Occurrence of malformed calves in April - May 2021 indicates an unnoticed 2020 emergence of Schmallenberg virus in Denmark

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Abstract

During the European emergence of Schmallenberg virus (SBV) in 2011, examination of *Culicoides* spp. showed that SBV infected midges were present across Denmark. However, SBV associated malformations in ruminant species have not been reported in Denmark. In April 2021, seven calves with severe congenital generalized arthrogryposis and reduced body weight originating from a narrow region of the Jutlandic peninsula were submitted for examination. Analysis of fetal brain tissue for SBV viral RNA and pleural effusion for fetal anti-SBV antibodies identified SBV as the cause of the congenital syndrome. Backwards calculation from the calving dates indicated the occurrence of an unnoticed emergence of SBV in Denmark from early August 2020 and during the late summer and autumn. As SBV associated malformations may lead to dystocia urging for fetotomy or Cesarean section, veterinarians performing obstetric intervention are first line personnel in recognition of SBV emergence in domestic ruminants.

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Summary

During the European emergence of Schmallenberg virus (SBV) in 2011, examination of *Culicoides* spp. showed that SBV infected midges were present across Denmark. However, SBV associated malformations in ruminant species have not been reported in Denmark. In April 2021, seven calves with severe congenital generalized arthrogryposis and reduced body weight originating from a narrow region of the Jutlandic peninsula were submitted for examination. Analysis of fetal brain tissue for SBV viral RNA and pleural effusion for fetal anti-SBV antibodies identified SBV as the cause of the congenital syndrome. Backwards calculation from the calving dates indicated the occurrence of an unnoticed emergence of SBV in Denmark from early August 2020 and during the late summer and autumn. As SBV associated malformations may lead to dystocia urging for fetotomy or Cesarean section, veterinarians performing obstetric intervention are first line personnel in recognition of SBV emergence in domestic ruminants.

Keywords: Schmallenberg virus, Peribunyavirus, ruminants, malformation, fetal infection

Introduction

Schmallenberg virus (SBV), an orthobunyavirus belonging to the Simbu serogroup that predominantly infects ruminants, emerged in Central Europe in 2011 (Hoffmann et al., 2012). The virus is transmitted between its mammalian hosts by hematophagous insect vectors (*Culicoides*biting midges) (Elbers, Meiswinkel, van Weezep, Sloet van Oldruitenborgh-Oosterbaan, & Kooi, 2013; Sick, Beer, Kampen, & Wernike, 2019). Examination of *Culicoides* spp. caught in Denmark during the initial spread of SBV in 2011 revealed that infected midges were present across the country (Rasmussen et al., 2014). However, outbreaks of SBV associated diseases in ruminants have not been published, nor have cases been diagnosed during a national bovine abortion surveillance program from 2015 to 2017 (Wolf-Jäckel et al., 2020). Hence, outbreaks of SBV infection in Denmark seem to be rare, but as official surveillance for SBV is not in force in Denmark and as the infection is not notifiable, knowledge is empiric.

In adult animals, SBV induces a short-lived viremia of two to six days, associated with either none or only mild unspecific clinical signs like fever, diarrhea or reduced milk yield (Beer & Wernike, 2021; Hoffmann et al., 2012). However, SBV may cause fetal malformation, when a susceptible pregnant ruminant species becomes infected during a vulnerable phase of gestation (Beer & Wernike, 2021). Like other viruses of the Simbu serogroup such as Akabane virus (Kirkland, 2015), SBV induces a syndrome mainly characterized by brain malformation and generalized arthrogryposis (Agerholm, Hewicker-Trautwein, Peperkamp, & Windsor, 2015; Bayrou et al., 2014). Generalized arthrogryposis, usually referred to as arthrogryposis multiplex congenita (AMC), is a severe neuromuscular condition characterized by angulation and ankylosis of the joints. In addition to SBV and a few other viruses, AMC may have other causes such as genetic defects (Agerholm, McEvoy, Menzi, Jagannathan, & Drögemüller, 2016). In Denmark, a nationwide surveillance system for bovine genetic diseases has been in force since 1989. As part of this program, selected cases of congenital malformations are submitted to the University of Copenhagen for necropsy and genetic assessment. In April-May 2021, seven AMC cases were reported by veterinary practitioners and acquired for necropsy. To rule out a viral etiology, these cases were examined for teratogenic viruses and found to be positive for SBV or anti-SBV antibodies. The results of this investigation are reported here.

