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Borna Disease Virus infection in young children

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INTRODUCTION

Borna Disease Virus infection (BDV) is a well known animal disease. It got its name from a disease outbreak among horses around the small town "Borna", 26 km south-east of Leipzig in the late 19th century.

The symptoms in horses have been described in detail and involve neurologic as well as mental and behavioral disorders. A long debate exists whether or not human infections do exist. Several papers reported an association with psyciatric diseases (1–4) but were disputed by others (5).

Reports on young animals are rare but the reports yielded interesting insights into disturbed adaptation to social interactions of the affected animals (6–8). Similar reports on children are lacking. The aim of the present study was therefore to investigate the prevalence of serological BDV-markers in children.

MATERIALS

From 1999 to 2006 we investigated 4226 blood samples from 2417 patients and healthy volunteers from 1 day to 80 years of age (mean value 9 years). A mean of 1,7 tests were performed per individual (1–54 examinations).

METHODS

All virological examinations were carried out at the Laboratory of Liv Bode at the Robert-Koch- Institute Berlin, Germany, and since mid 2006 at the IFLB laboratory, Berlin, by G. Czech and H. Ludwig.

Parameters determined were Borna Disease Virus (BDV) Antibodies (BDVAb), free BDV antigen (BDVAg) and circulating immune complexes (CIC) containing both BDVAb and BDVAg. Methods applied were an ELISA developed by Bode and coworkers. All results were given in a semiquantitative range from 0 (negative) to 4 (maximum values) (9) based on a double-sandwich format with monoclonal antibodies recognizing conformational epitopes of BDV N (p40) and P (p24) proteins.

In multiple examinations of individuals the maximum value of their BDV tests was used for further analysis.

RESULTS

Prevalence of BDV-markers in blood samples

BDVAg detection increased within the first weeks after birth starting at a rate of 8% positive samples which corresponds to the detection rate among adults (>18 years – 7%). A steep increase was found until the age of 4–6 months with a short decline afterwards (see Fig. 1). At the beginning of the first year detection rates again increased considerably to reach a second climax in the age group of 2–3 year old children. Afterwards a continous decline was evident.

In contrast to BDVAg a rather high prevalence of positive BDVAb tests was noted from the first months of life (Fig. 2). Their course was more uniforme with a peak of 75% detection rate in the 4–6 months old age group and a steadily slight decline later on. Anyhow, the rate always was much above the BDVAg rate and had its lowest values in adults with 44%.

The BDVAb profile resembled that of BDV-CIC, which had a constant higher rate but a

