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Borna disease virus and the evidence for human pathogenicity: a systematic review

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Summary

Background: Borna disease is a neurological viral disease of veterinary importance in central Europe, although Borna Disease virus (BDV) has been reported to be present in animals in most continents. The hypothesis that BDV is associated with human illness is controversial. However, should even a small fraction of mental illness be attributable to infection with BDV, this would be an important finding, not least because illness in that subpopulation would, theoretically, be preventable.
Methods: We systematically reviewed the evidence: that BDV infects humans; for the role of BDV in human neuropsychiatric illness; to assess the suitability of currently available laboratory methods for human epidemiological studies.
Results: We identified 75 documents published before the end of January 2000, describing 50 human studies for BDV. There were five case studies and 44 (sero)prevalence studies, in a variety of patient groups. Nineteen prevalence studies (43%) investigated seroprevalence, 11 (25%) investigated viral prevalence and 14 (32%) investigated both. Seroprevalence ranged from 0% to 48%, and prevalence of virus or viral footprints from 0% to 82%.
Discussion: Although agreed gold standard tests and evidence for test specificity are lacking, there is evidence that humans are exposed to the virus. Further epidemiological studies are required to establish whether there are associations with disease.

Introduction

Borna disease was first described in the early 19th century as an 'equine brain disease with signs of agitation'[1] and obtained its name following an epidemic in military horses in Borna, Saxony, in 1885. Since then, this naturally occurring meningoencephalitic disease has been described mainly in horses, but also in sheep, cattle and other domesticated and wild animals and in a variety of experimentally infected host species.[2] The aetiological agent, Borna disease virus (BDV), also causes clinically definable and reproducible behavioural abnormalities in rats and non-human primates, some of which resemble neuropsychiatric disorders in humans. Viral tropism for the limbic system, including excitatory fields in the hippocampus, has been reported.[3,4] These areas of the brain govern emotions important to survival, visceral responses and olfactory sensations, and processes involved in memory. The detection of BDV antibodies and viral RNA in peripheral blood mononuclear cells (PBMCs) from psychiatric patients has furthered the suggestion that BDV is linked to human neuropsychiatric illness.[5]

Routes of transmission are unproven, but thought to be via excreta or nasal, saliva and conjunctival secretions, either directly or indirectly through

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contaminated food and water. BDV RNA has been detected in secretions from horses by reverse transcriptase polymerase chain reaction (RT-PCR), both in the presence and absence of clinical illness.[6,7] The detection of viral markers in blood raises the possibility of blood-borne transmission. The virus itself is an enveloped, non-segmented negative-strand RNA virus, Order Mononegavirales, Family Bornaviridae (ICV Jerusalem). Several features of its molecular biology are remarkable, and some are relevant to questions regarding the detection and spread of the virus, and the epidemiology of disease. For example, the virus replicates at lower levels than many other viruses, producing low numbers of infectious particles, although cell to cell transmission of incomplete particles may occur.[8] Sensitive methods are therefore required for virus detection and identification. Demonstration of BDV antigens in populations of PBMCs from hospitalised psychiatric patients was first reported by Bode *et al.*, in 1994,[9] and the detection of BDV RNA in PBMCs of neonatally infected rats[10] stimulated investigation and detection in psychiatric patients.[11] The detection of antibodies in humans was first reported in 1985, in patients with depression and psychiatric disorders.[12] The s-antigen (p40 and p24 complex) initiates the major humoral response, and has been the main target for serological studies.[13]

The hypothesis that BDV is associated with human clinical illness is controversial. However, should even a small fraction of mental illness be attributable to infection with BDV, this would be an important finding, not least because illness in that sub-population would be potentially preventable. We performed a systematic review[14] to: evaluate the evidence that BDV infects humans; evaluate the role of BDV in human neuropsychiatric illness; and assess the suitability of currently available laboratory methods for human epidemiological studies.

Methods

On-line and library databases were used to identify any studies involving the detection of BDV or its markers in human subjects. The following sources were searched using the term 'Borna': Medline, 1966 to January 2000; Institute for Science Information (Current Contents and Science Citation Index); British Library Document Supply Centre (including System for Information on Grey Literature); BIDS, 1981 to January 2000; BIOSYS via Datastar on-line index system, 1970 to January 2000; and the Cochrane Collaboration on CD-Rom. The search was not limited by language or sources. Inclusion criteria were studies published prior to January 2000, where the study participants = human, investigation = antibody or antigen or viral markers, outcomes = prevalence data. This was followed by hand searching of the literature for further references, and by consultation with leading researchers, facilitated by the first UK workshop on Borna Disease Virus in March 2000.[15]

The primary studies were identified and assessed by the same researcher (RC). Due to the nature of the investigations and variation in methods and study groups found, meta-analysis was not undertaken. However, results were summarized to describe the studies undertaken, show the groups investigated, the tissue tested, laboratory methods used and results obtained. To facilitate cross-referencing, reports were allocated a study number, generated in chronological order. Seroprevalence (antibody) studies were allocated a number prefixed with 'Ab', studies for the prevalence of viral antigens or genetic markers were numbered with a 'V' prefix, and studies for both, were given an 'AbV' prefix. Individual case studies were described separately.

Results

We identified 75 publications that contained details of 50 human case or prevalence studies of Borna disease. The study designs identified were: five case studies of individual patients, and 44 (sero)prevalence studies of 138 groups of patients, including five studies of monozygotic twins (Tables 1–3). In 24/44 (55%) studies covering 64 groups of patients, a control or a comparison group was also tested to compare prevalence and to measure association between markers for infection and clinical illness. Epidemiological and exposure data was additionally gathered in just two studies. There were no studies in which the study design described the random or systematic recruitment of cases, selection of controls or systematic recruitment of a study cohort.

