



Novel Orthobunyavirus detected in cattle in Germany

Information of the Friedrich-Loeffler-Institut

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The Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health (FLI), has first detected a virus of the genus *Orthobunyavirus* in cattle in Germany. Comparative analyses of the genetic material lead to the assumption that the virus belongs to the Simbu serogroup (Shamonda, Aina, Akabane viruses). The virus could be cultivated and replicated on insect cells and later on also on a hamster cell line. Based on the geographic origin of the sample, the virus was provisionally named „Schmallenberg virus“. Further investigations for characterization of the virus and epidemiological investigations will follow.

So far, investigations have yielded 12 positive samples from cattle from 6 different holdings in Northrhine-Westphalia. Among them is a twin calf which died *in utero* 10 days prior to due date. „Schmallenberg virus“ was detected in the peritoneal fluid of the calves by *real-time* RT-PCR. Furthermore, the virus was detected in the brains of malformed lambs from 14 holdings, 7 of them located in Northrhine-Westphalia and 7 in Lower Saxony. The malformations are late sequelae of infection at an earlier stage of pregnancy in summer/autumn of 2011.

The detection method was made available to institutions in Belgium, France, England, the Netherlands, and Italy.

The Netherlands primarily have reported cases in sheep; so far more than 50 holdings are affected. Furthermore, malformed newborn calves from 126 cattle, sheep, and goat holdings are investigated for the virus. In Belgium, „Schmallenberg virus“ has also been detected in sheep. Meanwhile, an association between virus detection and the observed symptoms and damages is considered very likely.

It is still unclear whether this exotic virus has been newly introduced or whether orthobunyaviruses already have been present in ruminants in Europe for some time. Therefore, further investigations are necessary to assess this virus detection.

Orthobunyaviruses of cattle are widely distributed in Oceania, Australia and Africa and, as a rule, initially cause very mild clinical symptoms. If pregnant animals are infected, however, temporarily delayed, sometimes considerable congenital damages, premature births and reproductive disorders may occur. Akabane-like viruses are mainly transmitted by biting midges. These viruses which are relevant in cattle do not represent a risk for humans. They are no zoonotic agents. Due to the relationship of „Schmallenberg virus“ with Shamonda, Aino, and Akabane virus, a risk for humans is not to be expected (also see risk assessment of the European Center for Disease Prevention and Control: http://ecdc.europa.eu/en/publications/Publications/Forms/ECDC_DisForm.aspx?ID=795).



New introduction of bluetongue disease suspected at first

Since the summer months of 2011, the national reference laboratory for bluetongue disease at the Institute of Diagnostic Virology, FLI Isle of Riems, has been notified of clinical symptoms in dairy cows in North Rhine-Westphalia which indicated a new introduction of bluetongue disease. In several herds, some animals showed fever of over 40°C, reduced general condition, loss of appetite and a drop in milk yield (up to 50 %). The symptoms disappeared after a few days.

This disease, which had already been reported earlier in the Netherlands, spread further. In the Netherlands, cases were reported from over 80 holdings; the clinical picture (fever, drop in milk yield) sometimes included diarrhea and abortions. Since September 2011, the FLI received an increasing number of samples from affected German holdings.

Findings and data

All samples were tested for a series of viruses at the Institute of Diagnostic Virology of the FLI, Isle of Riems. The following pathogens could be excluded as causative agents: bluetongue virus, epizootic haemorrhagic disease (EHD) virus, foot-and-mouth disease (FMD) virus, bovine viral diarrhea (BVD) virus and other pestiviruses, bovine herpesvirus 1 and other herpesviruses as well as Rift Valley fever virus and bovine ephemeral fever virus. Cultivation of selected samples in bovine cell cultures did not yield a detectable virus replication.

As the number of cases increased and conventional diagnostic methods failed, a new procedure which had recently been established at the Institute of Diagnostic Virology was used, the so-called metagenomic analysis. This technique, which is very laborious and expensive, permits the non-targeted detection of genetic material (genome) of potential infectious agents or of genomic sequences in any kind of sample material. The detection rate strongly depends on the relation between the quantities of pathogen genome and host genome. For the analysis, the FLI uses the Roche Genome Sequencer FLX. Metagenomic analysis had already been carried out and validated by the FLI for several months in the frame of an EU project (EMPERIE) and a BMBF network (PHÄNOMICS).

The thus optimized procedures were used in early November 2011 for analyzing a pool of 3 samples from a holding in Schmallenberg (district Hochsauerlandkreis). The samples originated from animals with reported fever (>40°C) and a strong loss in milk yield (up to 50%).

Metagenomic analysis revealed the presence of viral genome sequences which showed the highest homology with the genus *Orthobunyavirus* in the family of *Bunyaviridae*. Further analyses showed that the sequences are most strongly related to the so-called Akabane, Aino and Shamonda viruses.

The genome of orthobunyaviruses consists of three segments (L, M, S); the detected sequences showed homologies to all of these three segments. Depending on the sequence section and the virus used for comparison, homologies ranged between approx. 60 and 95%.

Based on this sequence information, several *real-time* RT-PCR tests were developed and used for further investigation of the samples (blood or serum). It was shown that all three samples from the pool of the metagenomic analysis were positive. One sample showed a Ct value of approximately 26.

So far, investigation by PCR yielded at least 12 positive samples from 6 holdings, some of them showed Ct values below 30 and can be classified as clearly positive. More than 90 investigated samples from



cattle of non-affected areas (Southern Germany, Mecklenburg-Western Pomerania) were clearly negative.

By means of the virus isolate, a serum neutralization test was established, which is now used for first serological investigations. An ELISA test is being developed.

Conclusions

The described sequence findings and the data obtained by *real-time* RT-PCR are the first evidence for the presence of a virus of the genus *Orthobunyavirus* in cattle in Germany. The sequence homology indicates that the agent is a virus of the Simbu serogroup. Based on the geographic origin of the samples, the virus was provisionally named „Schmallenberg virus“.

The occurrence of the symptoms from August to the end of October and the now reported decrease in cases support the causal relationship with an Arbovirus. Akabane virus and similar viruses are mainly transmitted by biting midges. Similar transmission routes are possible for “Schmallenberg virus” and may be another indication of a causal relationship with the observed clinical disease.

Further investigations have been initiated, which include optimized and extended cultivation of the virus, inoculation of cattle, development of a serological assay, and testing of further samples from the affected area. In addition, further epidemiological investigations will be carried out.

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Therefore, the investigations will concentrate on the intensive surveillance of the disease situation in the region where the virus has been detected. For further clarification and particularly for the investigation of a possible causal relationship between the newly detected pathogen and the observed clinical picture, especially blood samples of acute suspect cases (fever $>40^{\circ}\text{C}$, reduced general condition, massive drop in milk yield) and of suspect newborn calves (stillbirths, malformations, abortions) should be sent to the Friedrich-Loeffler-Institut for further investigations (contact: PD Dr. Martin Beer, martin.beer@fli.bund.de).