



FOCUS ARTICLE: BORNA DISEASE

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INTRODUCTION

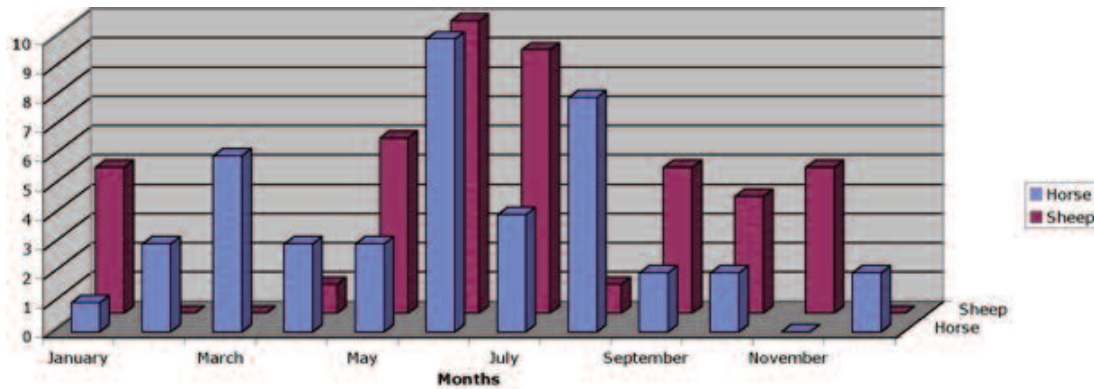
Borna disease virus (BDV) is an enveloped virus with a negative-stranded non-segmented RNA genome of approximately 8.9 kb. It replicates and transcribes its genome in the nucleus and uses the cellular RNA splicing machinery to regulate gene expression. Mainly because of these features, BDV has been classified as the prototype virus of a newly established family, Bornaviridae, within the order Mononegavirales. BDV is the causative agent of Borna disease (BD), a mostly fatal meningoencephalitis originally detected among horses of Germany. Natural hosts of BDV are horses, sheep and other farm animals. Many other warm-blooded vertebrates ranging from rodents to non-human primates are susceptible to experimental infection with BDV. In these animals, BDV infection may either remain clinically inapparent, or it may lead to severe neurological abnormalities and eventually to death. Numerous studies with experimentally infected rats and mice have conclusively demonstrated that BD is caused by immunopathological mechanisms in which the antiviral T cell response results in neurological disorder.

BORNA DISEASE IN HORSES AND SHEEP IS RESTRICTED TO CENTRAL EUROPE

BDV infections can result in neurological disease that mainly affects horses and sheep in certain areas of Germany. The endemic area also includes parts of the upper Rhine valley between Switzerland, Austria and the Principality of Liechtenstein. Between 1894 and 1896 a large epidemic of BDV-induced disease occurred among cavalry horses in the town of Borna in the state of Saxony (Germany). The disease and its inducing viral agent are named after the location of this first documented large outbreak. In recent years, the number of animals diagnosed with classical BD was relatively low, usually affecting less than a total of 100 horses and 100 sheep each year. To our knowledge, until very recently, no confirmed cases of BD had been reported in horses or sheep outside the endemic areas described above. The exception is a BD case in a horse from eastern Austria. Unlike infected animals from the classical endemic areas, this horse was shown to be infected with a novel genotype of BDV. Serological studies in horses outside the known endemic regions (e.g. Italy, Finland, Iran) indicate that BDV could be more widespread.

Regarding the seasonal incidence of natural BD cases, the figure below shows the distribution of 107 BD cases in Switzerland, which had been diagnosed at the Institute of Veterinary Pathology in Zurich (47 horses, 46 sheep; not included in the graphic are 4 donkeys, 2 mules, 3 goats, 2 cows, 2 rabbits and 1 cat) during the last 30 years (1976 until today).

The figure shows a peak during the summer months, but there are as well cases during the other months. So the question aroused if this appearance would correlate with the occurrence of possible vector populations. However the incubation time is supposed to be up to several months, which might explain BD cases occurring in wintertime.