Case studies

A case of encephalitis with neurological and psychiatric symptomology was attributed to BDV when an IgG titre of 1/10 was detected.[84] Two family cases of psychiatric disorders were detected among 200 seropositive psychiatric cases. In both families, in addition to the primary patient, a second family member also had psychiatric or neurological illness as well as BDV antibodies.[85] BDV antibodies

Table 1 Published Borna disease virus (sero)prevalence studies

Study code	Study description	References
Ab 1	Prevalence of BDV antibodies in patients with unipolar and bipolar depression in Philadelphia (US) and patients with heterogenous pychiatric disorders in Giessen and Wurzburg (Germany), and in local control subjects.	12, 16
Ab 2	Prevalence of BDV antibodies in patients with HIV, EBV, affective disorder, drug abusers and a control group in US and West Germany.	17
Ab 3	Prevalence of BDV antibodies in psychiatric in-patients, and newly admitted psychiatric, neurological and surgery patients in Germany. MRI scans of psychiatric and surgical patients.	
	CSF testing of further seropositive psychiatric patients, including virus isolation and infectivity in rabbits from two neurological and one psychiatric patient.	18–24
Ab 4	Retrospective prevalence of antibodies to BDV S-antigen amongst different groups of psychiatric, neurological and chronically infected people in different countries and in a control group from the population and blood donors in the US and Europe.	25, 26
Ab 5	Prevalence of antibodies to BDV S-antigen in patients with chronic fatigue syndrome in Germany.	27
Ab 6	Prevalence of antibodies to BDV 24 kDa and 38/40 kDa proteins in affective disorder patients and local healthy comparison subjects in Pennsylvania, USA.	28, 29
Ab 7	A longitudinal prevalence study of antibodies to BDV 38/40 kDa proteins in psychiatric patients in Germany.	30–32
Ab 8	Prevalence of BDV antibodies to gp18 and p40 in people exposed to farmed ostriches and a matched control group of blood donors in Israel.	33
Ab 9	Prevalence BDV antibodies in schizophrenic patients and community controls in Maryland, US and further investigation of deficit syndrome positive and negative patients.	34, 35
Ab 10	Prevalence of BDV antibodies in schizophrenic patients in Pennsylvania, US.	36
Ab 11	Prevalence of BDV antibodies in multiple sclerosis patients in Germany.	37
Ab 12	Prevalence of antibodies to BDV p24 in various HIV infected groups and AIDS patients in Thailand.	38
Ab 13	Prevalence of BDV antibodies in monozygotic twins in the US concordant and discordant for schizophrenia, bipolar depression and other psychoses.	39, 40
Ab 14	Prevalence of BDV antibodies in first episode schizophrenics in the US and seroconversion at 6-month follow-up, and comparison with unspecified controls.	41, 42
Ab 15	Prevalence of antibodies to BDV p40 in schizophrenic patients and healthy medical staff in Japan.	43
Ab 16	Prevalence of BDV antibodies in patients with panic disorder and healthy controls in Vienna, Austria.	44
Ab 17	Prevalence of antibodies to BDV p24 and p40 in Chinese schizophrenic patients, family members, mental health workers and non-psychiatric controls in Taiwan.	45
Ab 18	Prevalence of BDV antibodies in veterinarians practising in Styria, Austria.	46–48
Ab 19	Prevalence of BDV antibodies in patients with a variety of psychiatric, neurological and other disorders and blood donors in Japan.	49

were detected in a patient with motor neurone disease at a titre of 1/20.[86] Six blood samples were taken from a patient with somatization disorder and schizophrenia, and whole blood tested for p24 and p40 gene sequences by reverse transcriptive polymerase chain reaction (RT-PCR).[87] All samples contained both gene sequences.

Prevalence studies

Nineteen of 44 (43%) studies investigated seroprevalence, 11/44 (25%) investigated the prevalence of BDV, and 14/44 (32%) investigated both. Seroprevalence studies have been conducted in USA (5 studies), Germany (3 studies), Austria (2 studies),

Table 2 Summary of seroprevalence and prevalence of Borna disease virus in different study groups

Study group type	Seroprevalence		BDV prevalence	
	Positive studies/total studies	Median (range)	Positive studies/total studies	Median (range)
Unspecified psychiatric in-patients	9/11	7% (0–30%)	6/7	11% (0–54%)
Schizophrenia	10/13	5% (0–45%)	5/13	0% (0–82%)
Major depressive disorder	6/6	4% (1–12%)	1/8	0% (0–40%)
Neurotic, personality, adjustment and mood disorder	3/4	6% (0–15%)	1/1	4% (4%)
Mental disorders relating to alcohol and drugs	2/2	4% (2–5%)		NT
Mental retardation	0/2	0% (0%)		NT
Neurological patients	3/6	1% (0–5%)	1/2	1% (0–2%)
Dementia	0/3	0% (0%)	0/1	0% (0%)
Alzheimer's disease	1/1	1% (1%)	1/3	0% (0–11%)
Multiple sclerosis	1/3	5% (0–13%)	0/1	0% (0%)
Epilepsy	1/1	1% (1–1%)		NT
Parkinson's disease		NT	1/2	8% (0–17%)
Brain tumour		NT	1/1	14% (14%)
Chronic fatigue syndrome	2/4	5% (0–34%)	3/4	12% (0–24%)
HIV/AIDS	8/8	11% (1–48%)		NT
EBV	2/3	1% (0–6%)		NT
Parasitological infections	4/4	8% (5–19%)		NT
Occupational groups	2/3	10% (0–46%)		NT
Other groups	9/13	7% (0–36%)	3/8	0% (0–7%)

NT, none tested.

