

Detection of Borna Disease Virus-Reactive Antibodies from Patients with Psychiatric Disorders and from Horses by Electrochemiluminescence Immunoassay

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The prevalence of Borna disease virus (BDV)-specific antibodies among patients with psychiatric disorders and healthy individuals has varied in several reports using several different serological assay methods. A reliable and specific method for anti-BDV antibodies needs to be developed to clarify the pathological significance of BDV infections in humans. We developed a new electrochemiluminescence immunoassay (ECLIA) for the antibody to BDV that uses two recombinant proteins of BDV, p40 and p24 (full length). Using this ECLIA, we examined 3,476 serum samples from humans with various diseases and 917 sera from blood donors in Japan for the presence of anti-BDV antibodies. By ECLIA, 26 (3.08%) of 845 schizophrenia patients and 9 (3.59%) of 251 patients with mood disorders were seropositive for BDV. Among 323 patients with other psychiatric diseases, 114 with neurological diseases, 75 with chronic fatigue syndrome, 85 human immunodeficiency virus-infected patients, 50 with autoimmune diseases including rheumatoid arthritis and systemic lupus erythematosus and 17 with leprosy, there was no positive case except one case each with alcohol addiction, AIDS, and dementia. Although 19 (1.36%) of 1,393 patients with various ocular diseases, 10 (1.09%) of 917 blood donors, and 3 (4.55%) of 66 multitransfused patients were seropositive for BDV-specific antigen, high levels of seroprevalence in schizophrenia patients and young patients (16 to 59 years old) with mood disorders were statistically significant. The immunoreactivity of seropositive sera could be verified for specificity by blocking with soluble p40 and/or p24 recombinant protein. Anti-p24 antibody was more frequent than p40 antibody in most cases, and in some psychotic patients antibody profiles showed only p40 antibody. Although serum positive for both p40 and p24 antibodies was not found in this study, the p40 ECLIA count in schizophrenia patients was higher than that of blood donors. Furthermore, we examined 90 sera from Japanese feral horses. Antibody profiles of control human samples are similar to that of naturally BDV-infected feral horses. We concluded that BDV infection was associated in some way with psychiatric disorders.

Borna disease virus (BDV) is a noncytolytic, neurotropic, single-strand, negative-sense RNA virus that naturally infects a wide range of vertebrate species from birds and rodents to primates. BDV is experimentally transmissible to other animal species and can also cause encephalomyelitis in a wide range of experimental animals (6, 7, 17). Because BDV-induced behavioral disturbances in animals resemble some types of psychiatric disorders in humans, it is important to determine any possible role for BDV in human mental disorders. That BDV is pathogenic for humans was first suggested by antibodies in human sera that react with BDV-infected cells and subse-

quently with purified BDV proteins (1, 13, 24). Recently, the recovery of infectious BDV and the detection of BDV nucleic acids in human cells support the characterization of BDV as a newly identified human pathogen (8).

The epidemiological studies were carried out by serological assays, such as indirect immunofluorescence assay (IFA), immunoprecipitation, enzyme-linked immunosorbent assay (ELISA), and Western blot (WB) analyses. However, the prevalence of BDV-specific antibodies and BDV-related RNA among patients with psychiatric disorders has varied (from 3.7% by IFA to 23.3%) in several reports (4). These assay systems are sometimes problematic; for example, due to the existence of cell-specific autoantibodies, IFA has a variability of reader interpretation and a lack of sensitivity for detecting low anti-BDV titers. Although immunoprecipitation and WB analyses (10) may be more reliable and specific than IFA for

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TABLE 1. BDV seroprevalence among human patients by ECLIA with recombinant p40 and p24 proteins