DIAGNOSIS OF BORNA DISEASE

Reliable ante mortem diagnosis of BD is difficult. Horses and sheep with BD exhibit a variety of clinical symptoms, predominantly behavioural abnormalities, apathy and movement disorders, which are not specific for BD but may also be seen in animals infected with other microorganisms that invade the CNS. Cerebrospinal fluid (CSF) of animals with BD may display pathological alterations, such as increased protein content and mononuclear pleocytosis. However, these changes are not specific for BD but rather represent non-specific indicators of viral meningoencephalitis. BDV-specific antibodies in serum and/or CSF are better indicators. Among the currently used methods of detecting these antibodies, indirect immunofluorescence assay (IFA) appears most reliable. The percentage of horses and sheep with confirmed BD that scored positive in this serological assay varied considerably between different studies. For these reasons, ante mortem examination alone can usually not provide firm proof of BD. Confirmation by post-mortem examination with histological analysis of brain tissue is required.

Histologically, variable degrees of encephalitis are observed in brains of animals with BD. Lymphocytic infiltrations are usually most prominent in the hippocampus, the brain stem and in parts of the cerebral cortex. Inflammation is usually absent or less prominent in the cerebellum. CD4+ T cells are predominantly present at perivascular sites, whereas CD8+ T cells are found both in the perivascular cuffs and in the brain parenchyma. To clearly distinguish BD from encephalitis induced by other viruses, it is mandatory to prove that BDV infection of the CNS has occurred. Traditionally, Joest-Degen inclusion bodies in nuclei of infected neurons have served as BDV-specific markers, but they cannot consistently be seen by routine histology in brains of diseased animals.

BORNA DISEASE IN OTHER ANIMALS

BD is not strictly limited to horses and sheep, although the frequency at which other animals get the disease appears to be very low. BDV was found in donkeys, goats and cattle with neurological disease and strong lymphocytic infiltrations of the CNS. Some of the diseased bovines were from farms in regions of Germany in which BD is not endemic in horses and sheep. BDV antigen and infectious virus was shown to be present in the CNS of two rabbits with neurological disease which originated from the endemic region in Switzerland. An earlier report described the isolation of BDV from the brain of a rabbit with neurological disease. BDV antigen and RNA were further found in brains of several zoo animals in Erfurt that showed neurological disease. However, the identification of BDV in hosts such as cats, dogs, lynx, rabbits, and even ostriches indicates that the virus has a broad host repertoire of various birds and mammals.



VIRUS RESERVOIR?

The search for vectors for BDV has mainly concentrated on subclinically infected horses and small ruminants, wild rodents and other small mammals. We identified BDV-infections without evidence of disease in wild shrews (*Crocidura leucodon*) captured from an endemic area.

Shrews belong to the order insectivora. Their appearance can be described as something between a mouse and a mole. The bicoloured white-toothed shrew, *Crocidura leucodon*, occurs in the eastern part of Switzerland (Rhine Valley, Tessin), in the Rhône Valley, in the region of Basel, and the Principality of Liechtenstein and it belongs to the genus *Crocidura* together with two other species which occur in Europe (*C. russula* and *C. suaveolens*). Shrews have to be considered as a reservoir species, because virus antigen could be demonstrated in various organs using different techniques like immunohistochemistry and real-time RT PCR without showing any pathological lesions.

Studies in rats had shown that the clinical course and histopathology of Borna disease varies with the age of the animal at the time of infection. In adult Lewis rats, BDV infection results in severe encephalitis accompanied by clinical symptoms that include hyperactivity, aggressiveness, and ataxia. At a later stage of the disease, surviving animals are apathetic and show signs of dementia and behavioural abnormalities, and their brains show a dramatic loss of neuronal tissue.

Experimental infection of adult mice takes an asymptomatic course, an observation previously believed to indicate that this animal species is not suitable for pathogenesis studies. However, BDV frequently induces severe neurological disease in infected newborn mice.

In wild birds, fragments of the BDV p24 and p40 genes from faecal samples collected at a bird pond could be amplified. Recently, the existence of avian bornaviruses was demonstrated and provided as a compelling candidate in the search for an etiologic agent of proventricular dilation disease (PDD).

Ticks had been suspected to be possible vectors for BDV. This however has not been proven so far.