Table 3 Published studies of the prevalence of viral markers for Borna disease virus

Study code	Study description	Reference
V 1	Prevalence of p40 and p24 gene coding sequences in brain tumour patients in Japan.	50
V 2	Prevalence of p40 antigen and p40 gene coding sequences in brains of patients with histopathological characteristics on an Alzheimer Disease database in California, US.	51
V 3	Prevalence of P gene (p24) coding sequences in brain samples from neuropsychiatric patients and normal controls in North America and Europe.	52
V 4	Prevalence of P gene (p24) coding sequences in brain samples from neuropsychiatric patients and controls in Japan.	53
V 5	Prevalence of p24 gene coding sequences in normal brains at post mortem in Japan.	54
V 6	Prevalence of p40 gene coding sequences in whole peripheral blood and PBMCs from psychiatric patients in various regions of Germany with controls from one region.	55
V 7	Prevalence of p40 gene coding sequences in peripheral blood from blood donors and immunocompromised heart transfusion patients in Germany.	56
V 8	Prevalence of p24 gene coding sequences in PBMCs from patients with mood disorders or schizophrenia, and from age and sex matched blood donors, in Japan.	57
V 9	Prevalence of p40 gene coding sequences in brain, CSF and PBMCs of schizophrenics in US.	58
V 10	Prevalence of p24 gene coding sequences in PBMCs of psychiatric patients with schizophrenia or bipolar disorder in Korea.	59
V 11	Prevalence of unspecified virus gene coding sequences in brain tissue from neuropsychiatric and medical patients and healthy subjects at post mortem.	60

Japan (2 studies), Taiwan, Thailand and Israel (1 study each), and there were multicentre studies involving USA and Germany (2 studies), and USA, Europe and Africa (1 study). The location of one study was not specified. Prevalence studies have been conducted in Japan (4 studies), USA (3 studies), Germany (2 studies), Korea (1 study), and in USA and Europe as a muticentre study. Studies of both seroprevalence and prevalence have been conducted in Japan (8 studies), Germany (3 studies), UK (1 study), the Netherlands (1 study), and in one multicentre study in USA and Germany. Studies were predominantly of adults, but children were included in one study.

Prevalence in patient groups

A variety of patient groups have been studied (Tables 4 and 5): unspecified psychiatric patients, schizophrenia cases, major depressive disorder,

Table 4 Published studies combining seroprevalence and prevalence of Borna disease viral markers

Study code	Study description	References
AbV 1	Detection of BDV antigens in populations of blood monocytes by flow cytometry in samples from psychiatric patients. Detection of p24 and p40 gene coding sequences in selected antigen positive blood samples, and virus isolated and characterized from further selected samples.	9, 11, 61–63
AbV 2	Prevalence of p24 gene coding sequence in blood donors in two areas of Japan (Sapporo and Tokyo), and prevalence of antibodies to BDV p24 in the Sapporo blood donors.	64
AbV 3	Prevalence of p24 gene coding sequence and prevalence of antibodies to BDV p24 in Japanese psychiatric patients. Sequence variability investigated in cDNA cloned and sequenced from three patients.	65, 66
AbV 4	Prevalence of antibodies to BDV p24 in Japanese CFS patients and healthy controls, and prevalence of p24 gene coding sequences in a further group of CFS patients. (Preliminary studies were for prevalence and seroprevalence in a sub-cohort of 25 CFS patients.)	67, 68
AbV 5	Prevalence of p24 gene coding sequence and prevalence of BDV antibodies in psychiatric patients and blood donors in Kumamoto, Japan.	69
AbV 6	Prevalence of antibodies to BDV 16 kDa, 24 kDa and 40 kDa antigens inpsychiatric patients and controls in Hamburg, Germany, and prevalence of p24 and p40 gene coding sequences in a subset of those tested for antibodies.	70
AbV 7	Prevalence of antibodies to BDV p38 in psychiatric patients and controls in Japan and prevalence of p24 gene coding sequences in a subset of these.	71
AbV 8	Prevalence of BDV antibodies and prevalence of p24 gene coding sequence in schizophrenic patients and normal controls in Philadelphia.	72
AbV 9	Prevalence of BDV antibodies and prevalence of p24 gene coding sequences in British CFS patients, those with affective disorders and normal controls. Brain tissue examined for virus.	73, 74
AbV 10	Prevalence of antibodies to BDV p24 and p40 and prevalence of p24 coding sequences in Japanese schizophrenic patients and healthy controls. Some epidemiological data also collected and analysed.	75–78
AbV 11	Prevalence of BDV antibodies and prevalence of BDVp24 and p40 gene coding sequences in CSF of depressive patients and MS patients and healthy controls in Munich, Germany.	79, 80
AbV 12	Prevalence of antibodies to BDV and prevalence of BDV p40 gene coding sequences in Alzheimer and vascular dementia patients and healthy blood donor controls.	81
AbV 13	Only prevalence of BDV antibodies results given.	82
AbV 14	Prevalence of antibodies to BDV p24 in blood donors in towns near Thoroughbred horse farms. 7 antibody positive donors also tested for BDV RNA. Prevalence of BDV p24 gene coding sequence also tested in one group of blood donors.	83

