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SHORT COMMUNICATION

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Complete genome sequence of bluetongue virus serotype 4 that emerged on the French island of Corsica in December 2016

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Summary

In November 2016, sheep located in the south of Corsica island exhibited clinical signs suggestive of bluetongue virus (BTV) infection. Laboratory analyses allowed to isolate and identify a BTV strain of serotype 4. The analysis of the full viral genome showed that all the 10 genomic segments were closely related to those of the BTV-4 present in Hungary in 2014 and involved in a large BT outbreak in the Balkan Peninsula. These results together with epidemiological data suggest that BTV-4 has been introduced to Corsica from Italy (Sardinia) where BTV-4 outbreaks have been reported in autumn 2016. This is the first report of the introduction in Corsica of a BTV strain previously spreading in eastern Europe.

KEYWORDS

bluetongue, diagnosis, emergence, serotype 4

1 | INTRODUCTION

Bluetongue virus (BTV) is the causative agent of bluetongue (BT), an insect-transmitted disease of domestic and wild ruminants (Maclachlan, Drew, Darpel, & Worwa, 2009; Verwoerd & Erasmus, 2004). Bluetongue virus is the prototype member of the *Orbivirus* genus in the *Reoviridae* family (Mertens, Maan, Samuel, & Attoui, 2005) with 27 serotypes currently described (Zientara et al., 2014) and at least four putative new serotypes (Maan et al., 2015; Savini et al., 2017; Sun et al., 2016).

In Corsica, BT first appeared in 2000 with the introduction of BTV-2 that most likely originated from the island of Sardinia (Zientara et al., 2002). Forty-nine sheep flocks were infected during this year. In 2001, BTV-2 continued to spread throughout the island (335 infected flocks), but after two widespread winter campaigns of vaccination of sheep with the South African attenuated BTV-2 vaccine, no other outbreaks or evidence of virus circulation were observed (Gerbier et al., 2008). In 2003, the same course of events occurred with another serotype, BTV-4, that was recorded on the island and caused 17 outbreaks. After confirmation of the emergence of this serotype, an attenuated vaccine against BTV-4 was included in the extensive vaccination campaign carried out in sheep in Corsica during the 2003–2004 winter. In contrast to the widespread of BTV-2 before the start of the vaccination programme, outbreaks of BTV-4 were limited to the south-west of Corsica (Breard et al., 2004). The following year, a new serotype was detected (BTV-16). A total of 39 outbreaks caused by BTV-4, and 16 were then recorded on the island with a predominance of BTV-16 (Zientara, MacLachlan, Calistri, Sanchez-Vizcaino, & Savini, 2010). In 2013, a new serotype (BTV-1) was detected in the south of Corsica (Sailleau et al., 2015). The location of the first outbreaks and the molecular data strongly suggested that the virus has been introduced to Corsica from the island of Sardinia, where many outbreaks of BTV-1 have been reported during autumn 2012 (Lorusso et al., 2013) and summer 2013. To eradicate BTV-1 from Corsica, the French veterinary authorities have organized a comprehensive and compulsory vaccination campaign targeting all susceptible species of domestic ruminants (cattle, sheep and goats) based on the use of available inactivated vaccines. During the BTV vaccination and monitoring programme, a novel bluetongue virus (BTV-27), with unknown origin, was discovered in clinically healthy goats in 2014 (Zientara et al., 2014). Since then, no other

outbreak has been reported on the island of Corsica. Full genome sequences of all these BTV-1, 2, 4 and 16 corsican strains have been determined and revealed an African origin (Nomikou et al., 2015).

2 | MATERIALS AND METHODS

From the end of October 2016, clinical signs suggestive of BTV infection (ptyalism, facial oedema, nasal discharge and increased temperature) were reported in a mixed herd (sheep and goats) located in the extreme south of the island. The veterinary practitioner was only called at the end of November. In November, three blood samples were collected from sick sheep and sent to the National Reference Laboratory (ANSES) for virological analyses. No other outbreak was notified in the area. After the confirmation of the BTV infection and to estimate the seroprevalence in this infected herd, serum samples were taken from all animals (86 sheep and 21 goats).

2.1 | Molecular diagnosis

Molecular amplification of viral RNA was achieved by RT-qPCR using the two commercial kits Adiavet™ BTV REAL TIME (Bio-X Diagnostics) and LSI VetMAX™ European BTV Typing (LSI-Thermo Fisher Scientific) for BTV group detection and genotyping, respectively.