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FOCUS ARTICLE: THE IMPORTANCE OF WEST NILE VIRUS LINEAGE

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Since 1996 West Nile Virus (WNV) has re-emerged as an arbovirus of both public health and veterinary concern. WNV is a non-contagious viral disease transmitted predominantly by *Culex* mosquitoes in a bird and ornithophilic mosquito enzootic amplification cycle. Incidental infections occur in horses, humans and other mammals as a result of bites from haematophagus bridging vectors.

Though initially thought to be two lineages, phylogenetic analysis of West Nile Virus has defined five distinct lineages that differ from each other by 20-25% at the complete genome level.

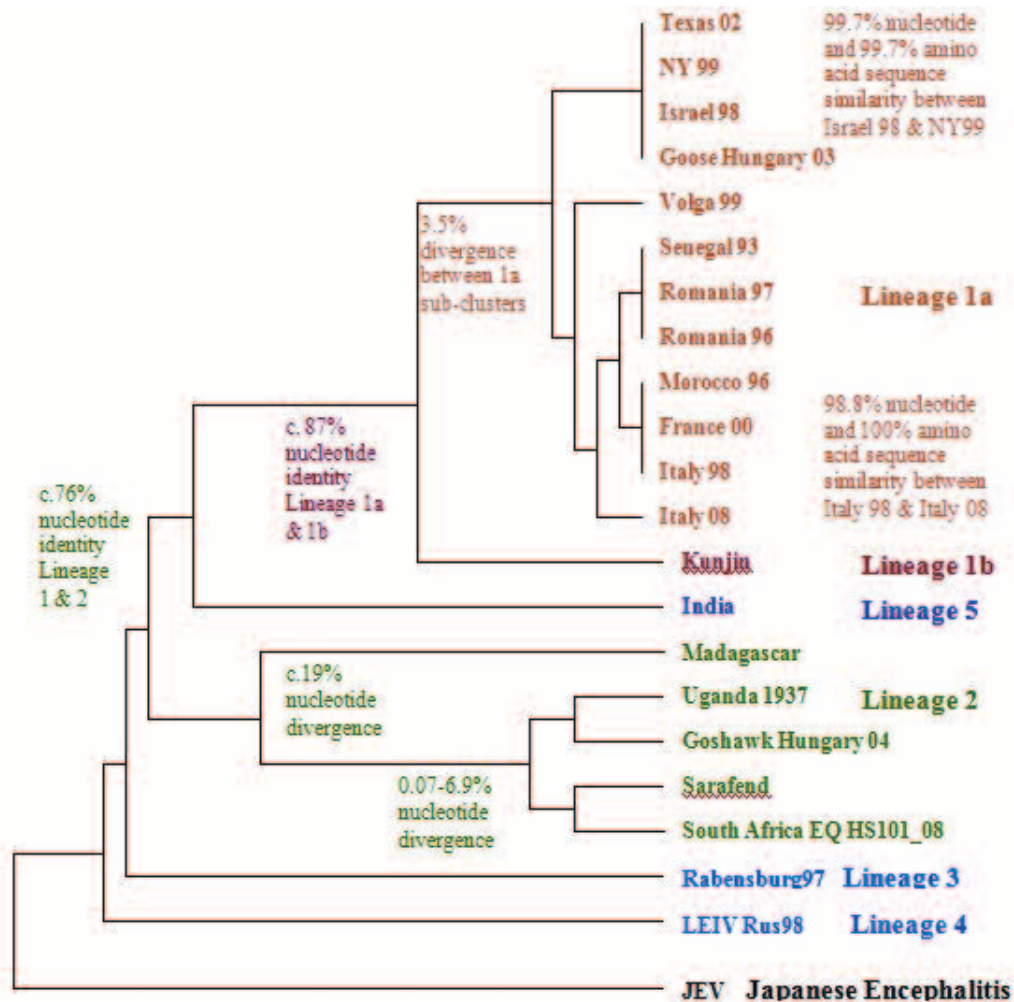


Figure 1: Phylogenetic analysis of West Nile Virus lineages (E-protein genome)

Lineage 1 is the most geographically widespread (1a Europe, Africa, Americas & Asia; 1b Kunjin Australia). Lineage 2 has been found in Africa and recently related to avian mortality in Central Europe (Hungary 2004 & 2005, Austria 2008). Lineages 3 and 4 have been identified in Russia and lineage 5 (formerly classified as 1c) in India.