Patient diagnosis	Mean age + SD	% Males/ % females	No. of positive sera/total no. (%) ^a
Schizophrenia	49.3 + 12.3	49/51	26/845 (3.08)
Mood disorders	55.6 + 14.0	39/61	9/251 (3.59)
Alcohol addiction	51.3 + 6.7	88/12	1/42 (2.38)
Mental retardation	38.6 + 13.4	60/40	0/25 (0)
Alzheimer's disease	82.9 + 8.2	36/64	1/89 (1.12)
Vascular dementia	78.5 + 8.5	49/51	0/46 (0)
Other psychiatric diseases	57.0 + 17.2	48/52	0/164 (0)
Subtotal			2/366 (0.55)
Neurological diseases	37.2 + 16.1	64/36	0/114 (0)
Multiple sclerosis			0/67 (0)
Encephalitis			0/33 (0)
Degenerative diseases			0/14 (0)
Epilepsy	43.9 + 13.7	56/44	3/214 (1.40)
Chronic fatigue syndrome	32.0 + 9.8	60/40	0/75 (0)
HIV infection (including AIDS)	31.9 + 13.4	97/2	1/85 (1.18)
Autoimmune diseases	47.2 + 17.3	4/96	0/50 (0)
Leprosy	67.2 + 10.4	53/47	0/17 (0)
Multitransfused patients	50.6 + 18.6	52/48	3/66 (4.55)
Ocular diseases	50.1 + 19.1	42/58	19/1,393 (1.36)
Blood donors	35.1 + 13.1	69/31	10/917 (1.09)

^a Statistical analysis data are as follows: schizophrenia versus other psychiatric diseases ($\chi^2 = 6.16$; $P > 0.01$ and < 0.025), schizophrenia versus blood donors ($\chi^2 = 8.67$; $P < 0.005$), schizophrenia versus ocular diseases ($\chi^2 = 7.82$; $P > 0.01$ and < 0.01), mood disorders versus other psychiatric diseases ($\chi^2 = 6.21$; $P > 0.025$ and < 0.01), mood disorders versus donors ($\chi^2 = 7.67$; $P > 0.01$ and < 0.005), mood disorders versus ocular diseases ($\chi^2 = 6.27$; $P > 0.025$ and < 0.01), and multitransfused patients versus donors ($\chi^2 = 3.30$; P , not significant).

evaluating BDV serology, these methods are time-consuming and expensive and, thus, are less suitable for rapid, economical, large-scale serological screening. Standard ELISA or capture ELISA methods (11) have been reported, but their limited utility for detecting low-affinity, low-titer anti-BDV antibodies typical of some species remains problematic. Furthermore, a marked discrepancy between reverse transcription-PCR and immunoassay has been reported. The screening of large numbers of humans with clinical diseases for evidence of BDV infection will require a rapid, economical, and reliable assay.

We developed a new electrochemiluminescence immunoassay (ECLIA) system that uses BDV p40 and p24 recombinant proteins, which has the sensitivity and specificity to serve as a serological screening method for measuring BDV antibodies. A seroepidemiological study on two species believed to have low-affinity and low-titer antibody to BDV, humans and horses, was conducted in Japan by using this ECLIA system.

MATERIALS AND METHODS

Study population. (i) **Humans.** Basic data on patients and healthy individuals are summarized in Table 1. Patients with psychiatric disorders from 11 hospitals in six prefectures in Japan were clinically diagnosed according to *Diagnostic and Statistical Manual of Mental Disorders IV* criteria on the basis of interviews and medical records. Anti-BDV antibody detection was performed only after obtaining informed consent from the patients or from the family when the patients did not have the capacity to provide consent. Almost all of the 4,393 sera are from Japanese patients. Some sera were collected between 1995 and 1998 and were provided retrospectively for this study. They were stored at -70°C . All samples were coded before use and tested in a double-blind manner.

(ii) **Horses.** Serum was obtained from 90 feral horses from Japan (Misakiuma, Miyazaki prefecture), living in an isolated area as a closed colony for 300 years.

These horses do not receive any medical care or vaccinations but are observed daily for general health and social behaviors by veterinarians.

ECLIA methods. Two kinds of BDV protein corresponding to full-length p40 and p24 were expressed as recombinant protein in *Escherichia coli*, by using the pGEX-5X-3 vector system (Pharmacia, Uppsala, Sweden), which generated the fusion protein with glutathione *S*-transferase (GST) and recombinant p40 or p24 protein. Each fusion protein was bound on glutathione-Sepharose 4B (Pharmacia) and then released by factor Xa (Sigma, St. Louis, Mo.), which cleaved between GST and p40 or p24 of the fusion protein. Further, to remove the contaminating protein derived from *E. coli*, each recombinant protein was purified by ion-exchange chromatography with Mono Q (Pharmacia) followed by affinity chromatography with Sepharose 4B (Pharmacia) combined with anti-*E. coli* immunoglobulin G (IgG) obtained from a rabbit immunized with *E. coli*.