2.2 | Virus isolation and serological analysis

The blood samples were inoculated to KC cells (*Culicoides sonorensis* cell line) (Wechsler, McHolland, & Tabachnick, 1989). Briefly, a confluent monolayer of KC cells was inoculated with washed and lysed red blood cells. After incubation at 28°C for 7 days, cells and supernatant were used to inoculate BSR cells (a clone of BHK-21, from baby hamster kidney).

The detection of BTV antibodies was performed using VP7 competition enzyme-linked immunosorbent assay (cELISA, ID SCREEN_ Bluetongue Competition Kit, IDVET, France).

Each goat and sheep ELISA-positive serum was tested by seroneutralization test (SNT) against BTV-1 and BTV-4 for the detection of specific neutralizing antibodies (NA) as previously described (Breard et al., 2011). Titres were expressed as the log10 of the reciprocal endpoint serum dilution. The positive–negative cut-off was 0.9 log 10 (titre <0.9: negative; titre ≥ 0.9: positive).

2.3 | Sequence analysis

For a more comprehensive characterization, the BTV-4 strain designated BTV4-16-03 was used for full genome sequencing (Proton Ion Torrent). Next-generation sequencing was performed on RNA extracts from infected cultures. RNA extraction was performed using TRIzol LS reagent (Life Technologies, Germany), as described by the manufacturer. After cleaning by Trimmomatic 0.36 (Bolger, Lohse, & Usadel, 2014), reads were aligned with bowtie2 2.2.5 (Langmead & Salzberg, 2012) on a local copy of NT databank (Coordinators, 2016) to find a reference for each segment. An accurate BWA (Li & Durbin, 2009) alignment on these references selected 43,393 reads of BTV-4 and provided us a good estimation of the coverage depth.

We downsampled sample reads to fit a mean theoretical coverage depth of 80. De novo assemblies were performed with Spades 3.10.0 (Bankevich et al., 2012) on cleaned reads and Mira 4.0.2 (Chevreux et al., 2004) on raw reads.

An alignment of the de novo contigs on local NT databank using megablast 2.4 (Altschul, Gish, Miller, Myers, & Lipman, 1990) gave new references genomes with the best homologies. BWA reads alignments on those references helped us to polish 5′ and 3′ ends of the de novo assembly obtained by combining spades and Mira results. The obtained sequences were then manually curated to check for any discrepancies between the de novo assembly and the alignment on references. These eventual discrepancies were then resolved by manually checking the alignment of the raw reads on the proposed sequence. Sequencing permitted the determination of the full-length genome.

3 | RESULTS AND DISCUSSION

The three sheep blood samples tested were BTV PCR positive (Ct range 21–24). By serotype-specific RT-PCR, serotype 4 was determined (Ct range 20–22) while BTV-1, BTV-2, BTV-8, BTV-9, BTV-11 and BTV-16 were not detected. All BSR cell cultures inoculated with RT-PCR-positive KC cell cultures showed a cytophatic effect after 3 days at 37°C. Confirmation of the serotype (BTV-4) of isolates was given by serotype-specific RT-PCR kits.

Only 23 of 107 (21.4%) animals were tested positive by ELISA. SNT performed on ELISA-positive samples showed BTV-1 NA only in one animal (titre: 1.4) while 19 animals were tested positive against BTV-4 (titre range 0.9 to >2.4). Thirteen of 19 showed NA titres superior to 2.1. These last results confirm the recent infection of the herd by BTV-4.

The complete coding genome sequences of the BTV-4 have been deposited in GenBank under accession numbers KY654328–KY6543377. Full-length sequences, with the exception of the 10 nucleotides present at the 5′ end of Seg-9, of all BTV genome segments from viral RNA purified from BSR cell cultures were obtained. Sequence analysis of the 10 double-stranded RNA segments showed a close relationship with the BTV-4 isolated in Hungary in 2014 (Hornyak et al., 2015) and involved in a large BT outbreak in the Balkan Peninsula and in Italy from 2014 (see Table 1 and Figure 1). By contrast, the comparison with BTV-4 that circulated in the Corsica island in 2003 clearly shows that the two strains are genetically distinct. These results showed the first introduction in Corsica of a BTV strain that previously spread in eastern Europe (Nomikou et al., 2015).

