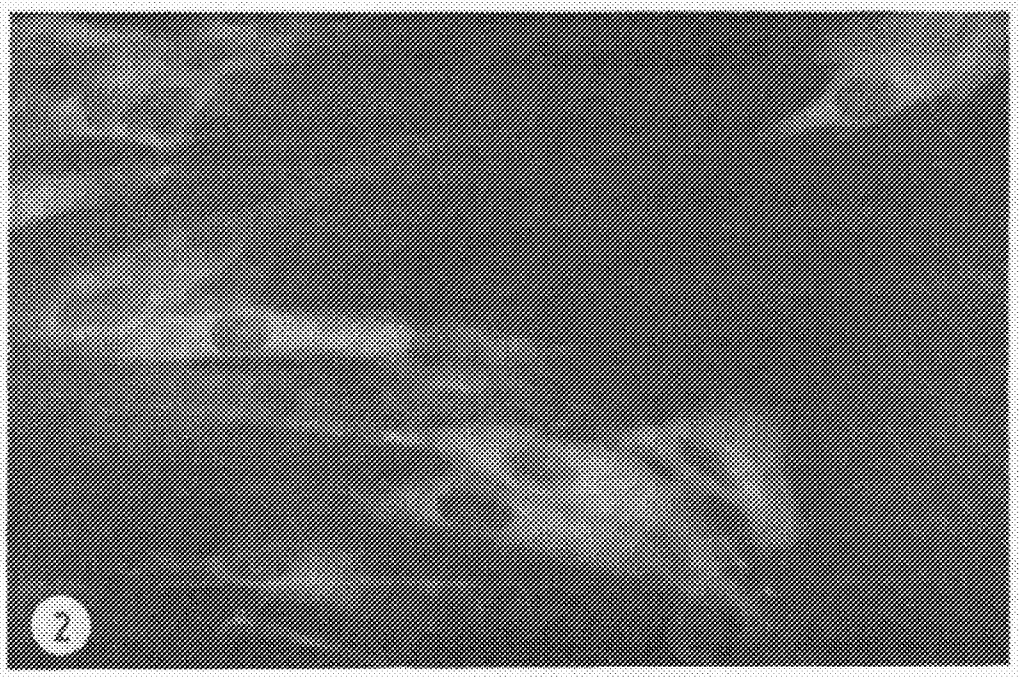
To determin the accuracy of serological methods in detecting Borna-disease(BD) viral antibodies, 273 experimentally infected rabbit sera were compared by using indirect immunofluorescence antibody test(IFA), serum neutralization test(SN) and enzyme-linked immunosorbent assay(ELISA). One hundred twenty-three serum samples had BD viral antibodies detected by IFA. CELISA antibodies to BD virus were also present in the same one hundred twenty-three serum samples. However, neutralization test antibodies to BD virus were present in 27 of the in rabbit serum samples. Neutralization test was sensitive in comparison with KFA and CELISA. In comparison with IFA, CELISA was both sensitive and specific in detecting BD viral antibodies. These results extend observations made with laboratory animals to the diagnosis of naturally infected animals.

Legends for figures

Fig 1. Positive reaction for BD virus in CELISA after treatment with rabbit sera.
Specific dark brown color was otained in the nucleus and cytoplasm(arrows) of persistently infected JKG cells with BD virus. × 800

Fig 2. Immunofluorescence staining of persistently infected JDG cells after treatment with rabbit sera.
The cells were tested at 6 days p.i.. × 800





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