The recombinant proteins (50 μg in 0.05 M borate buffer, pH 9.5) were mixed with 2×10^8 microbeads overnight at 37°C . Then the beads were washed three times with the washing buffer (0.05 M Tris buffer, pH 8.0, including 0.15 M NaCl and 0.01% Tween 20), and the recombinant protein-coated beads were suspended in the bead buffer (0.05 M Tris, pH 8.0, including 0.001% Tween 20 and 10% normal chicken serum) at the concentration of 10^7 beads.

Twenty microliters of each serum sample diluted 1:10 with 200 μl of the normal rabbit serum was incubated with 3×10^7 recombinant protein-coated beads for 9 min at 30°C . After the beads were washed three times with the washing buffer, 200 μl of the second antibody (0.1 $\mu\text{g}/\text{ml}$), anti-human IgG (Fc) mouse monoclonal antibody (Wako Junyaku, Osaka, Japan) coupled with ruthenium(II) Tris (bipyridyl)-*N*-hydroxysuccinimide ester [$\text{Ru}(\text{bpy})_3^{2+}$] (IGEN International Inc., Gaithersburg, Md.) (3), for the human samples, or anti-horse IgG polyclonal antibody (EY Laboratories, Inc.) coupled with $\text{Ru}(\text{bpy})_3^{2+}$, for the horse samples, was added to the beads and incubated for 9 min at 30°C . After the beads were washed three times with the washing buffer, the beads were conducted into the electrode and the photon (wavelength, 620 nm) emitted from the $\text{Ru}(\text{bpy})_3^{2+}$ coupled with the second antibody was counted with a photomultiplier tube. The above ECLIA procedures were carried out with the automatic ECLIA analyzer (Picolumi 8220; Sanko Junyaku, Tokyo, Japan).

The sample was considered positive if the ECLIA count was higher than the cutoff counts, i.e., the mean plus 3 standard deviations (SD) of the ECLIA counts for 200 human blood donor samples as controls. The cutoff count was 1,101 (mean plus 3 SD, 267 + 834) when both recombinant p40- and p24-coated beads were used for the ECLIA assay. Similarly, the cutoff count was 868 for p40-coated beads and 1,341 for p24-coated beads.

Confirmation test. The ECLIA-positive samples were confirmed in specificity with the following inhibition test. Twenty microliters of the serum sample was preincubated with 200 μl of normal rabbit serum containing 1 μg each of recombinant p40 and/or p24 protein for 15 min at room temperature. Similarly, the serum sample preincubated with normal rabbit serum alone served as the control. After the preincubations, the count of each sample was measured by ECLIA. The sera were finally judged antibody positive, if the ECLIA counts were specifically inhibited by more than 50% of the original counts.

RESULTS

(i) **Detection of antibodies to BDV p40 and p24 recombinant proteins in human sera.** A total of 917 sera from voluntary blood donors were tested for anti-BDV p40 and p24 antibodies by ECLIA. By using the specific inhibition paradigm, 10 sera were judged seropositive (1.09%).

Twenty-six (3.08%) of 845 schizophrenia patients and 9 (3.59%) of 251 patients with mood disorders were seropositive for BDV. The seroprevalence in young (16 to 59 years old) patients with schizophrenia (17 of 631 [2.69%]) and mood disorders (8 of 136 [5.88%]) was significantly higher than that of blood donors (10 of 917 [1.09%]) and patients with ocular diseases (6 of 916 [0.66%]) (chi-square test). Among 366 patients with other psychiatric diseases, 114 with neurological diseases, 75 with chronic fatigue syndrome, 85 human immunodeficiency virus (HIV)-infected patients including those with AIDS, 50 with autoimmune diseases, and 17 with leprosy, there were three positive cases, one patient each with alcohol addiction, AIDS, and dementia. Although 19 (1.36%) of 1,393 patients with various ocular diseases and 3 (4.55%) of 66 multitransfused patients were seropositive for BDV-specific antigen, high seroprevalence in schizophrenia patients was statistically significant.