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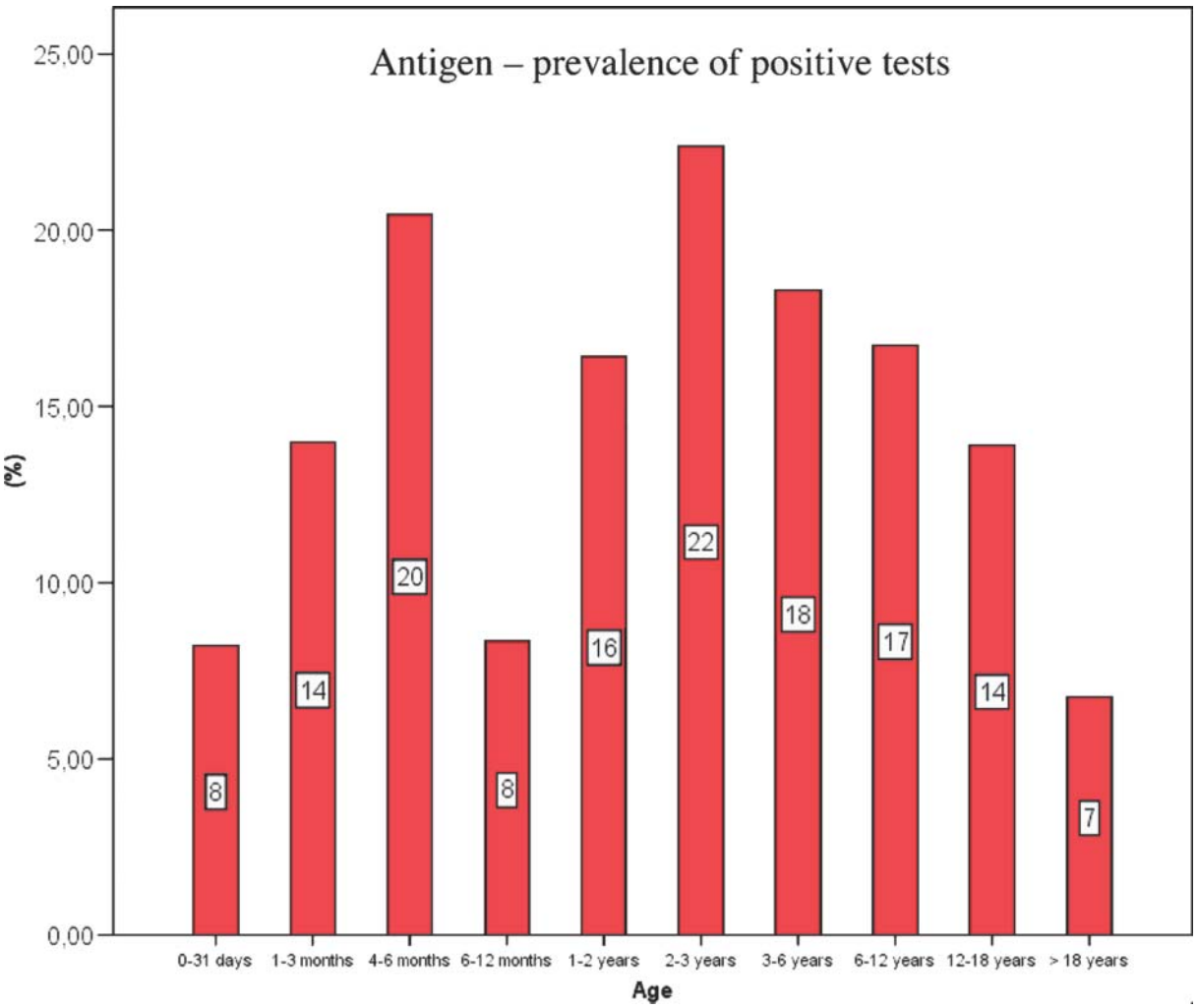

Fig. 1. Prevalence of BDV-antigen in blood samples from patients of various age groups.

similar profile increasing to 84% between 4 months and two years. Later titres declined to 48% in adults.

The level of BDV markers all showed a rapid increase within the first months of life. BDVAb and CIC could be detected in higher concentrations than BDVAg and had their climax at 4–6 months after birth (Fig. 4).

Among 118 mother (M) –newborn (NB)-pairs the following constellation of positive BDV markers was found at the day of birth Table 1:

	M	NB
Ag	3	2
Ab	19	24
CIC	20	27

DISCUSSION

BDVAg, BDVAb and CIC were detected in all age groups by means of a double sandwich ELISA. Even in the newborn age group (day 1–31) BDVAg was observed. The prevalence was nearly identical to the adult age group. This supports the assumption that a vertical transmission of BDVAg may occur. Three of 114 mothers were antigen positive at labor (level 1, 2 and 3 respectively), two of their children (of mothers with level 2 or 3) had BDVAg at level 2. The level of antigenemia in mothers at labor were thus less than in the general population (2,6% vs. 7%). The mother with level 1 BDVAg had nevertheless a child with level 1 CIC whereas the other mothers had children with

