RAPID RISK ASSESSMENT

Novel zoonotic Borna disease virus associated with severe disease in breeders of variegated squirrels in Germany

First update, 5 May 2015

Main conclusions and options for actions

A recently identified cluster of acute fatal encephalitis in three breeders of variegated squirrels in the German state of Saxony-Anhalt is an unusual public health event with a potentially high impact on the small group of people who are exposed to this particular squirrel species. Further investigations are ongoing to describe these cases. The role of a newly identified Borna disease virus (BDV) isolate in the aetiology of these cases remains to be confirmed. Further work is required to identify natural hosts, reservoirs, vectors, transmission routes, and distribution.

Serological investigations as well as retrospective and prospective testing of human cases of encephalitis potentially caused by BDV, particularly in areas where BDV infection is documented in animals, will contribute to a better understanding of the risk of BDV infection in humans. Further comparative phylogenetic analytic investigations of BDV in Old World animals versus New World animals might provide additional information. The clear association between direct exposure to variegated squirrels and developing encephalitis observed in Saxony-Anhalt justifies a recommendation to avoid direct/close contact with living or dead variegated squirrels and exposure to dust particles contaminated with squirrel excretions (as a precautionary measure) until more is known about this potential zoonosis.

Source and date of request

ECDC internal decision on 17 April 2015 to update the Rapid Risk Assessment dated 25 February 2015.

Public health issue

The investigation of a cluster of three fatal cases of encephalitis in Germany with a history of exposure to variegated squirrels (*Sciurus variegatoides*) led to the detection of the genome of a previously unknown type of Borna disease virus (BDV). The genome was sequenced after isolation from organ samples from a squirrel kept by one of the dead breeders. Tests on brain samples from the three human cases identified gene sequences of what

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Erratum: On 8 May 2015 the word 'potentially' was added to the first sentence of the second paragraph in the conclusions: 'Serological investigations as well as retrospective and prospective testing of human cases of encephalitis potentially caused by BDV, particularly in areas where BDV infection is documented in animals, will contribute to a better understanding of the risk of BDV infection in humans.'

appears to be the same BDV. This raises the possibility that the squirrel breeders died from a zoonotic infection caused by a previously unknown BDV.

Consulted experts

Experts from ECDC: Denis Coulombier, Céline Gossner, Niklas Danielsson, Katrin Leitmeyer and Hervé Zeller. External experts from Germany: Mirko Faber (Robert Koch Institute), Martin Beer (Friedrich Loeffler Institute), Jonas Schmidt-Chanasit (Bernhard Nocht Institute for Tropical Medicine)

Disease background information

Borna disease virus

Borna disease viruses (BDV) are enveloped, negative-sense, single-stranded RNA viruses [1]. The neurotropic BDV is a member of the *Bornaviridae* family within the *Mononegavirales* order. BDVs replicate inside the host cells' nuclei, and the close association with the host's chromosomes facilitates its spread from cell to cell during mitosis [2]. The uniquely limited amplification of the virus' genome favours non-cytolytic persistence in the host cells and evasion from antiviral immune responses [3]. Phylogenetic analysis of exogenous bornaviruses and mammalian endogenous bornavirus-like (EBLs) elements shows that BDV are likely to have co-existed with primates for as long as 40 million years [4].

Borna disease virus infection in animals

Borna disease virus infection was first described in the 18th century and later named after the German town of Borna in Saxony, where an outbreak of a fatal neurological disease among military horses occurred in 1885 [5]. BDV can infect a wide range of vertebrates, including rhesus monkeys, horses, sheep, cattle, goats, rabbits, deer, llamas, alpacas, cats, rats, mice, shrews, gerbils, dogs and ostriches. In addition, avian bornaviruses have been described in psittacine birds [6], Canada geese, trumpeter and mute swans [7], and canary birds [8,9].

BDV infections in animals have been described in central and northern Europe, Japan, Australia, and the United States [10,11].

In animals, BDV infection may cause acute or subacute meningoencephalitis and mild neurological manifestations [5]. Paralysis is common in classic BDV infections, and the majority of the affected animals die within five weeks of onset. Recovery is possible but with lifelong altered behaviour [13].

Small wild mammals are the most likely reservoirs and vectors of BDV, although the epidemiology is not fully understood [11]. Experimental animals are most efficiently infected with BDV via intranasal or intracerebral routes and via intramuscular, intradermal or subcutaneous injections. Intravenous and intragastric administration does not result in infection [12]. Transmission may occur via excretions (urine or faeces). BDV spreads intra-axonally from the inoculation site to the central nervous system, and animal experiments have shown that the incubation period is determined by the distance from the inoculation site to the central nervous system [12].

Borna disease virus infection and disease in humans

Several studies have reported higher seroprevalence of BDV antibodies and other BDV markers in patients with a diverse range of psychiatric conditions [13,14]. A few BDV isolates were recovered from severely depressed patients [15], but transmissibility of these BDV isolates remain unknown. A causal relationship between BDV infection and psychiatric disease has not yet been established. The frequency of BDV infections in humans and the significance of endogenised BDV genes in humans remain uncertain [5].

Event background information

On 19 February 2015, Germany posted a message on EWRS reporting three cases of fatal encephalitis in the state of Saxony-Anhalt. The first case developed symptoms in 2011, and the second and third cases became symptomatic in 2013. They did not live close to each other and were admitted to different hospitals. All three patients were males, between 62 and 72 years of age, and of age-typical health status. They all bred variegated squirrels (*Sciurus variegatoides*), a tree squirrel species common to Central America that can be kept as an exotic outdoor pet. The three breeders, who were acquainted, exchanged animals, either directly or via a third party.