(ii) **Age and sex distribution of anti-BDV antibody.** From a serological survey on BDV antibody-positive schizophrenia and mood disorder patients, the positive rate did not increase

TABLE 2. BDV antibody profiles in human sera

Patient diagnosis	ECLIA count (p24 + p40)	Inhibition (%)	ECLIA count		
			p24	p40	
Schizophrenia	1,868	511 (27.6)	2,624	606	
	1,353	340 (24.9)	2,184	277	
	1,231	271 (22.0)	2,014	622	
	1,559	244 (15.6)	178	2,974	
	1,312	305 (23.3)	1,063	628	
	8,070	1,115 (13.8)	13,383	644	
	1,182	354 (30.0)	1,698	509	
	1,177	509 (43.3)	1,501	735	
	1,617	225 (14.0)	150	2,992	
	3,796	177 (4.7)	5,863	149	
	5,078	2,202 (43.4)	8,825	578	
	2,050	362 (17.7)	3,823	71	
	2,616	916 (35.0)	4,396	239	
	3,185	927 (29.1)	4,208	94	
	10,382	892 (8.6)	999	18,147	
	1,207	394 (32.6)	1,525	534	
	16,648	3,882 (23.3)	29,144	947	
	17,682	4,772 (27.0)	32,933	1,060	
	Mood disorder	19,740	7,326 (37.1)	501	31,172
		1,182	354 (30.0)	1,698	509
1,794		502 (27.9)	3,050	350	
1,168		212 (18.2)	1,635	167	
1,129		382 (33.8)	1,426	575	
1,701		491 (28.8)	2,675	410	
7,283		693 (9.5)	227	12,631	
Dementia	6,191	596 (9.6)	15,144	158	
	Multitransfused	2,159	900 (41.7)	2,718	726
2,816		668 (23.7)	3,985	511	
5,706		2,254 (39.5)	11,608	610	
Epilepsy	3,044	433 (14.2)	4,651	186	
	3,448	551 (16.0)	5,329	433	
	3,126	740 (23.7)	5,610	231	
Donor	2,251	608 (27.0)	3,086	263	
	2,655	354 (13.2)	3,821	202	
	1,350	641 (47.5)	1,428	257	
	6,997	570 (8.1)	10,738	200	
	1,770	306 (17.3)	2,397	261	
	4,156	869 (20.9)	5,403	201	
	1,557	507 (32.6)	2,059	208	
	3,747	314 (8.4)	6,976	264	
Cutoff	1,101	(more than 50%)	1,341	868	

with age. The BDV antibody positivity rate in donors also did not increase with age. There was no difference between females and males among these psychosis patients. The BDV antibody-positive rate in males (1.16%) was higher than that in females (0.65%) among blood donors.

(iii) Profiles of antibodies to BDV recombinant proteins in humans. Although p24 antibody was more frequent than p40 antibody in most cases, in some psychosis patients antibody profiles showed only p40 antibody (Table 2). ECLIA counts of p40 antibody in most patients were higher than in healthy donors, even when ECLIA counts were under the cutoff level. The immunoreactivity of seropositive sera was verified for specificity by blocking with soluble p40 and/or p24 protein.

(iv) Detection of antibodies to BDV recombinant proteins in horses. As a first step, 21 of 90 (23.3%) Japanese feral horses had an ECLIA count of more than 1,000 by using both p40 and p24 recombinant proteins. In order to enhance specificity, an inhibition test was also used in these ECLIA. Sixteen sera were determined to be BDV seropositive (17.8%) when the addition of both BDV p40 and p24 recombinant proteins in-

TABLE 3. BDV antibody profiles in Japanese feral horse sera^a

Misakiuma antibody	ECLIA count (% inhibition) (p24 + p40)	ECLIA count	
		p24	p40
7	7,190 (93.9)	12,415	391
11	9,124 (89.9)	14,457	573
22	2,689 (73.1)	4,831	483
26	1,924 (52.7)	2,934	421
36	14,710 (93.6)	25,856	254
41	46,270 (98.1)	69,733	690
49	1,150 (92.7)	1,539	125
50	5,960 (92.6)	10,026	114
64	1,642 (90.4)	2,509	188
65	9,614 (95.9)	15,713	345
75	19,948 (87.7)	27,704	561
77	46,734 (93.8)	80,185	2,747
79	1,799 (54.1)	2,796	634
82	2,930 (88.5)	5,115	378
84	1,521 (91.0)	2,390	166
99	1,374 (58.2)	1,463	994

^a BDV seropositivity, 16 of 90 (17.8%).

hibited more than 50% of the original value obtained (Table 3). BDV antibody profiles in these horses were similar to that of humans; two horses had both p40 and p24 antibodies but the remainder had only the p24 antibody. There was no clinical evidence of neurological or behavioral disease observed in any of the Japanese feral horses. Misakiuma horses 77 and 41 are parent and child: the mother of horse 41 (a two-day-old baby) is horse 77. Their ECLIA counts are very similar, and the ECLIA count of horse 41 fell rapidly from 46,270 (inhibition, 98.1%) to 1,048 (95.6%) after 3 months.