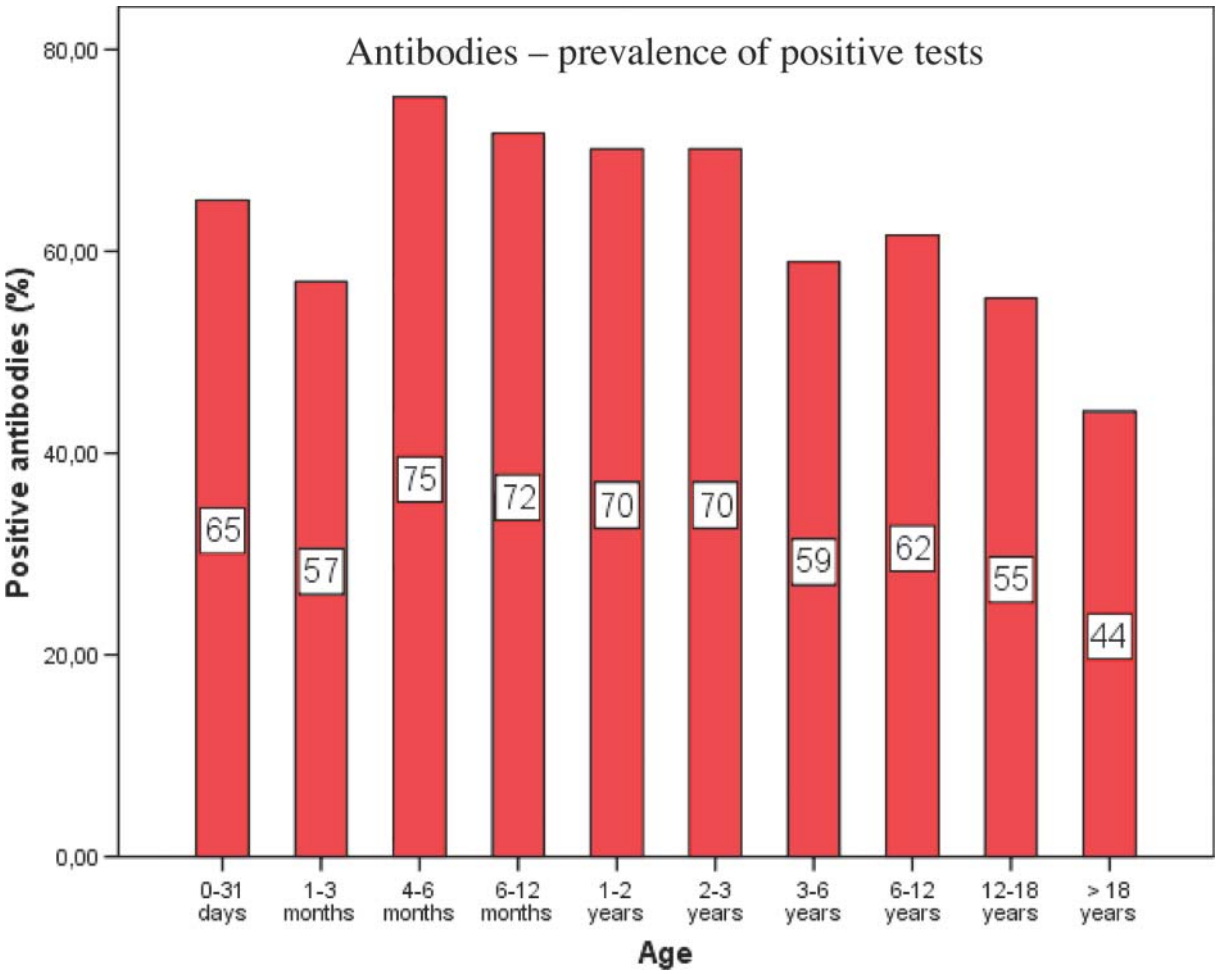

Fig. 2. Prevalence of BDV-antibodies in blood samples from patients of various age groups.

level 4 (mother BDVAg level 3) and 2 (mother BDVAg level 2). Among the mothers without antigenemia 24 newborns were CIC positive (level 1–3, mean 1,46).

