

1 **Emergence of bluetongue virus serotype 3 in western Germany, October 2023, and *ad-***
2 ***hoc* monitoring in *Culicoides* biting midges**

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4 Anja Voigt (ORCID 0009-0005-3272-2272), Helge Kampen, Elisa Heuser, Sophie Zeiske, Bernd
5 Hoffmann, Dirk Höper, Mark Holsteg, Franziska Sick, Sophia Ziegler, Kerstin Wernike,
6 Martin Beer*(ORCID 0000-0002-0598-5254), Doreen Werner

7

8 Leibniz-Centre for Agricultural Landscape Research, Muencheberg, Germany (A. Voigt, D.
9 Werner)

10 Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Greifswald – Insel
11 Riems, Germany (H. Kampen, E. Heuser, S. Zeiske, F. Sick, S. Ziegler, K. Wernike, M. Beer)
12 Chamber of Agriculture for North Rhine-Westphalia, Bovine Health Service, Bad Sassendorf,
13 Germany (M. Holsteg)

14

15 *Corresponding author: Martin Beer, Friedrich-Loeffler-Institut, Federal Research Institute
16 for Animal Health, Suedufer 10, 17493 Greifswald – Insel Riems, Germany; email.
17 martin.beer@fli.de

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19 **One sentence summary line:**

20 Coinciding with the emergence and low-level circulation of bluetongue virus serotype
21 3 in ruminants in Germany in October 2023, the virus was detected in a pool of *Culicoides*
22 biting midges of the Obsoletus Group, while enzootic Schmallerberg virus was found in
23 numerous midge pools.

24

25 **Running title:**

26 Emergence of BTV-3 in Germany, 2023

27 **Abstract**

28 Bluetongue virus serotype 3 emerged in October 2023 in Germany, where
29 Schmallenberg virus is enzootic. BTV-3 was detected in a pool of *Culicoides* biting midges at
30 the same time as infections were reported from ruminants. Schmallenberg virus was
31 frequently found in vector pools, reflecting the epidemiological situation.

32

33 **Main text**

34 Bluetongue virus (BTV), an orbivirus of the *Sedoreoviridae* family, may cause
35 epizootic disease in domesticated and wild ruminants (1). Bluetongue (BT) is a WOAHA listed
36 disease and regulated within the European Union (EU) in accordance with Regulation (EU)
37 2016/429 (“Animal Health Law”) and its delegated regulations (2), with outbreaks requiring
38 trade restrictions in EU member states to prevent the etiologic agent from spreading.

39 BTV-3 emerged for the first time in continental Europe in early September 2023, when
40 clinical disease was observed on four sheep farms in the Netherlands. By mid-October, more
41 than 1,000 outbreaks had been detected throughout the Netherlands, increasing to 5,884 by
42 mid-December (3). Meanwhile, BTV-3 has also reached Belgium and the UK (4, 5). In
43 contrast to the newly emerging BTV-3, the orthobunyavirus Schmallenberg virus (SBV) is
44 enzootic in continental Europe since its first appearance near the German-Dutch border in
45 2011 (6). BTV and SBV share major epidemiological characteristics, as both are transmitted
46 by *Culicoides* biting midges (Diptera: Ceratopogonidae) and affect mainly ruminants (1, 7).

47

48 **The Study**

49 Shortly after Germany was officially declared free of BTV (BTV-8) in June 2023 (7),
50 the first German case of BTV-3 was confirmed on 12 October in a sheep in the German
51 district of Kleve, close to the Dutch border. Until February 15th, 2024, 38 additional BTV-3
52 cases were reported from sheep and cattle farms in the German federal states of North Rhine-

53 Westphalia and Lower Saxony (Fig. 1). An isolate was obtained from a BTV-3 positive blood
54 sample and further characterized on both *Culicoides* cells (KC cells) and baby hamster kidney
55 cells (BHK cells). Whole genome sequencing resulted in a nearly complete genome sequence
56 (available from the INSDC databases under project ID PRJEB72862). Overall, the obtained
57 genome is 99.94% at the nucleotide level and 99.95% at the amino acid level, respectively,
58 identical with the sequence of a recent BTV-3 isolate from the Netherlands (OR603992.1,
59 OR603993.1, OR603994.1, OR603995.1, OR603996.1, OR603997.1, OR603998.1,
60 OR604000.1, OR603999.1, OR604001.1).