DISCUSSION

The ECLIA method using the conjugate labeled with ruthenium(II) Tris (bipyridyl) and magnetic microbeads was developed to provide higher sensitivity, wider dynamic range, improved precision, and shorter testing time than other conventional immunoassay methods. We used this ECLIA system to detect antibodies to BDV p40 (viral nucleoprotein) and p24 (viral phosphoprotein) in sera from two species believed to have low affinity for and low titers against BDV, humans and horses. p40 and p24, expressed at high levels in the rat brain and infected cells, represent good markers with which to search for evidence of BDV infection in animal and human sera (12). The ECLIA was able to accurately identify experimentally infected rats and horses (26). In addition, the ECLIA corresponded to IFA in domestic horses that were also seropositive by a sensitive, specific WB assay.

A possible association between BDV infection and major psychiatric disorders was initially proposed after finding BDV-reactive antibodies in the sera of a small, but significant, percentage of people with these disorders, as compared to controls (13). The first studies reporting that patients with psychiatric diseases, e.g., unipolar or bipolar affective disorder, showed a higher prevalence of anti-BDV antibodies (1.6%) than healthy controls (0%) were performed by using the IFA (19). Fu et al. (10) confirmed the earlier report by using a WB assay with two BDV proteins. Waltrip et al. (25) reported a significantly higher prevalence of anti-BDV antibodies in patients with schizophrenia (13.3%) than in controls (0%), by using a WB assay.

Following the identification of BDV RNA in peripheral blood mononuclear cells (PBMC) of experimentally infected

rats (23), Bode et al. (5) reported finding BDV protein in CD14⁺ PBMC and RNA in PBMC from psychiatric patients. However, there continues to be conflicting data in this area, as Richt et al. (18) failed to detect BDV in PBMC from 42 seropositive humans with psychiatric disorders in Germany and the U.S. This latter group also identified contamination in their laboratory and another laboratory of their collaborators and concluded, on that basis, that the reports of reverse transcription-PCR identification of BDV in human PBMC may reflect contamination from laboratory strains.

In addition to reports of BDV in human PBMC, the recovery of BDV from human brain tissue has been reported (8). BDV-like antigens and RNA sequences were detected by immunohistochemistry, in situ hybridization, and reverse transcription-PCR in postmortem tissue from four of five human brains (from patients with non-Alzheimer's dementia) selected for hippocampal sclerosis and astrogliosis.

There have been attempts to correlate BDV RNA detection with BDV seropositivity in the same individual. Sauder et al. (22) described a similar assay used in the detection of human anti-BDV antibodies. Using this assay, Sauder et al. reported finding a BDV seroprevalence of 9.6% among 416 neuropsychiatric patients, versus 1.4% among 203 healthy controls. The majority of these positive sera recognized only the BDV p40 antigen. The authors also reported that three of the 13 patients whose PBMC were BDV RNA positive were also BDV seropositive, whereas one patient with serum antibodies to BDV p40 was BDV RNA negative.

However, since the overall difficulty in recovery of BDV from humans carries with it some significant false-positive and false-negative technical issues, for the foreseeable future and certainly for mass screening attempts, serological assays are likely to remain the major BDV diagnostic tests.

The ECLIA identified 10 of 917 blood donors whose sera specifically recognized BDV antigens in three different areas of Japan, although at this point we are unable to independently confirm that these individuals are infected with BDV. However, if BDV infection is present in even a small percentage of blood donors, then we have to consider initiating the screening of blood products for BDV to prevent possible iatrogenic transmission. The prevalence we observed in multitransfused patients is higher than that of blood donors, although this is not statistically significant (Yates' correction [$\chi^2 = 3.30$]; $P > 0.05$ and < 0.1). Three BDV antibody-positive patients receiving large quantities of blood products (erythrocytes and platelets) for many years (more than 12 years) are suffering from severe bone marrow dysfunction. They have been diagnosed with aplastic anemia, acute myelogenous leukemia, and paroxysmal nocturnal hemoglobinuria. Additional data from a large-scale study are needed to resolve this problem.