During the two or more weeks from first symptoms to hospital admission, the patients experienced fever, chills, fatigue, weakness and walking difficulties. Due to increased confusion and psychomotor impairment they were admitted to neurology wards where they subsequently developed ocular paresis. Their condition deteriorated rapidly after admission, and they died despite intensive care and mechanical ventilation. Laboratory investigations on samples from cerebrospinal fluid and brain tissue performed at the Bernhard Nocht Institute for Tropical Medicine in Hamburg failed to produce evidence of any recognised aetiology of non-purulent encephalitis.

The Friedrich Loeffler Institute (a German federal research institute for animal health) investigated the carcass of one of the variegated squirrels that had belonged to the third patient. A genetic analysis of a tissue sample pool of the animal using a metagenomics approach produced gene sequences of a previously unknown type of BDV. The tested squirrel had not demonstrated symptoms suggestive of BDV infection.

Further molecular and immunohistochemical analysis of brain tissue from the three deceased patients confirmed the presence of gene sequences identical to sequences found in the genome of the squirrel. The genome of the newly identified virus isolate clearly differs from known BDVs, and the homology with compared viruses was below 77%.

Despite active case finding (through an online survey and veterinary departments), no additional cases of encephalitis or suspicious deaths among breeders of variegated squirrels or their household contacts could be found.

The Bernhard Nocht Institute for Tropical Medicine has recently developed a serological test for antibodies against this novel BDV strain and began offering laboratory testing for breeders of variegated squirrels and their household contacts. Further information (in German) is available at the Robert Koch Institute's website: http://www.rki.de/DE/Content/InfAZ/Z/Zoonosen/Bornavirus_Hinweise-zur-Diagnostik.html.

The Friedrich Loeffler Institute has developed a test with sufficient sensitivity to detect BDV in mouth swabs and blood samples from variegated squirrels. The new test means that animals do not have to be killed in order to diagnose BDV infection.

ECDC threat assessment for the EU

The compelling epidemiological link between the three human cases (same symptomatology, same uncommon activity of breeding variegated squirrels in captivity) and the confirmatory microbiological test results (BDV gene sequences found in brain samples from all human cases matched the BDV genome identified in a variegated squirrel bred by one of the cases) suggest a zoonotic transmission from the variegated squirrels to the breeders.

The possibility of human-to-human transmission remains unclear.

The first case precedes the second and third cases by more than one year, which could be explained by the fact that 1) the breeders imported infected squirrels at different times, 2) the incubation period has a wide range depending on the individual susceptibility, 3) the intensity and frequency of the exposure of the cases was different (i.e. different viral shedding of squirrels, different risk behaviour of the breeders), or 4) the breeders exchanged infected animals at different points in time.

The clustering of human cases in Saxony-Anhalt may be explained by the fact that breeders exchanged animals but it could also reflect the distribution of variegated squirrels (and breeders) in Germany. In addition, the increased awareness of the health authorities has most likely triggered additional testing, leading to the detection of the following human cases.

There is so far no definitive evidence of a causal relationship between the presence of BDV gene sequences in the brain tissue of the human encephalitis cases and the clinical presentation.

Variegated squirrels are native to central and northern America and were introduced to Europe as pet animals. The number of animals and breeders, and their distribution in Europe, is unknown. It is possible that infected squirrels were imported, but it is just as likely that the animals became infected in Germany through contact with other infected animals.

Data on the distribution of the virus in the variegated squirrel population are not available, and it is not known if variegated squirrels are reservoirs or vectors. It is unclear whether other mammals can be infected with this newly identified BDV isolate. To date, there is no evidence that other species of squirrels, either native to Europe or imported, can become infected.

BDV transmission routes have not been determined for the three human cases. A plausible route would be through bites or scratches, but it cannot be excluded that BDV can be transmitted through direct deposition on the mucous membranes or inhalation of particles contaminated by faeces or urine of infected animals.

Assuming that the variegated squirrel is a reservoir or a vector for this novel BDV and that there is no evidence so far of human-to-human transmission of the disease, the number of people who may be exposed to this novel BDV appears to be very limited, and the probability of infection remains very low.

The probability of infection for people who breed variegated squirrels or keep them as pets is higher. It is currently difficult to quantify that risk because there is a lack of information regarding most factors that influence this probability, e.g. information about the new virus's biology and pathophysiology in humans, transmission routes, size and distribution of the captive variegated squirrel population, prevalence of infection in variegated squirrels in Europe. It is also unclear whether variegated squirrels are a reservoir or a vector for the newly identified virus isolate. But since all three cases were fatal, future compelling evidence that the newly identified BDV isolate causes disease in humans would necessitate a range of different countermeasures.

Conclusions and options for action

This cluster of acute fatal encephalitis in three breeders of variegated squirrels in the German state of Saxony-Anhalt is an unusual public health event with a potentially high impact on the small group of people who are exposed to this particular squirrel species. Further investigations are ongoing to describe these cases. The role of a newly identified Borna disease virus isolate in the aetiology of these cases remains to be confirmed. Further work is required to identify natural hosts, reservoirs, vectors, transmission routes, and distribution.

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