The location of the infected farm and the sequence data suggest that BTV-4 has been introduced to Corsica from Italy, probably from Sardinia, where BTV-4 outbreaks have been reported during autumn 2016 (Animal Disease Notification System). This introduction could have occurred either by wind-borne movements of adult *Culicoides* midges (12 km between the two islands) or by the introduction of

TABLE 1 Greatest sequence identity of BTV4-16-03 Corsica to BTV isolates in GenBank and comparisons with the sequences of the last BTV-4 strain isolated in Corsica in 2003

Genome segment	Greatest sequence identity (%) nucleotides (amino acids)	Strain in GenBank (Acc. numbers)	Sequence identity (%) with corsican BTV-4 2003 nucleotides (amino acids)	Acc. numbers
S1	99.9 (99.7)	BTV-4 HUN (KP68814)	95 (98.8)	KP820928
S2	99.8 (99.9)	BTV-4 HUN (KP68815)	95 (97.8)	KP821048
S3	99.9 (99.8)	BTV-4 HUN (KP68816)	96 (99.4)	KP821170
S4	99.8 (99.7)	BTV-4 HUN (KP68817)	98 (98.4)	KP821290
S5	99.9 (99.8)	BTV-4 HUN (KP68818)	96 (99)	KP821410
S6	99.8 (99.8)	BTV-4 HUN (KP68819)	99 (99.4)	KP821530
S7	99.9 (100)	BTV-4 HUN (KP68820)	94 (99.7)	KP821652
S8	99.9 (100)	BTV-4 HUN (KP68821)	94 (99.4)	KP821772
S9	99.6 (100)	BTV-4 HUN (KP68822)	96 (96.9)	KP821892
S10	100 (100)	BTV-4 HUN (KP68823)	97 (100)	KP822013

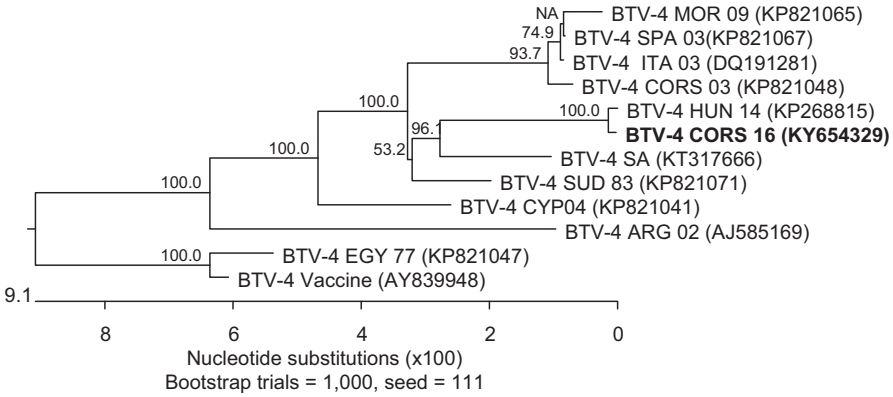

FIGURE 1 Phylogenetic tree of full-length segment 2 showing relationships between BTV-4 Corsica isolate and homologous BTV serotypes (GenBank). The phylogenetic tree of nucleotide sequences was constructed using MegAlign-Clustal V method (DNAstar software-Lasergene 8)

an infected animal. The same events occurred few years ago with the incursion of BTV-2, 4, 16 and 1 (Breard et al., 2004; Gerbier et al., 2008; Sailleau et al., 2015; Zientara et al., 2002). Since the report of BTV-4 in this herd, no other outbreak has been reported in the island. This could be due to the preventive compulsory vaccination campaign against BTV-4 carried out since May 2016 that may have helped to avoid disease spread. Also, as the outbreak occurred in November, it can be assumed that climatic conditions were not optimal for a widespread through the island. Investigations conducted in the infected herd showed that animals were in bad health. Moreover, the serological results (seroprevalence rate: 21.4%) suggest that the animals were not all properly vaccinated.

In the next months, to prevent the spread of BTV-4 (new introduction or a phenomena of overwintering), the compulsory vaccination targeting all susceptible species of domestic ruminants will be maintained. Moreover, active surveillance should be reinforced to early detect any new outbreak.

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REFERENCES

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of molecular biology*, *215*, 403–410.

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., … Pevzner, P. A. (2012). SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. *Journal of Computational Biology*, *19*, 455–477.

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics*, *30*, 2114–2120.

Breard, E., Belbis, G., Hamers, C., Moulin, V., Lilin, T., Moreau, F., … Zientara, S. (2011). Evaluation of humoral response and protective efficacy of two inactivated vaccines against bluetongue virus after vaccination of goats. *Vaccine*, *29*, 2495–2502.

Breard, E., Hamblin, C., Hammoumi, S., Sailleau, C., Dauphin, G., & Zientara, S. (2004). The epidemiology and diagnosis of bluetongue with particular reference to Corsica. *Research in Veterinary Science*, *77*, 1–8.

Chevreux, B., Pfisterer, T., Drescher, B., Driesel, A. J., Muller, W. E., Wetter, T., & Suhai, S. (2004). Using the miraEST assembler for reliable and automated mRNA transcript assembly and SNP detection in sequenced ESTs. *Genome Research*, *14*, 1147–1159.