Table 5 Prevalence of antibodies to Borna disease virus by study group

Location	Study number and study group description	Study group seroprevalence	Test sample	Test method	Control group sero-prevalence	Quoted *p* value
Unspecified psychiatric in-patients						
Japan	AbV 3 Unspecified psychiatric in-patients	18/60 (30%)	Blood plasma	Western blot		
Japan	AbV 5 Unspecified psychiatric in-patients	13/33 (24%)	Blood plasma	Indirect IFA		
Germany	AbV 6 Mental disorders (organic factors)	7/34 (21%)	Blood sera	Western blot	3/203 (2%)	
Germany	AbV 1 Unspecified psychiatric in-patients	12/68 (18%)	Blood sera	Double stain IFA		
Germany	AbV 6 Unspecified mental disorders on admission	2/20 (10%)	Blood sera	Western blot	3/203 (2%)	
Germany	Ab 3 Unspecified psychiatric in-patients	68/1003 (7%)	Blood sera	Indirect IFA		
Germany	Ab 3 Newly admitted psychiatric in-patients	140/2377 (6%)	Blood sera	Indirect IFA		
Germany	AbV 11 Unspecified psychiatric in-patients	2/128 (2%)				
Germany	Ab 1 Unspecified psychiatric in-patients	4/694 (1%)	Blood sera	Indirect IFA	0/95 (0%)	
Japan	Ab 19 Unspecified psychiatric in-patients	0/164 (0%)	Blood sera	ECL immunoassay		
Japan	AbV 7 Unspecified psychiatric in-patients	0/44 (0%)	Blood plasma	Indirect IFA	0/70 (0%)	
Schizophrenic patients						
Japan	AbV 10 Schizophrenics	30/67 (45%)	Blood plasma	Western blot	0/26 (0%)	
USA	Ab 9 Schizophrenic outpatients	13/90 (14%)	Blood sera	Western blot	0/20 (0%)	0.06
Germany	AbV 6 Schizophrenia and other psychotic disorders	16/114 (14%)	Blood sera	Western blot	3/203 (2%)	

USA	Ab 14 First episode schizophrenia	10/82 (12%)	Blood sera	Western blot	0/22 (0%)	
Taiwan	Ab 17 Chronic schizophrenia	38/314 (12%)	Blood sera	Western blot	8/277 (3%)	
Netherlands	AbV13 Male schizophrenic immigrants	3/27 (11%)	Not stated	IFA	6/29 (21%)	
USA	Ab 10 Schizophrenics	2/41 (5%)	Blood sera	Western blot		
USA	Ab 13 Monozygotic twins concordant schizophrenia	1/26 (4%)	Blood sera	Western blot		
Japan	Ab 19 Schizophrenics	26/845 (3%)	Blood sera	ECL immunoassay	10/917 (1%)	<0.005
Japan	Ab 15 Chronic schizophrenia	2/70 (3%)	Blood sera	ELISA	1/40 (3%)	
USA	Ab 2 Schizophrenics	0/49 (0%)	Blood sera	Double stain IFA	11/540 (2%)	
Japan	AbV 7 Schizophrenics	0/179 (0%)	Blood plasma	Indirect IFA	0/70 (0%)	
USA	AbV 8 Schizophrenics	2/10 (2%)	Blood sera	Western blot	0/10 (0%)	
Major depressive disorder						
Germany	AbV 6 Affective psychoses and disorder	6/52 (12%)	Blood sera	Western blot	3/203 (2%)	
USA	Ab 6 Major depressive disorder	9/138 (7%)	Blood sera	Western blot	1/117 (1%)	<0.025 *q*
USA	Ab 1 Unipolar and bipolar depression	12/285 (4%)	Blood sera	Indirect IFA	0/105 (0%)	
USA	Ab 4 Bipolar depression	7/187 (4%)	Blood sera	Double stain IFA	11/483 (2%)	
USA	Ab 2 Depression	13/593 (2%)	Blood sera	Double stain IFA	11/540 (2%)	
USA	Ab 4 Unipolar depression Neurotic, personality, adjustment and mood disorder	5/363 (1%)	Blood sera	Double stain IFA	11/483 (2%)	
Germany	AbV 6 Neurotic, personality, adjustment disorder	8/54 (15%)	Blood sera	Western blot	3/203 (2%)	

(*Continued*)

Table 5 Continued

Location	Study number and study group description	Study group seroprevalence	Test sample	Test method	Control group sero-prevalence	Quoted *p* value
Austria	Ab 16 Outpatients with panic disorder	4/55 (7%)	Not stated	Not stated	2/34 (6%)	>0.05 *q*
Japan	Ab 19 Mood disorder	9/251 (4%)	Blood sera	ECL immunoassay	10/917 (1%)	<0.05 *q*
Japan	AbV 7 Mood disorder	0/123 (0%)	Blood plasma	Indirect IFA	0/70 (0%)	
Mental disorders relating to alcohol and drugs						
Germany	AbV 6 Alcohol/drug related mental disorders	1/22 (5%)	Blood sera	Western blot	3/203 (2%)	
Japan	Ab 19 Alcohol addiction	1/42 (2%)	Blood sera	ECL immunoassay		
Mental retardation						
Japan	Ab 19 Mental retardation	0/25 (0%)	Blood sera	ECL immunoassay		
Germany	AbV 6 Mental retardation	0/2 (0%)	Blood sera	Western blot		
Neurological patients						
Germany	Ab 3 Newly admitted unspecified patients	87/1791 (5%)	CSF	Indirect IFA		
USA	Ab 4 Peripheral neurology	4/92 (4%)	Blood sera	Double stain IFA	11/483 (2%)	>0.05 *q*
Germany	AbV 11 Neurological inpatients	1/102 (1%)	CSF	Indirect IFA	None	
Germany	Ab 4 Purulent non-viral encephalitis	0/54 (0%)	Blood sera	Double stain IFA		
Japan	Ab 19 Encephalitis	0/33 (0%)	Blood sera	ECL immunoassay		
Japan	Ab 19 Degenerative diseases	0/14 (0%)	Blood sera	ECL immunoassay		