61 Following the emergence of BTV-3 in the Netherlands, i.e. before any clinical
62 suspicions had been announced in ruminants in Germany, biting midge traps were installed in
63 animal stables in western Germany to collect putative BTV-3 vectors and test them for virus
64 infection. Between September 24 and 26, UV-light-traps were set up in cattle, sheep and goat
65 sheds on 18 German farms along the Dutch border (North-Rhine Westphalia and Lower
66 Saxony). The traps were placed close to the animals and were protected from wind and rain.
67 Insects were sampled daily until 9 or 11 November, depending on the location, and then once
68 a week for 24 hours. Collected biting midges were morphologically identified according to
69 ‘Obsoletus Group’, ‘Pulicaris Complex’ and ‘other *Culicoides*’. Obsoletus Group and
70 Pulicaris Complex, which are considered to contain the main virus vectors in Europe (8), were
71 screened in pools of up to 50 individuals for RNA of BTV and epizootic haemorrhagic disease
72 virus (EHDV), another culicoid-borne orbivirus that recently emerged in Europe (9). To
73 include a virus that is enzootic in the ruminant population in the region, and that is
74 consequently expected to be present in the insect vectors at detectable prevalences, biting
75 midges were also tested for Schmallenberg virus (SBV). The SBV-specific RT-qPCR system
76 used has been described earlier (10), and for BTV and EHDV detection a multiplex RT-qPCR
77 was used (11). Pools that tested positive for BTV were subsequently analyzed by a BTV-3-
78 specific RT-qPCR (12). Biting midge pools positive for viral RNA were retrospectively

79 examined for the biting midge species contained in the pools
80 (13,<https://biorxiv.org/cgi/content/short/2024.01.23.576915v1>).

81 In a first approach, 1,603 biting midge pools collected at nine sites from 26 September
82 to 09 November were tested for BTV, SBV and EHDV, with the number of pools per site
83 ranging from 27 to 466. One pool collected October 12 in the district of Kleve (Fig. 1) was
84 tested positive for BTV-RNA (quantification cycle [Cq] value: 35.6), and was subsequently
85 confirmed as BTV-3 positive (Cq 37.5). The pool consisted of a mixture of *C. obsoletus* clade
86 O1, *C. scoticus* and *C. chiopterus*. Another pool with Obsoletus Group biting midges
87 collected on the same day and at the same site tested positive for SBV. In addition, SBV was
88 detected in 534 further samples of this 6.5-week-period from all nine locations, one site in
89 Lower Saxony in the district of Grafschaft-Bentheim and eight sites in North Rhine-
90 Westphalia (three in the district of Kleve, two in the district of Wesel, two in the district of
91 Borken and one in the district of Heinsberg) (Fig. 1; Appendix). With the exception of two
92 Pulicaris Complex pools, all SBV-RNA-positive pools belonged to the Obsoletus Group (for
93 taxon specification see Appendix). No pool was found positive for both BTV- and SBV-RNA,
94 and all pools tested were negative for EHDV.

95

96 **Conclusions**

97 BTV-3 was first confirmed in Germany on 12 October 2023 in a sick sheep, and
98 further spread was detected in two German federal states until winter 2023/2024. The isolated
99 BTV-3 is almost identical to the virus of the outbreaks in the Netherlands. A pool of biting
100 midges of the Obsoletus Group – collected on the day of the first BTV-3 confirmation in
101 Germany – was found positive for BTV-3-RNA on a cattle farm in the same district. The
102 detection of the virus in their putative vectors confirms an ongoing transmission cycle at that
103 time, albeit circulating at a very low level, as only a single insect pool tested positive and only
104 single animals were positive in infected farms. In contrast, the SBV genome was found in

105 numerous *Culicoides* pools, reflecting the situation in ruminant populations, as SBV-infected
106 cattle, sheep and goats have been reported in Germany since 2011, albeit with varying
107 prevalence between years (6). The Cq values of the SBV RT-qPCR in some of the
108 investigated *Culicoides* pools (Appendix) indicate substantial virus loads, reflecting extensive
109 regional SBV circulation in autumn 2023. While SBV has become enzootic in Germany, the
110 new outbreak of BTV-3 is expected to continue, intensify and spread with the seasonal onset
111 of biting midge activity in spring 2024.

