CVI Journals.ASM.org

Surveillance of Batai Virus in Bovines from Germany

Miriam Hofmann,[a] Anke Wiethölter,[b] Irena Blaha,[c] Hanna Jöst,[a,d] Patrick Heinemann,[a,e] Maria Lehmann,[a] Thomas Miller,[c] Daniel Cadar,[a] Tohru Yanase,[f] Nils Kley,[b] Martin Eiden,[b] Martin Groschup,[b] Jonas Schmidt-Chanasit[a,d]

Bernhard Nocht Institute for Tropical Medicine, WHO Collaborating Centre for Arbovirus and Hemorrhagic Fever Reference and Research, Hamburg, Germany[a]; Friedrich-Loeffler-Institut, Institute for Novel and Emerging Infectious Diseases, Greifswald, Germany[b]; Staatliches Tierärztliches Untersuchungsamt-Diagnostikzentrum, Aulendorf, Germany[c]; German Centre for Infection Research (DZIF), Hamburg-Luebeck-Borstel partner site, Hamburg, Germany[d]; Charite Medical School, Institute of Medical Virology, Berlin, Germany[e]; Kyushu Research Station, National Institute of Animal Health, NARO, Kagoshima, Japan[f]

To estimate the veterinary importance of Batai virus (BATV), we investigated the presence of BATV-specific antibodies and BATV RNA in 548 bovines from southwest Germany, and we demonstrated that 3 cattle serum samples contained BATV-neutralizing antibodies, resulting in a seroprevalence of 0.55%. Thus, our results confirm local transmission and indicate cattle as potential hosts of BATV in southwest Germany.

Batai virus (BATV) belongs to the genus *Orthobunyavirus* of the family *Bunyaviridae* and is an arthropod-borne virus (arbovirus) that is widely distributed in Africa and Eurasia (1). BATV was originally isolated from *Culex gelidus* mosquitoes aspirated from cattle that were used as bait (because they attract mosquitoes) in the Batai outskirts of Kuala Lumpur, Malaysia, in 1955 (2). In Europe, BATV is mainly transmitted by the zoophilic mosquitoes *Anopheles maculipennis sensu lato* and *A. claviger* (1). Natural foci of BATV infection occur in agroecosystems in a domestic animal-mosquito cycle (1). Vertebrate hosts are domestic pigs, horses, and ruminants (1). BATV has been reported to cause mild illness in sheep and goats (3). In Japan and China, BATV was isolated from the blood of sentinel cattle that showed no signs of disease or illness (4, 5). In humans, BATV infection is associated with influenza-like febrile illness (6). The German arbovirus surveillance program demonstrated the presence of BATV in southwest Germany in anopheline and culicine mosquitoes in 2009 (BATV strain 53.2; GenBank accession number HQ455791) and 2012 (BATV strains 5593, 5676, and 5677) (7, 8). To estimate the veterinary importance of BATV, we investigated the presence of BATV-specific antibodies and viral RNA in bovines from the administrative district of Karlsruhe, southwest Germany. Therefore, sera from 548 healthy bovines (535 cattle [*Bos primigenius taurus*]), 11 yak [*Bos mutus*], and 2 zebus [*Bos primigenius indicus*]; ages 8 to 216 months), collected in 2011 and 2012, were analyzed for the presence of BATV RNA by BATV-specific real-time reverse transcription-PCR (RT-PCR) (7) with the AgPath-ID one-step RT-PCR kit (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. In addition, the sera were analyzed for BATV-specific IgG antibodies using an in-house immunoblot (IB) with a recombinant nucleocapsid (rN) protein of BATV strain 53.2 as the antigen and an in-house indirect immunofluorescence assay (IIFA) with BATV strain 53.2-infected Vero E6 cells as the antigen. The BATV rN was expressed in Sf9 insect cells using the flashBac baculovirus expression system (Oxford Expression Technologies, Oxford, England) and purified by nickel affinity chromatography. All samples were further investigated with a virus neutralization test (VNT) using BATV strain 53.2 and Vero E6 cells (7). All samples tested negative for BATV RNA by specific real-time RT-PCR. In contrast, two serum samples from cattle (1534-7 and 1534-8) from 2011 tested positive for BATV-specific IgG antibodies with the IB (Table 1). In addition, two serum samples from cattle (1534-7 and 1534-6) from 2011 tested positive for BATV-specific IgG antibodies with the IIFA (Table 1). The 3 reactive serum samples (from cattle 1534-6 [age 24 months], 1534-7 [age 13 months], and 1534-8 [age 16 months]) contained BATV-neutralizing antibodies in the VNT, thus resulting in a seroprevalence of 0.55%. All positive tested samples originated from a single dairy farm on which animals were kept inside and on pastures. All analyzed animals were resident in that area during the study period, and importation of animals into the study group did not take place.

Our results confirm local transmission and indicate cattle as potential hosts of BATV in southwest Germany. This is in line with a recent study from northern Italy that demonstrated serological evidence (by IIFA and VNT) of BATV infections in cattle from an area with previous evidence of BATV circulation in mosquitoes (9, 10). However, the observed BATV seroprevalence in cattle from northern Italy was significantly higher (7%) than the BATV seroprevalence in southwest Germany (9). In addition, our study highlighted the advantage of using two different assays (IB and IIFA) in parallel. Each one would have missed 1 out of the 3 positive tested samples. This observation is confirmed by the BATV seroprevalence study from Italy: the comparison of data generated by the IIFA and VNT suggests that the VNT is more sensitive for the detection of BATV antibodies through neutralizing activity in samples with low antibody titers (9). However, IB and IIFA are easier to handle than VNT; thus, large numbers of samples can be processed much faster. We suggest that the tested animals were not

Received 12 February 2015 **Returned for modification** 10 March 2015
Accepted 6 April 2015

Accepted manuscript posted online 15 April 2015

Citation Hofmann M, Wiethölter A, Blaha I, Jöst H, Heinemann P, Lehmann M, Miller T, Cadar D, Yanase T, Kley N, Eiden M, Groschup M, Schmidt-Chanasit J. 2015. Surveillance of Batai virus in bovines from Germany. Clin Vaccine Immunol 22:672–673. doi:10.1128/CVI.00082-15.