Dementia						
Japan	AbV 12 Vascular dementia patients	0/10 (0%)	Blood plasma	Indirect IFA	4/36 (11%)	
Japan	Ab 19 Vascular dementia	0/46 (0%)	Blood sera	ECL immunoassay		
Japan	AbV 12 Dementia patients	0/10 (0%)	Blood plasma	Indirect IFA	4/36 (11%)	
Alzheimer's disease						
Japan	Ab 19 Alzheimer's disease	1/89 (1%)	Blood sera	ECL immunoassay		
Multiple sclerosis						
Germany	Ab 4 Multiple sclerosis	15/114 (13%)	Blood sera	Double stain IFA	11/483 (2%)	<0.0001 q
Germany	Ab 11 Multiple sclerosis	0/50 (0%)	CSF and blood sera	Indirect IFA		
Japan	Ab 19 Multiple sclerosis	0/67 (0%)	Blood sera	ECL immunoassay		
Epilepsy						
Japan	Ab 19 Epilepsy	3/214 (1%)	Blood sera	ECL immunoassay		
Chronic fatigue syndrome						
Japan	AbV 4 Chronic fatigue syndrome	30/89 (34%)	Blood plasma or sera	ELISA	1/100 (1%)	<0.001
UK	AbV 9 Chronic fatigue syndrome	2/21 (10%)	Blood sera	Western blot	0/13 (0%)	
Germany	Ab 5 Chronic fatigue syndrome	0/50 (0%)	Blood sera	Double stain IFA	None	
Japan	Ab 19 Chronic fatigue syndrome	0/75 (0%)	Blood sera	ECL immunoassay		
HIV/AIDS						
Thailand	Ab 12 HIV Clade E with unspecified STD	24/50 (48%)	Blood sera	ELISA	2/103 (2%)	
Thailand	Ab 12 AIDS patients	12/67 (18%)	Blood sera	ELISA	None	
Thailand	Ab 12 Asymptomatic HIV carriers	9/60 (15%)	Blood sera	ELISA	None	
Germany	Ab 4 HIV lymphadenopathy	34/244 (14%)	Blood sera	Double stain IFA	11/483 (3%)	

(*Continued*)

Table 5 Continued

Location	Study number and study group description	Study group seroprevalence	Test sample	Test method	Control group sero-prevalence	Quoted *p* value
Germany	Ab 4 HIV antibody peak	61/751 (8%)	Blood sera	Double stain IFA	11/483 (3%)	
Germany	Ab 2 HIV antibody	36/460 (8%)	Blood sera	Double stain IFA	11/540 (2%)	
Germany	Ab 4 HIV antibody start	12/273 (4%)	Blood sera	Double stain IFA	11/483 (3%)	
Japan	Ab 19 HIV including AIDS	1/85 (1%)	Blood sera	ECL immunoassay		
Epstein Barr syndrome						
Germany	Ab 4 Children	9/160 (6%)	Blood sera	Double stain IFA	11/483 (3%)	
Germany	Ab 4 Adults	1/89 (1%)	Blood sera	Double stain IFA	11/483 (3%)	
Germany	Ab 2 EBV	0/24 (0%)	Blood sera	Double stain IFA		
Parasitological infections						
East Africa	Ab 4 Children with schistosomiasis and malaria	9/48 (19%)	Blood sera	Double stain IFA	11/483 (3%)	<0.0001
East Africa	Ab 4 Children with schistosomiasis or malaria	1/12 (8%)	Blood sera	Double stain IFA	11/483 (3%)	<0.05
East Africa	Ab 4 Adults with schistosomiasis and malaria	10/145 (7%)	Blood sera	Double stain IFA	11/483 (3%)	<0.01
East Africa	Ab 4 Adults with schistosomiasis or malaria	4/81 (5%)	Blood sera	Double stain IFA	11/483 (3%)	<0.05
Occupational groups						
Israel	Ab 8 Workers exposed to ostriches	19/41 (46%)	Blood sera	ELISA	4/41 (10%)	<0.0001
Taiwan	Ab 17 Mental health workers	8/82 (10%)	Blood sera	Western blot		
Austria	Ab 18 Veterinarians	0/137 (0%)	Blood sera	Indirect IFA		