The data presented here allow several conclusions:

1. There is a vertical transmission of BDVAg in human pregnancies.
2. 80% of the population under investigation acquired BDVAg within the first six months of life (Fig. 3).
3. There are two age intervals of BDV acquisition. The first with a peak at 6th month points to the mother (and/or the father) as a source of BDV. The second period with a peak value around 2–3 years could reflect other children as a source of infection since the contact to children in kindergardens and day care facilities usually intensifies at this age.

Thus acquisition of BDV is thus a general and common process during early childhood. Wether the BDV causes symptoms that hide among the many respiratory and other often febrile infections of this age group needs further studies. The strong reaction of the young immune system may be reflected by the contrast of detection rates of free BDVAg compared to BDVAb and BDVCIC. This could be interpreted as the effective activation of the infants' humoral immuneresponse to capture and eventually neutralize the replicating BDV thus leading to relatively low detection rates of free BDVAg (Figs. 1–4). Nevertheless it must be kept in mind that these tests does not detect complete virus particles but viral proteins only (p40,

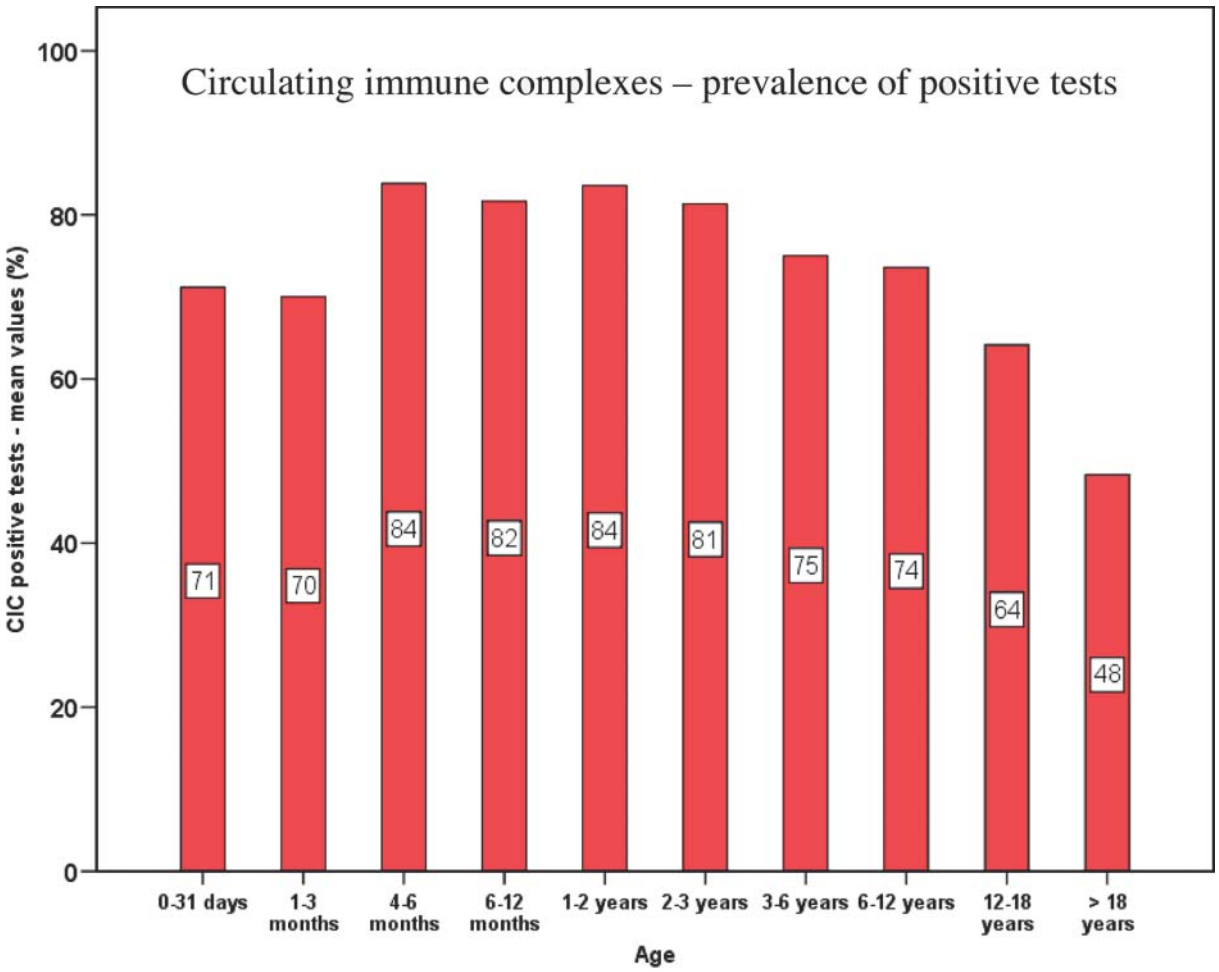

Fig. 3. Prevalence of BDV-immune compelexes in blood samples from patients of various age groups.

p24). It seems justified however to correlate their titre to the activity of viral replication. They should reflect viral activity and are candidates as markers for correlating symptoms to their occurrence in human blood samples. Such studies would be the next step in elucidating the nature of BDV infection in man.

Vertical transmission of BDV was reported in horses already (10). In this case an euthanized mare with a febrile condition showed characteristically reduced appetite, ataxia and paresis. The brain, showing multiple neuronal degeneration and necrosis with hemorrhage, and the histologically normal brain of the fetus were both positive for BDV RNA. In mice a vertical transmission was found under experimental conditions as well (11). These authors used RT-nested PCR techniques for BDV p24-RNAs to detect BDV in brains of 7 days old newborn mice. Our study is the first to detect BDVAg by means of an ELISA in peripheral blood of human newborns whose mothers demonstrated free BDVAg in their blood samples at the day of delivery but prior to delivery.

The time of infection is crucial for development of symptomatic BDV infection in some species. Rats infected as neonates showed no inflammatory cerebral infection in contrast to animals inoculated at 1 or 2 months of age (12). Purkinje cells of the cerebellum were a distinct target of BDV in neonatal rats (13). This could be the basis of neurodevelopmental delay in these affected individuals, a situation which is rather frequently encountered in human babies and remains unclear in most cases (personal observation). While in adult rats BDV replication is restricted to neural cells, neonatally infected rats have infectious virus or viral antigens in the cells of most organs (14). If this distribution pattern is valid for human beings too the poss-