In our ECLIA system, the profiles of BDV antibody differed between patients with psychiatric disorders and control donors. Three of 15 patients with schizophrenia and bipolar disorder showed the antibody to only the p40 protein, and the anti-p40 antibody was not demonstrated in blood donors. Although antibodies from humans that react with both antigens have not yet been observed, ECLIA counts of p40 antibody in psychiatric patients are higher than those of blood donors. BDV p40 is a major target of the CD8⁺ T-cell-mediated immune response in Lewis rats (16). The immune response and pathogenesis of these p40-positive psychiatric patients may be different from psychiatric patients and blood donors positive only for p24 antibodies.

Seropositive patients presented a broad spectrum of mental disorders with a predominance of deficit syndrome of schizophrenia, recurrent unipolar depression, and bipolar affective

disorders. Based on these data, there is no evidence for a major clinical manifestation of infection with BDV. Since genetic background plays a role in some types of BDV-associated diseases in animals, some variability in clinical disease expression is not surprising in an outbred human population (21).

We could demonstrate very low-level BDV antibody positivity among patients with HIV infection and chronic fatigue syndrome, although there are some reports of high seroprevalence among patients with these diseases. Auwanit et al. (2) reported an unusually high seroprevalence of BDV in HIV-infected patients by ELISA with GST-BDV p24 (full-length) fusion protein as the antigen, and Nakaya et al. (15) also reported that six of 25 chronic fatigue syndrome patients were positive for BDV by WB with the same GST-BDV p24 fusion protein. In our ECLIA system, some sera from HIV-infected patients have had high counts over the cutoff level at first screening, but all except one were judged negative by a specific inhibition test. These assay systems remain problematic for specificity due to contamination of *E. coli* components and the use of the GST-BDV p24 fusion protein, which did not release GST. A confirmation test, such as an inhibition test with a soluble antigen, is needed to rule out a nonspecific reaction.

More than 5,000 sera from psychiatric patients and patients with unclear neurological diagnoses were investigated under blind conditions in Europe and the U.S. by IFA and immunoblot assays (20). In 4 to 7% of these sera BDV-specific antibodies could be demonstrated with titers from 1:10 to 1:640, depending on the geographic region from which the patients came. The highest percentage of seropositive patients came from a region in southern Germany where Borna disease has been known to be endemic in horses and sheep. However, approximately 1% of the 1,000 control specimens also showed antibodies to BDV irrespective of their origin.

In Japan, the high prevalence (29.8%) of BDV p24 RNA in PBMC from 57 healthy horses was demonstrated, and about 60% of the BDV RNA-positive animals showed seropositivity by WB with GST-BDV p24 fusion protein (14). However, there has so far been no report of clinically apparent Borna disease in horses. In our assay system, the feral horses in Japan also showed 18% BDV seropositivity. These horses in the Miyazaki prefecture have not been subjected to veterinary care, including vaccination or medication, for a long period of time. Thus, we can rule out the possibility that these feral horses were infected by BDV contamination of a vaccine used in domestic horses. Furthermore, we did test the detection of BDV antibody in 200 domestic horses in Japan, and 112 (56%) of these horses were BDV antibody positive (p24-positive horses, 104; p40-positive horses, 1; both p24- and p40-positive horses, 7) (unpublished data). The BDV seroprevalence of domestic horses is apparently higher than that of feral horses. The difference of seroprevalence among these two groups (feral and domestic) may depend on the circumstances of close housing and origin.

It is not known whether BDV can be transmitted from infected horses, or other animals, to humans. However, the similarity in sequence between animal and human BDV isolates (9) and experimental data showing a broad species preference for this agent suggest that animal-to-human transmission is a distinct possibility.

There is a need for epidemiological and pathological studies to rigorously evaluate the contribution of BDV to human psychiatric disorders. The ECLIA is a new, highly sensitive, and specific serological screening tool based on recombinant proteins for the measurement of anti-BDV antibody to p40 and p24 for use in investigations of human and animal BDV infections. In particular, this rapid, economical technique will be

useful for the large-scale screening required of serious epidemiology studies of BDV in humans. The ECLIA will also be useful in evaluating the transmission of BDV by blood products and will prove helpful in investigating the pathogenesis of disease states associated with BDV infection.

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