Bluetongue Virus Serotype 8 Reemergence in Germany, 2007 and 2008

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Reemerging bluetongue virus serotype 8 (BTV-8) in Germany was detected first in May 2007 in a sentinel cow and in February 2008 in an export heifer. Reemergence was confirmed by retesting the samples, experimental inoculation, fingerprinting analysis, and virus isolation. Overwintering of BTV-8 and continuous low-level infections are assumed.

Bluetongue virus (BTV) is a double-stranded RNA virus of the genus *Orbivirus.* BTV is transmitted to its hosts by the bite of *Culicoides* spp. midges. It causes a noncontagious, arthropod-borne disease of domestic and wild ruminants and camelids (*1*); disease can be serious, particularly in sheep (*2–4*). BTV had never been reported in any European country north of the Alps until August 2006, when outbreaks of BTV serotype 8 (BTV-8) were almost simultaneously discovered in the Netherlands, Belgium, Germany, and France (*5–7*). In 2006, a total of 893 cases were detected in Germany, but the source of initial virus introduction remains unknown. Subsequently, BTV-8 overwintered in the region, spread over most of the country, and led to almost 21,000 new cases in 2007 in Germany. BTV-8 infections spread to additional European countries, e.g., the United Kingdom, Switzerland, the Czech Republic, Denmark, and Spain (*8–11*).

To detect reemerging BTV-8 in Germany, a sentinel animal program was started in March 2007. Each month, the program tested 150 BTV-naive cattle per 2,025 km². Screening was performed by ≈30 regional laboratories located in the 16 federal states of Germany. Diagnostic testing was performed with commercially available BTV-blocking-ELISAs (Pourquier ELISA Bluetongue Serum, Institut Pourquier, Montpellier, France; Bluetongue Virus Antibody Test Kit, VMRD, Pullman, WA, USA: ID Screen Bluetongue Competition ELISA kit, ID Vet, Montpellier, France; INGEZIM BTV, INGENASA, Madrid, Spain) and validated BTV real-time PCRs (*12*; B. Hoffmann et al., unpub. data). All positive results were confirmed by the National Reference Laboratory for BTV at the Friedrich-Loeffler-Institut, Insel Riems.

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The Cases

On May 3, 2007, the first sentinel animal in the program (4-year-old Holstein Friesian cow, North-Rhine Westphalia, Germany) tested positive for BTV antibodies and genomes in a regional laboratory (Krefeld, Germany). This animal had tested negative for BTV on March 3 and April 3. After the positive test result, the original samples as well as new samples were transferred to the reference laboratory for further investigation. The sample collected on May 3 and all further samples of this animal tested positive by BTV ELISA as well as by BTV real-time reverse transcription–PCR (RT-PCR). According to Deutscher Wetterdienst, available from www.dwd.de, the daily temperatures in the region of North-Rhine Westphalia during this period ranged from minimums of 13.5°C to –1.3°C to maximums of 9.5°C to 28.1°C. A period with daily maximum temperatures >22°C from April 12 to April 16 suggests increased likelihood for activity of *Culicoides*. Similar prognoses for the reemergence of BTV-8 were published by Gloster et al. (*13*).

Samples taken on May 3 had positive ELISA titers of 8–16, and the cycle threshold (Ct) values of the real-time RT-PCR analysis were 25.6–27.9 (Figure, panel A). Therefore, new BTV-infection of a BTV-naive animal was suggested. To confirm the presence of infectious BTV, fresh EDTA-blood samples from the positive animal were experimentally injected into 2 bovids (40 mL intravenously and 10 mL subcutaneously). To mimic natural exposure by the bite of a midge, only 20 μL was injected subcutaneously into a 3rd bovid (Table). Both animals inoculated with 50 mL had positive real-time PCRs at day 2 postinoculation and reached Ct values of 22.9 and 24.3 by day 13. In contrast, the animal that had received the smaller volume remained negative for BTV antibodies and genomes. In addition, 1 of the animals that had received the larger volume showed late seroconversion according to BTV-blocking ELISA at day 30 (Table). Furthermore, the virus was typed as BTV-8 by using the BTV-8–specific primers BT8/101/F (*14*) and BT8/765/R (5′-CCACTGTCTATGTG CGTAA-3′, kindly provided by P. Mertens, Institute for Animal Health, Pirbright, UK) to amplify a 1,993-bp fragment of the VP2 gene by conventional RT-PCR (Figure, panel B). Testing of 80 contact animals from the same herd showed 38 antibody-positive cattle but no additional RT-PCR–positive case.