Coordinators, N. R. (2016). Database resources of the National Center for Biotechnology Information. *Nucleic Acids Research*, *44*, D7–D19.

Gerbier, G., Biteau-Coroller, F., Grillet, C., Parodi, J., Zientara, S., Baldet, T., ... Roger, F. (2008). Description of the outbreak of bluetongue in Corsica in 2003, and lessons for surveillance. *Veterinary Record*, *162*, 173–176.

Hornyak, A., Malik, P., Marton, S., Doro, R., Cadar, D., & Banyai, K. (2015). Emergence of multireassortant bluetongue virus serotype 4 in Hungary. *Infection, Genetics and Evolution*, *33*, 6–10.

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nature Methods*, *9*, 357–359.

Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*, *25*, 1754–1760.

Lorusso, A., Sghaier, S., Carvelli, A., Di Gennaro, A., Leone, A., Marini, V., ... Savini, G. (2013). Bluetongue virus serotypes 1 and 4 in Sardinia during autumn 2012: new incursions or re-infection with old strains? *Infection, Genetics and Evolution*, *19*, 81–87.

Maan, S., Maan, N. S., Belaganahalli, M. N., Rao, P. P., Singh, K. P., Hemadri, D., ... Mertens, P. P. (2015). Full-genome sequencing as a basis for molecular epidemiology studies of bluetongue virus in India. *PLoS One*, *10*, e0131257.

Maclachlan, N. J., Drew, C. P., Darpel, K. E., & Worwa, G. (2009). The pathology and pathogenesis of bluetongue. *Journal of Comparative Pathology*, *141*, 1–16.

Mertens, P., Maan, S., Samuel, A., & Attoui, H. (2005). Orbivirus Reoviridae. In C. M. Fauquet, M. A. Mayo, J. Maniloff, U. Desselberger, & L. A. Ball (Eds.), *Virus Taxonomy VIIIth Report of the ICTV* (pp. 466–483). London: Elsevier/Academic Press.

Nomikou, K., Hughes, J., Wash, R., Kellam, P., Breard, E., Zientara, S., ... Mertens, P. (2015). Widespread reassortment shapes the evolution and epidemiology of bluetongue virus following European invasion. *PLoS Pathogens*, *11*, e1005056.

Sailleau, C., Viarouge, C., Breard, E., Perrin, J. B., Doceul, V., Vitour, D., & Zientara, S. (2015). Emergence of bluetongue virus serotype 1 in French Corsica Island in September 2013. *Transboundary and Emerging Diseases*, *62*, e89–e91.

Savini, G., Puggioni, G., Meloni, G., Marcacci, M., Di Domenico, M., Rocchigiani, A. M., ... Lorusso, A. (2017). Novel putative Bluetongue virus in healthy goats from Sardinia, Italy. *Infection, Genetics and Evolution*, *51*, 108–117.

Sun, E. C., Huang, L. P., Xu, Q. Y., Wang, H. X., Xue, X. M., Lu, P., ... Wu, D. L. (2016). Emergence of a novel bluetongue virus serotype, China 2014. *Transboundary and Emerging Diseases*, *63*, 585–589.

Verwoerd, D., & Erasmus, B. J. (2004). Bluetongue. In J. A. W. Coetzer & R. C. Tustin (Eds.), *Infectious diseases of livestock*, 2nd ed. (pp. 1201–1220). CapeTown: Oxford University Press.

Wechsler, S. J., McHolland, L. E., & Tabachnick, W. J. (1989). Cell lines from *Culicoides variipennis* (Diptera: Ceratopogonidae) support replication of bluetongue virus. *Journal of Invertebrate Pathology*, *54*, 385–393.

Zientara, S., MacLachlan, N. J., Calistri, P., Sanchez-Vizcaino, J. M., & Savini, G. (2010). Bluetongue vaccination in Europe. *Expert Review of Vaccines*, *9*, 989–991.

Zientara, S., Sailleau, C., Dauphin, G., Roquier, C., Rémond, E. M., Lebreton, F., ... Bréard, E. (2002). Identification of bluetongue virus serotype 2 (Corsican strain) by reverse-transcriptase PCR reaction analysis of segment 2 of the genome. *Veterinary Record*, *150*, 598–601.

Zientara, S., Sailleau, C., Viarouge, C., Hoper, D., Beer, M., Jenckel, M., ... Breard, E. (2014). Novel bluetongue virus in goats, Corsica, France, 2014. *Emerging Infectious Diseases*, *20*, 2123–2125.

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