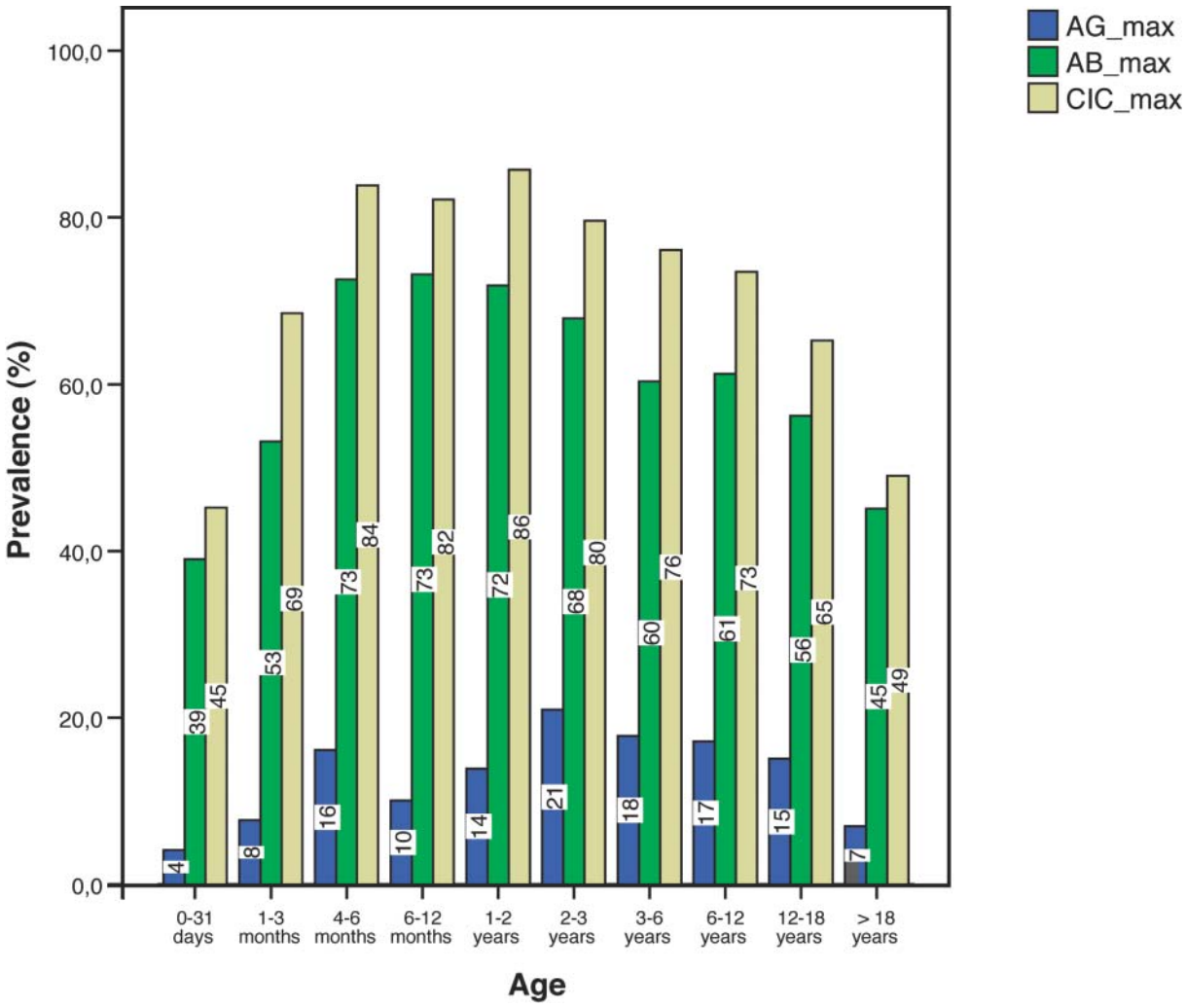

Fig. 4. Course of activity markers from patients of various age groups.

ible spread of BDV to extracerebral organs would prompt us to search for symptoms not primarily related to cerebral and neural structures. Therefore it is necessary to clarify the time of BDV acquisition in man. Our results indicate a bimodal time course of acquistion. This should alert future researchers to differentiate possible symptoms in these age groups: neonates and toddlers.

Our results showed a fairly high rate of positive BDVAb tests pointing to a high infection rate in young children. This corresponds rather well to the infection rates in sheep (15). According to these authors BDV prevalences by immunoblotting and/or reverse transcriptase PCR were 0% (0 of 19) in newborns (<1 month old), 51.7% (15 of 29) in lambs (1 to 6 months old), and 36.7% (11 of 30) in adults (>2 years old). Among animals positive for BDV, 60% of lambs and 45.5% of adults contained BDV RNA in PBMCs while 46.7% of lambs and 90.9% of adults contained specific antibodies to BDV. They suggest that virus replication in the blood is usually reduced in adulthood by raising immune responses to BDV.

In conclusion, our results are well in line with observations concerning prevalence and acquisition times in animals and raising the question if there should not be similar symptoms in humans as observed in animals, namely behavioural disturbances, neurological as well as psychiatric disturbances as previously reported by several groups (1, 16–18). Moreover, future research should also expand the knowledge on extracerebral symptoms since in young age, affection of other organs was incidentally re-

ported (19, 20) pointing to a possibly broader spectrum of BDV symptoms in newborns, babies, young children and toddlers.

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