Horses are susceptible to infection by both lineage 1 and 2, however it is not the individual WNV lineage, but the neuroinvasiveness of the individual virus serotypes that influence the development of clinical signs.

WNV presentation is a function of lesion location and produces a neurological syndrome characterised by a combination of clinical signs. The majority of WNV cases are subclinical or present with non-specific signs such as depression, anorexia and stiffness that resolve within 24-48hrs. Mild transient pyrexia (38-39oC) may occur, but is often undetected. Serological investigations in a number of WNV outbreaks indicate that many horses produce antibodies but few develop clinical signs or mortality. WNV infection can however be life threatening to clinically affected horses.

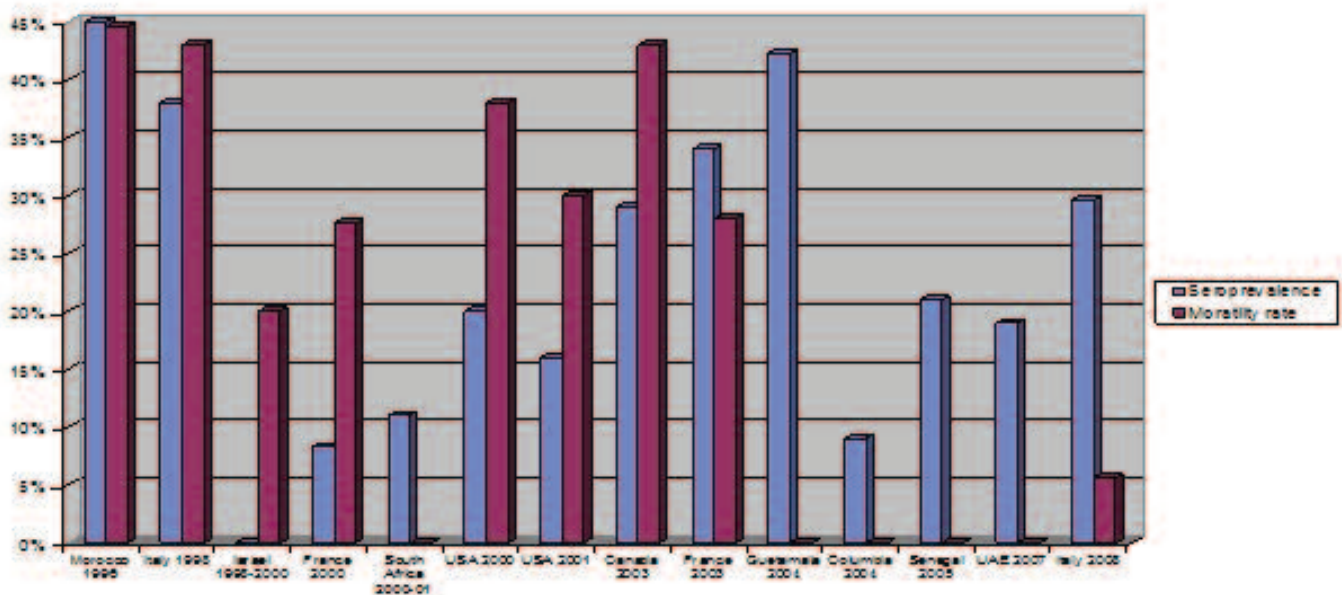


Figure 2: Comparison between observed seroprevalence and mortality rates for WNV lineage 1 and lineage 2 (South Africa 2000-01).

Mortality and recovery rates differ both between and within lineages. In the Italy 1998 lineage 1a outbreak the mortality rate was 43%, but all of the horses that did recover did so fully and within a 5-15 day clinical course. This contrasts with the North American lineage 1a WNV outbreaks, where mortality rates ranged up to 43%, but had mean clinical courses >21 days and recovery rates of only 79-93%. This North American lineage 1a WNV serotype then spread into the Caribbean in 2004, with a seroprevalence of 9-42.3% in the absence of clinical disease. A neuroinvasive Lineage 2 strain in South African 2008 killed 5/7 clinically affected horses. The two surviving horses did fully recover after protracted rest over several months. Wider seroprevalence of this strain in South Africa is unknown, however in endemic regions equine WNV may reach high seroprevalence rates. In Romania lineage 1a WNV seroprevalence was reported at 56% in the absence of clinical signs, while maximum seroprevalence of African lineages has been reported at 75-86% with an annual infection rate of 11-21%.