Other groups						
USA	Ab 13 Twenty five pairs of monozygotic twins discordant for schizophrenia	9/25 (36%) affected subjects 5/25 (20%) unaffected subjects	Blood sera	Western blot		
Taiwan	Ab 17 Family members of schizophrenic patients	16/132 (21%)	Blood sera	Western blot		
Germany	Ab 7 Sequentially tested psychiatric in-patients	14/71 (20%)	Blood sera	Double stain IFA	8/22 (36%) major depression	
Japan	AbV 14 Blood donors from horse rearing area	36/428 (8%)	Blood sera	ELISA	1/100 (1%)	<0.05
Japan	Ab 19 Multi-transfused patients	3/66 (5%)	Blood sera	ECL immunoassay		
Germany	Ab 2 HIV-antibody-negative drug abusers	4/106 (4%)	Blood sera	Double stain IFA	2/125 (2%) HIV negative non-drug abusers	
Germany	Ab 3 Newly admitted surgery patients	20/569 (4%)	Blood sera	Indirect IFA		
Japan	Ab 19 Ocular diseases	19/1393 (1%)	Blood sera	ECL immunoassay	10/917 (1%)	>0.05
Japan	AbV 2 Blood donors	1/100 (1%)	Blood sera	ELISA		
USA	Ab 13 Eight pairs monozygotic twins discordant for bipolar disorder	0/8 (0%) affected subjects 0/8 (0%) unaffected subjects	Blood sera	Western blot		
USA	Ab 13 Eight pairs normal monozygotic twins	0/16 (0%)	Blood sera	Western blot		
Japan	Ab 19 Autoimmune disease	0/50 (0%)	Blood sera	ECL immunoassay		
Japan	Ab 19 Leprosy	0/17 (0%)	Blood sera	ECL immunoassay		

other psychiatric disorders, neurology patients, dementia/Alzheimer's disease, multiple sclerosis, epilepsy and chronic fatigue syndrome, HIV/AIDS, EBV, parasitological infections and occupational groups. Five groups of monozygotic twins, concordant and discordant for schizophrenia and other disorders, have been studied. Patient groups were defined by internationally recognized criteria (Research Diagnostic Criteria, ICD codes, DSM codes or CDC guidelines) in 18/44 (41%) studies. Control or comparison groups were either from the local community or blood donors.

Prevalence and seroprevalence varied widely (Table 6) from 0% (no evidence of infection), while the highest seroprevalence in a single study group was 48% in 50 HIV Clade E patients with STDs in Thailand. The highest prevalence of virus was 82% in 11 brains of schizophrenic patients. However this is an outlying result, and the median for this patient group was 0%. Median seroprevalence figures were highest (11%) in HIV/AIDS patients, and the highest median prevalence was 13% in astrocytic brain tumours, although this was a single study.

Laboratory methods

Seroprevalence was mainly investigated by the detection of antibodies in serum or plasma (28 studies) but also in CSF and sera (2 studies), CSF only (1 study) and unspecified material (2 studies) (Table 4). Studies for genetic markers and viral antigens have been sought mainly in peripheral blood and PBMCs or leukocytes (PBLs) (15 studies), brain tissue (7 studies), CSF (1 study) and CSF, brain and blood (2 studies) (Table 5).

The laboratory methods used to test for BDV antibodies were: indirect immunofluorescence antibody/assay (IFA) (10 studies), double stain IFA (5 studies), Western blot using viral proteins (6 studies), Western blot using recombinant proteins (4 studies), enzyme-linked immunosorbent assay using recombinant proteins (recombinant ELISA) (4 studies), recombinant reverse type (RT) sandwich ELISA (1 study), electrochemiluminescence assay (1 study), and direct IFA (1 study); in one study, the method was not stated.

Test methods for antigens or genetic markers were immunohistochemistry with nested PCR (1 study), flow cytometry (1 study), enzyme immunoassay (EIA) (1 study), EIA with nested RT-PCR (1 study), and RT-PCR (3 studies). The most frequently used was nested RT-PCR for p24 and/or p40 gene sequences (17 studies).

Issues of sensitivity and specificity, reproducibility and reliability were rarely addressed. A second test was usually used for confirmation of antibody tests (Western blotting), and genetic markers were confirmed by Southern blotting, immunohistochemistry, competition experiments, *in-situ* hybridization and direct sequencing.

Discussion

The neurological or psychiatric symptoms of human patients studied for Borna disease are clinically diverse, and given the frequently multifactorial aetiology of mental illness, an alternative explanation would be possible for all of these cases. The presence of markers of BDV infection in a clinically ill patient cannot infer causation (see reference 88). Indeed, individuals may be predisposed to BDV infection because of altered immunocompetence during repeated affective episodes.[12] Clinical case studies, however, are valuable for generating hypotheses that should be tested further in systematic epidemiological studies. These patients also provide the opportunity for molecular studies and characterisation of virus isolates.

Seroprevalence studies provide information and evidence of exposure to BDV, while prevalence studies measure the proportion of current infection. Many such studies have been carried out, in a variety of patient groups most usually selected by a clinical illness. However, while simple in their design, the validity of these studies is dependent on the sensitivity, specificity and reproducibility both of the diagnostic tests performed and of the diagnostic criteria used in the classification of patients. A wide range of seroprevalence even within specific clinical groups has been demonstrated, which may reflect geographical variation in prevalence, selection bias, or the different laboratory methods used. Median seroprevalence was highest for unspecified psychiatric in-patients (7%), neurotic, personality adjustment and mood disorder patients (6%), schizophrenics (5%) and patients with chronic fatigue syndrome (5%). These data might indicate a direction for future epidemiological studies. It is also of note that at least one person tested seropositive in every clinical group examined, except for those patients suffering from mental retardation and dementia. Other groups have been examined, probably opportunistically, such as children and adults with parasitological infections and HIV patients. Any other rationale for screening such patient groups is unclear.