Materials and Methods

The calves were either stillborn or were euthanized for animal welfare reasons on the farms in connection to the delivery, which occurred in a narrow time period from April 12 – 26, 2021. The calves were submitted for necropsy as part of the Danish surveillance system for bovine genetic diseases. Two cases were frozen to - 20 °C before being shipped and were necropsied after thawing, while the other five cases were shipped directly to the laboratory and necropsied within 2-8 days after parturition. The calves underwent a necropsy and samples from cerebrum and cerebellum were taken as was a pleural effusion fluid. A blood sample from the dam was taken in a plain vacutainer tube and either followed the carcass or was requested afterwards. The blood was centrifuged at 1160 g for 15 min to separate the serum. All samples were stored at - 20 °C in plastic tubes before being shipped for virological and serological analyses at the Friedrich-Loeffler-Institut.

Upon arrival at the Friedrich-Loeffler-Institut, tissue samples were homogenized in 1 ml serum-free Minimum Essential Medium (MEM). Nucleic acid was extracted from 140 µl of the tissue homogenates, serum or pleural effusion fluid by using the QIAamp Viral RNA Mini kit (Qiagen, Hilden, Germany) and subsequently tested by an SBV S-segment based real-time RT-PCR (Bilk et al., 2012). To exclude an infection with further viral pathogens, especially viruses with a teratogenic potential, the samples have been additionally tested by a multiplex real-time RT-PCR for the detection of bovine viral diarrhea virus (BVDV), bluetongue virus (BTV), foot-and-mouth disease virus (FMDV), epizootic hemorrhagic disease virus (EHDV), and Rift Valley fever virus (RVFV) (Wernike, Hoffmann, & Beer, 2015).

In addition, sera and pleural effusion fluids were analyzed by a commercially available enzyme linked immunosorbent assay (ELISA) for the detection of anti-SBV antibodies (ID Screen® Schmallenberg virus Competition, ID.vet, France).

Data on calving outcomes in the herds +/- 3 months around the date for birth of the malformed calf were obtained from the Danish Cattle Database.

Results

All cases were diagnosed as AMC based on their state of congenital generalized arthrogryposis (Figure 1). Generally, the cervical spine had torticollis, while the thoracic spine showed either scoliosis or kyphoscoliosis associated with aberrant course of the ribs. This had let to narrowing of the thoracic cavity and consequently pulmonary hypoplasia and abnormal shape of the heart. The skeletal muscles had undergone lipomatous atrophy, especially affecting muscles of the limbs, pelvis and axis. The joints of the limbs had various combinations of flexure and extension and both bilateral symmetric and asymmetric arthrogryposis was found (Figure 1). All cases had reduced body weight but normal amount of fat depots thus reflecting the severe muscular atrophy. Mild to severe hydrocephalus was observed in four cases. The mesencephalic duct was malformed in two (Figure 2) and the cerebellum grossly hypoplastic in one case. Additional malformations were present in some calves (Supplementary table S1). None of the calves had received colostrum.

Real-time RT-PCR and ELISA analyses revealed that one case was positive for SBV viral RNA (case 1), one case was positive for both viral RNA and fetal anti-SBV antibodies (case 2) and five cases were positive only for fetal anti-SBV antibodies. The pleural effusion fluid of one calf scored in the doubtful measuring range of the antibody ELISA (case 7). All dams were seropositive for SBV (Table 1). Indication of other concomitant viral infections were not found.

The cases originated from 7 herds located in South-West of the Jutlandic peninsula (Figure 3) and within a distance of less than 150 km. Five cases clustered within a radius of only around 20 km (Figure 3). Two cases originated from Holstein dairy herds while five cases were beef cattle breeds. An insemination date was recorded for four cows and ranged from July 17th to August 7th2020, while the other three females were mated naturally during summer grazing. Additional case data are shown in Supplementary table S1.

Discussion

Bovine fetuses are expected to be vulnerable to the teratogenic properties of SBV from about 30 to 150 days of gestation (König et al., 2019; Wernike, Holsteg, Schirrmeier, Hoffmann, & Beer, 2014). By using the recorded insemination date or by backwards calculation from the calving dates, the fetuses presented in this study were probably exposed to SBV from early August 2020 and during the late summer and autumn. Five out of the six cases were born within a 14 days period and all cases were found within a narrow geographic region of the Jutlandic peninsula (Figure 3). This indicates the occurrence of a local unnoticed emergence of SBV during a short period in late summer or autumn of 2020. In Central Europe, SBV has established an endemic status with cyclic re-occurrence every two to three years (Larska, 2018; Wernike & Beer, 2017, 2020), but also resurgence of SBV in a given area after longer periods of epidemiological silence has been reported previously (Bayrou et al., 2021; Delooz et al., 2017). SBV was detected in Denmark in 2011 for the first time (Rasmussen et al., 2014) and although the knowledge on SBV infection of domestic ruminants in this country is sparse, it is likely that the reported cases represent such a resurgence of the virus.