112

113 **Acknowledgments**

114 We would like to thank all farmers who supported the study and volunteered in
115 attending biting midge traps. The study was funded by the German Federal Ministry of Food
116 and Agriculture (BMEL) through the Federal Office for Agriculture and Food (BLE), grant
117 numbers 28N207601 and 28N207602, as well as EU Horizon 2020 program project Versatile
118 Emerging infectious disease Observatory (VEO), grant number 874735.

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120 **Author biography**

121 Anja Voigt is a Ph.D. student at the Leibniz-Centre for Agricultural Landscape
122 Research, Muencheberg, Germany. Her research interests include behavior and habitat
123 binding of biting midge vectors.

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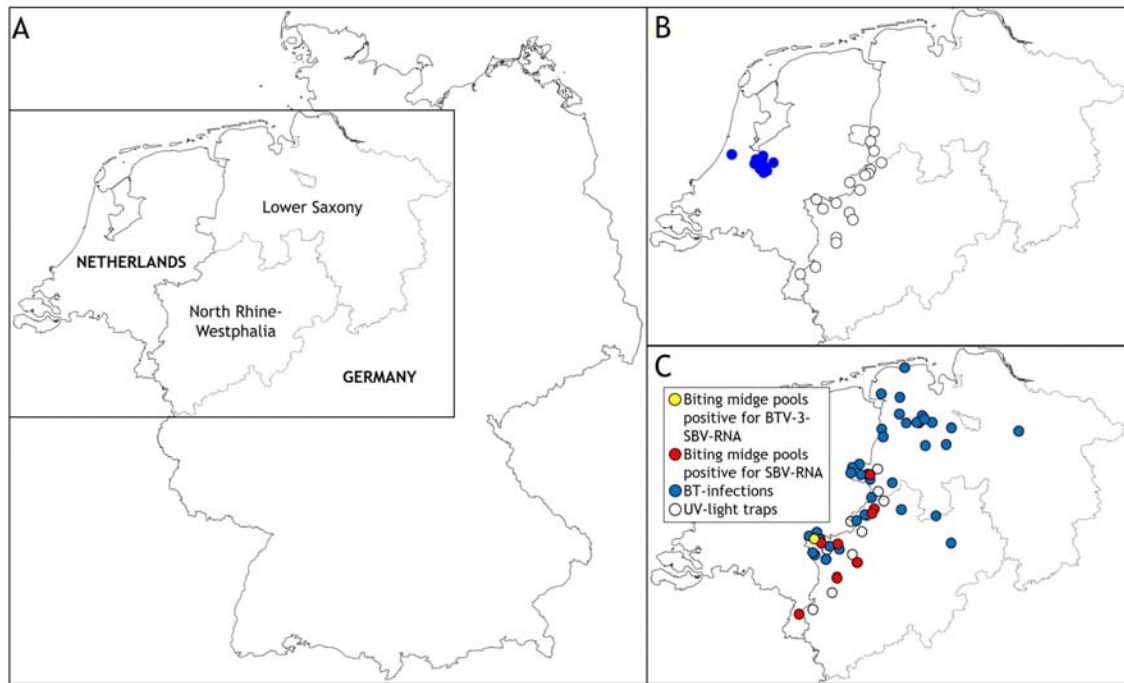
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156 Ceratopogonidae) belonging to the *Culicoides* subgenus *Culicoides*, including recently
157 discovered species and genetic variants. *Diversity*. 2023;15:699
158

159 **Figures**



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161

162 **Figure 1:** (A) Overview Germany – Netherlands – German federal states of North Rhine
163 Westphalia and Lower Saxony. (B) Dutch BTV-3 cases (blue dots) as of 08 Sept 2023 and
164 locations of UV-light-traps along the German-Dutch border (white dots). (C) BT infections in
165 ruminants (blue dots) as reported to the German animal disease reporting system as of 15 Feb
166 2024 and geographic assignment of BTV-3-/SBV-RNA- (yellow dot) and SBV-RNA- (red
167 dots) positive biting midge pools.

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