WNV infections have been confirmed in horses ranging from four months to 38 years. Age prevalence does not seem to be lineage related, though a number of equine case studies have shown an age-related incidence in WNV lineage 1a infection in the 6-10 and 10-16 year age brackets. This may not be a true risk but a factor of equine use and mosquito exposure, or biased dependent on the economic or emotional value of the equines and whether reporting of suspect clinical signs is compulsory in that country.



Concurrent disease does not seem to play a major role in the pathogenesis of lineage 1a WNV infection in horses; however co-infection in South Africa with African Horse Sickness, even in vaccinated horses, or Equine encephalosis may facilitate the central neuroinvasive quality of WNV lineage 2 leading to a greater case prevalence of blindness and seizures.

Ataxia and weakness are the major presenting clinical signs of WNV. Ataxia and muscle fasciculation may indicate a better prognosis than paresis, paralysis or central nervous signs. Though this is predominantly a function of diffuse lesions and less severe neuronal damage, recumbent horses have a poor prognosis due to intensive nursing requirement and associated secondary problems.

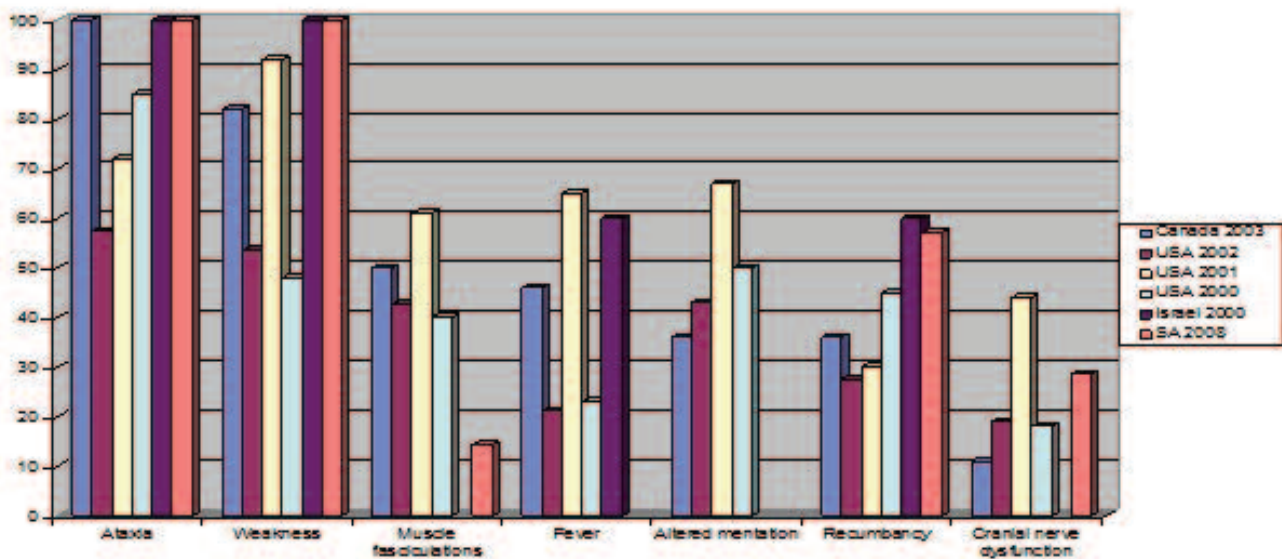


Figure 3: Clinical presenting signs associated with lineage 1 and lineage 2 (South Africa 2008) outbreaks

The risk of WNV introduction into the UK by horses remains very low (please see Defra-webpage for published risk assessment). Syndromic surveillance and the timely reporting of equine neurological disease can help detect any initial WNV case, regardless of WNV lineage, and provide a vital early warning of WNV circulation in the UK. Timely reporting and diagnosis also allows for more accurate assessment of the geographic distribution of WNV and will guide public health and veterinary preventative control measures with the aim of minimising any subsequent morbidity and mortality.

In the UK WNV is a notifiable disease. Any suspicion of WNV should be reported immediately to your local Animal Health office.

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