Editor: T. S. Alexander

Address correspondence to Jonas Schmidt-Chanasit, jonassi@gmx.de.

doi:10.1128/CVI.00082-15

TABLE 1 Results for 3 cattle serum samples that tested positive for BATV-specific antibodies

Sample ID	Immunoblot result[a]	Titer	
		Indirect immunofluorescence assay[b]	Virus neutralization test[c]
1534-6	Negative	1:40	1:80
1534-7	Positive	1:80	1:80
1534-8	Positive	<1:20	1:40
Negative control[d]	Negative	<1:20	<1:10
Positive control[d]	Positive	1:40	1:80

[a] In-house immunoblot with recombinant nucleocapsid protein of BATV strain 53.2 as the antigen was used with bovine sera diluted 1:100 in phosphate-buffered saline with 1% milk powder.
[b] In-house indirect immunofluorescence assay with BATV strain 53.2-infected Vero E6 cells as the antigen. Titers of <1:20 for serum were considered negative. Titers represent the dilution of serum at which no cytopathic effect was observed.
[c] Virus neutralization test using BATV strain 53.2 and Vero E6 cells. Titers of <1:10 for serum were considered negative. Titers represent the dilution of serum at which no cytopathic effect was observed.
[d] Cattle sera for controls were previously characterized (4) by a virus neutralization test.

viremic during the period of sampling, so BATV RNA was not detected with the real-time RT-PCR in the serum samples. The recent discoveries of BATV in anopheline and culicine mosquitoes captured in Germany and Italy confirmed the circulation of BATV in central and southern Europe (7, 10). However, BATV infection rates in mosquitoes from Germany are low, thus leading to a rather low seroprevalence in bovines. Nevertheless, it will be important to further investigate the ecology of BATV and other orthobunyaviruses in Germany and Europe, as there is a lack of information about hosts, vectors, and medical importance.

REFERENCES

1. **Hubálek Z.** 2008. Mosquito-borne viruses in Europe. Parasitol Res **103**(Suppl 1):S29–S43. http://dx.doi.org/10.1007/s00436-008-1064-7.
2. **American Committee on Arthropod-Borne Viruses.** Accessed 6 May 2015. The international catalog of arboviruses. https://wwwn.cdc.gov/arbocat/.
3. **Pavri S.** 1969. Activity of Chittoor virus in India, p 191–197. *In* Bárdős V, Blaskovic D, Libikova H (ed), Arboviruses of the California complex and the Bunyamwera supergroup. SAS, Bratislava, Czechoslovakia.
4. **Yanase T, Kato T, Yamakawa M, Takayoshi K, Nakamura K, Kokuba T, Tsuda T.** 2006. Genetic characterization of Batai virus indicates a genomic reassortment between orthobunyaviruses in nature. Arch Virol **151:**2253–2260. http://dx.doi.org/10.1007/s00705-006-0808-x.
5. **Liu H, Shao XQ, Hu B, Zhao JJ, Zhang L, Zhang HL, Bai X, Zhang RX, Niu DY, Sun YG, Yan XJ.** 2014. Isolation and complete nucleotide sequence of a Batai virus strain in Inner Mongolia, China. Virol J **11:**138. http://dx.doi.org/10.1186/1743-422X-11-138.
6. **Sluka F.** 1969. The clinical picture of the Čalovo virus infection, p 337–339. *In* Bárdős V, Blaskovic D, Libikova H (ed), Arboviruses of the California complex and the Bunyamwera group. SAS, Bratislava, Slovakia.
7. **Jöst H, Bialonski A, Schmetz C, Gunther S, Becker N, Schmidt-Chanasit J.** 2011. Isolation and phylogenetic analysis of Batai virus, Germany. Am J Trop Med Hyg **84:**241–243. http://dx.doi.org/10.4269/ajtmh.2011.10-0483.
8. **Becker N, Krüger A, Kuhn C, Plenge-Bönig A, Thomas SM, Schmidt-Chanasit J, Tannich E.** 2014. Mosquitoes as vectors for exotic pathogens in Germany. Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz **57:**531–540. (In German.) http://dx.doi.org/10.1007/s00103-013-1918-8.
9. **Lambert AJ, Huhtamo E, Di Fatta T, De Andrea M, Borella A, Vapalahti O, Kosoy O, Ravanini P.** 2014. Serological evidence of Batai virus infections, bovines, northern Italy, 2011. Vector Borne Zoonotic Dis **14:**688–689. http://dx.doi.org/10.1089/vbz.2014.1596.
10. **Huhtamo E, Lambert AJ, Costantino S, Servino L, Krizmancic L, Boldorini R, Allegrini S, Grasso I, Korhonen EM, Vapalahti O, Lanciotti RS, Ravanini P.** 2013. Isolation and full genomic characterization of Batai virus from mosquitoes, Italy 2009. J Gen Virol **94:**1242–1248. http://dx.doi.org/10.1099/vir.0.051359-0.