Prevalence studies are usually of limited value in aetiological research, since prevalence reflects both the incidence and probability of surviving the disease.[88] In these studies, incident mental illness

is the outcome and previous exposure to BDV is the exposure of interest. To conclude that BDV has a role in any of these clinical syndromes requires that the prevalence in these groups (cases) be compared with the prevalence in another 'healthy' or 'non-case' group (controls). The outcome of such a study would be a measure of the strength of association between having a marker for BDV exposure and/or infection and being in a certain clinical group, conventionally expressed as an odds ratio. However, the odds ratio calculated in a case-control study will be a biased estimate unless the controls selected are representative of the population that are well, but could become cases. We have carried out a quantitative systematic review but have not attempted any meta-analysis methods (see reference 89). As it was unlikely that the same disease diagnostic criteria were applied across studies, and because of the variation in selection of controls, no attempt has been made to combine data from individual studies to calculate summary odds ratios. In many published BDV seroprevalence studies, there was not enough information provided on the selection of controls for the reader to assess whether they represented well individuals belonging to at-risk populations. In some studies the 'control' group should be considered a 'comparison' group, and was often small. In most studies, odds ratios were not calculated. Chen *et al.*[45] reported a positive association between presence of BDV antibodies and schizophrenia in Chinese patients from Taiwan and presented their results as an odds ratio (odds ratio 4.63, 95%CI 2.25–9.52).

Few studies have attempted to investigate possible sources of human exposure to BDV. One published study[33] found high seroprevalence in those exposed to ostriches (46%), but did not investigate possible confounding factors that may be associated both with handling ostriches and exposure to BDV, such as contact with other farm animals. Takahashi *et al.*[83] found a high seropositive rate in people living near horse farms. For some individual cases, animal exposure histories have been reported together with the results of the sampling of animal contacts (e.g. reference 84). Histories of exposure to seropositive animals or animals with a history of Borna disease is important in assessing putative human cases. Further epidemiological studies could be carried out to identify possible exposures to BDV. Further animal studies would also prove useful to establish the host range and assess possible modes of transmission of the virus, including vectors or reservoirs of infection. Also of interest would be any links between reported human cases. Chen *et al.*[45] also found higher BDV seroprevalence in first degree relatives of schizophrenic patients and mental health workers. This raises the possibility of person-to-person transmission, but could also be the result of bias in selection of controls and/or confounding factors. Family clusters of Borna disease have been reported from Germany[84] and the identification and follow-up of such clusters could prove useful in providing further information on the zoonotic potential of BDV. Cases in the published literature attributed to infection with BDV have been from the geographical areas in central Europe where Borna disease is endemic in animals. Whilst this would appear consistent with the hypothesis that Borna disease is a zoonosis, it may simply be the result of ascertainment or publication bias.

There does appear to be evidence that people are exposed to BDV, or at least have antibodies that are reactive to BDV antigens. However, while a number of seroprevalence studies have been done, it is still difficult to conclude much about which population groups are most at risk and the natural history of human infection. Longitudinal studies are necessary to follow the serological response and clinical course in patients identified as antibody positive. Evidence that humans are infected with BDV, or a BDV-like virus, rather than exposed, is even more controversial. Conflicting results have arisen and evidence of infection or exposure by the detection of viral markers or antibodies, is not consistently linked to one currently defined disease in humans. Some results show that BDV RNA is detected in the absence of antibodies in serum, further complicating matters.

An agreed gold standard diagnostic test for BDV is lacking, and there is speculation concerning the specificity of the tests currently used. It has been suggested that PCR positives are the result of laboratory contamination.[90,91] Further investigations have revealed novel isolates that may not amplify with previously described markers, and variation may be greater than previously thought.[92] Multi-centre studies have tested the same samples in a number of different laboratories by a number of different methods but this has been performed in order to confirm a result rather than to validate tests in terms of their sensitivity and specificity, reproducibility and reliability. Of course, it is not possible to validate tests using populations of known cases and controls, because is not yet clear what criteria should be used to define a human case. A well-validated blood test would appear to be the most suitable test for epidemiological studies. Certainly there are practical difficulties in using brain tissue or CSF. In the absence of consensus as to the validity, it would appear that an antibody test

Table 6 Prevalence of Borna disease virus, by study group

Location	Study number, study group description	Tissue tested	Prevalence in study group	Test method	Prevalence in control group
Unspecified psychiatric in-patients					
Germany	AbV 1 Chronic mental illness	PBMCs	37/68 (54%)	Flow cytometry	
Germany	AbV 6 A subset of psychiatric patients	PBMCs	13/26 (50%)	Nested RT-PCR	0/23 (0%)
Japan	AbV 3 Psychiatric inpatients	PBMCs	22/60 (37%)	Nested RT-PCR	
Japan	AbV 5 Psychiatric patients	PBMCs	6/55 (11%)	Nested RT-PCR	
Germany	AbV 11 Psychiatric inpatients	CSF	3/128 (2%)	EIA	
Japan	AbV 7 A subset of psychiatric patients	PBMCs	2/106 (2%)	RT-PCR	0/12 (0%)
Germany	V 6 Psychiatric disorders	Whole blood	0/39 (0%)	Nested RT-PCR	
Schizophrenia and other psychotic disorders					
Europe and US	V 3 Schizophrenic	Brain	9/11 (82%)	Nested RT-PCR	0/10 (0%)
Japan	V 4 Schizophrenic	Brain	3/9 (33%)	Nested RT-PCR	2/31 (7%)
Japan	AbV 10 Schizophrenia patients	Whole blood	6/67 (9%)	Nested RT-PCR	
Japan	V 8 Schizophrenic	PBMCs	3/77 (4%)	Nested RT-PCR	
Germany	V 6 Schizophrenic	Whole blood	0/59 (0%)	Nested RT-PCR	
Germany	V 6 Schizoaffective disorder	Whole blood	0/10 (0%)	Nested RT-PCR	
Germany	V 6 Schizophrenic	Blood/PBMCs	0/20 (0%)	Nested RT-PCR	
US	V 9 Schizophrenic	CSF	0/48 (0%)	Cell culture and nested RT-PCR EIA	