The observed malformations as well as the significantly reduced body weight are similar to SBV-induced alterations reported previously in infected bovine fetuses (Bayrou et al., 2014; Herder, Wohlsein, Peters, Hansmann, & Baumgartner, 2012; Peperkamp et al., 2012).

An initial recognition of AMC with post mortem findings of brain malformations like hydrocephalus and cerebellar dysplasia should lead to a presumptive etiological diagnosis of fetal SBV exposure, which is to be verified by laboratory diagnostics. SBV is usually associated with severe dystocia in cattle urging for either delivery by fetotomy or Cesarean section. Bovine practitioners are therefore first line personnel in recognition of spread of SBV and other teratogenic viruses in cattle and surveillance systems should be in place to allow proper laboratory investigation of such cases. In the present Danish cases, the malformed calves were submitted as part of the national surveillance of bovine genetic diseases and examined for SBV exposure as part of the diagnostic workout.

Ethics Statement: The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. The fetuses and clinical specimens were submitted in the context of a Danish nationwide surveillance system for bovine genetic diseases, no further permissions were necessary.

Conflict of Interest: None.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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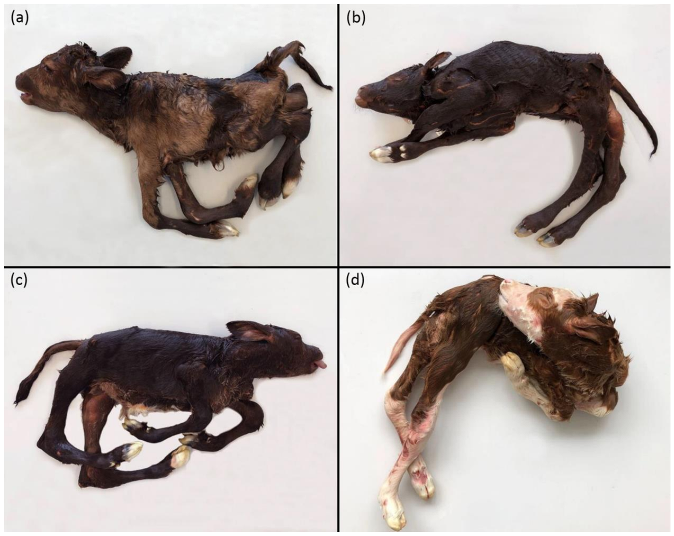
Figure legends

Figure 1 Morphological appearrence of four calves exposed to Schmallenberg virus during fetal development. All calves have generalized arthrogryposis affecting both the limbs and the spine, but the morphological expression of the condition varies among cases

Figure 2 Brain lesions in calves exposed to Schmallenberg virus during fetal development. (a) Hydrocephalus (asterisk) and dilation of the mesencephalic duct (arrow), case 3. (b) The forebrain developed bilateral collapse (circles) upon removal of the brain from the cranial vault due to decreased intraventricular pressure, case 1 (c) Severe hydrocephalus (asterisk) and ventral opening of the mesencephalic duct (arrow), case 1. (a-c): bar = 2 cm

Figure 3 Map of Denmark indicating the location of herds, where a malformed Schmallenberg virus exposed calf was born in April – May 2021. Cases positive for viral RNA by real-time RT-PCR are shown in red, while cases positive for fetal antibodies against Schmallenberg virus are shown in green. A case being negative for viral RNA and doubtful for fetal antibodies is shown in yellow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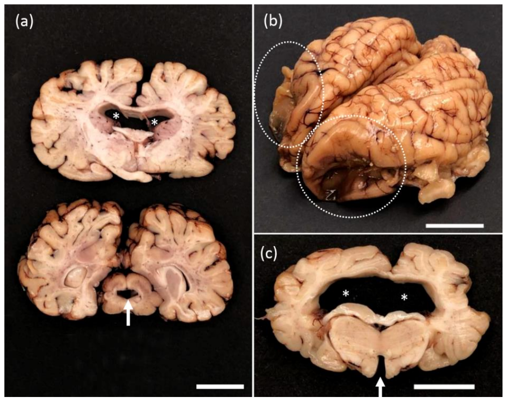
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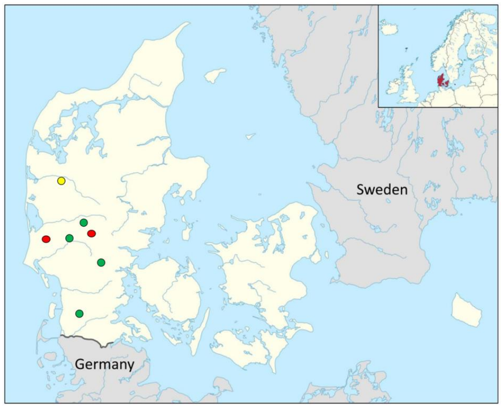

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