US	V 9 Schizophrenic	Brain	0/3	Nested RT-PCR EIA	
Europe and US	V 3 Psychotic disorder	Brain	0/2	Nested RT-PCR	
Korea	V 10 Schizophrenic	Not stated	0/39 (0%)	Nested RT-PCR	
US	V 11 Schizophrenic	Brain	0/16 (0%)	RT-PCR	
US	AbV 8 Schizophrenic patients	PBLs	2/10 (20%) in 1 lab, 0/10 (0%) in 2 other labs	RT-PCR	
Major depressive disorder					
Europe and US	V 3 Bipolar disorder	Brain	2/5 (40%)	Nested RT-PCR	0/10 (0%)
Europe and US	V 3 Major depressive disorder	Brain	0/6 (0%)	Nested RT-PCR	0/10 (0%)
Germany	V 6 Major depressive disorder	Whole blood/PBMCs	0/41 (0%)	Nested RT-PCR	
Germany	V 6 Bipolar disorder	Whole blood/PBMCs	0/10 (0%)	Nested RT-PCR	
US	V 9 Bipolar disorder	CSF/Brain/PBMCs	0/33 (0%)	Nested RT-PCR	
Korea	V 10 Major depression	Not stated	0/9 (0%)	Nested RT-PCR	
US	V 11 Affective disorder	Brain	0/5 (0%)	RT-PCR	
UK	AbV 9 Severe depression	PBMCs	0/8 (0%)	Nested RT-PCR	
Neurotic, personality, adjustment and mood disorder					
Japan	V 8 Mood disorder patients Neurological patients	PBMCs	2/49 (4%)	Nested RT-PCR	
Germany	AbV11 Neurological inpatients	CSF	2/102 (2%)	EIA	
US	V 11 Neurological disorder	Brain	0/17 (0%)	RT-PCR	

(*Continued*)

Table 6 Continued

Location	Study number, study group description	Tissue tested	Prevalence in study group	Test method	Prevalence in control group
Dementia					
Japan	AbV 12 Vascular dementia patients Multiple sclerosis	PBMCs	0/10 (0%)	Nested RT-PCR	0/36 (0%)
Europe and US	V 3 Multiple sclerosis	Brain	0/5 (0%)	Nested RT-PCR	0/10 (0%)
Alzheimer's disease					
US	V 2 Alzheimer's disease	Brain	4/34 (12%)	Immunohistochemistry	0/2 (0%)
Europe and US	V 3 Alzheimer's disease	Brain	0/19 (0%)	Nested RT-PCR	0/10 (0%)
Japan	AbV 12 Alzheimer's disease	PBMCs	0/10 (0%)	Nested RT-PCR	0/36 (0%)
Parkinson's disease					
Japan	V 4 Parkinson's disease	Brain	1/6 (17%)	Nested RT-PCR	2/31 (7%)
Europe and US	V 3 Parkinson's disease	Brain	0/11 (0%)	Nested RT-PCR	0/10 (0%)
Brain tumours					
Japan	V1 Astrocytic tumours	Brain tumours	5/37 (14%)	Nested RT-PCR	None
Chronic fatigue syndrome					
Japan	AbV 4 Sub-cohort of CFS patients	PBMC	6/25 (24%)	Nested RT-PCR	

Japan	AbV4 Further cohort of CFS patients	PBMC	7/57 (12%)	Nested RT-PCR	
UK	AbV 9 Subset of CSF from CFS patients	CSF	1/10 (10%)	Nested RT-PCR	0/10 (0%) CSF
UK	AbV 9 CFS patients	Sera/PBMC	0/21 (0%)	Nested RT-PCR	0/31 (0%)
Other groups					
Japan	V 5 Normal brains at post mortem	Brain	2/30 (7%)	Nested RT-PCR	
Japan	AbV 2 Blood donors	PBMCs	5/100 (5%)	Nested RT-PCR	
US	AbV 2 Blood donors	PBMCs	3/72 (4%)	Nested RT-PCR	
Germany	V 7 Blood donors	Blood	0/200 (0%)	RT-PCR	
US	V 9	CSF	0/18 (0%)	Cell culture and nested RT-PCR EIA	
	Nine sets monozygotic twins discordant for Schizophrenia	PBMCs	0/18 (0%)		
US	V 11 Medical patients	Brain	0/27 (0%)	RT-PCR	
US	V 11 Healthy subjects	Brain	0/20 (0%)	RT-PCR	
Germany	V 7 Immunosuppressed heart transplant patients	Blood	0/500 (0%)	Nested RT-PCR	

would be the most suitable test by which to screen large population samples. A 'gold-standard' serological test for Borna disease virus should be developed that is suitable for testing animal and human samples in epidemiological studies. Clinical specimens should be swapped between experts, and data should be gathered on the sensitivity, specificity and reproducibility of any such 'gold-standard' developed. This might be most feasible using sera from clinical cases of Borna disease in animals.

The hypothesis that Borna disease is a zoonosis merits serious consideration and is worthy of further investigation.

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