Dobrava-Belgrade Virus Spillover Infections, Germany

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We present the molecular identification of Apodemus agrarius (striped field mouse) as reservoir host of the Dobrava-Belgrade virus (DOBV) lineage DOBV-Aa in 3 federal states of Germany. Phylogenetic analyses provided evidence for multiple spillover of DOBV-Aa to A. flaviellus, a crucial prerequisite for host switch and genetic reassortment.

European hantaviruses are emerging viruses that can cause hemorrhagic fever with renal syndrome (HFRS) of differing severities. Dobrava-Belgrade virus (DOBV) is a hantavirus that appears in 3 distinct lineages hosted by different Apodemus species. The DOBV-Af lineage associated with the yellow-necked mouse (A. flavicollis) has caused serious HFRS in southeast Europe with a case-fatality rate \( \leq 12\% \) (1,2). Human infections with Caucasian wood mouse (A. ponticus)–associated DOBV-Ap have resulted in more severe than HFRS in the southern part of European Russia (3). Mild-to-moderate human DOBV disease in central and eastern Europe has been connected with infection by DOBV-Aa lineage carried by the striped field mouse (A. agrarius) (3–5). Other A. agrarius–associated strains, found in Estonia and called Saaremaa virus, have been proposed to form a distinct hantavirus species (6). In Germany, human DOBV cases with mild to moderate clinical outcomes have been detected by serologic investigations (4,7) but only 1 short DOBV-Aa small (S) segment sequence derived from a patient in northern Germany has been identified (8). The natural host and the geographic distribution of DOBV in its reservoir host has remained unknown in Germany.

The Study

During 2002 through 2008, a total of 366 Apodemus mice were trapped at 7 different sites in Germany (Figure 1). Serologic screening of transudates collected from these rodents by using an in-house DOBV immunoglobulin (Ig) G-ELISA, with a yeast-expressed nucleocapsid protein of DOBV-Af as antigen, identified 16 reactive and 5 equivocal samples of 114 A. agrarius trapped at 7 trapping sites in 3 federal states of Germany (Figure 1; online Technical Appendix, available from www.cdc.gov/EID/content/15/12/2017-Techapp.pdf). Additionally, of 237 A. flavicollis mice, 1 equivocal sample and 4 DOBV-reactive samples were detected at 4 trapping sites (Figure 1; online Technical Appendix). In contrast, of 15 wood mice (A. sylvaticus) originating from 3 trapping sites, none were found to be DOBV-seroreactive. A subsequent focus-reduction neutralization test showed a higher endpoint titer with DOBV-Aa than DOBV-Af (online Technical Appendix) for 6 of the 8 investigated transudates independently, whether originating from A. agrarius or A. flavicollis.

An initial screening by a large (L) segment–specific nested reverse transcription–PCR (RT-PCR) (3) of 67 lung samples, representing all seroreactive (n = 20) and equivocal (n = 6) as well as 36 selected seronegative and 5 serologically not-analyzed animals, showed a 390-nt amplification product for 21 samples representing 16 seroreactive, 4 seronegative, and 1 serologically not-investigated animals (online Technical Appendix). To enable a comparison with the only available DOBV sequence from Germany (H169), an S segment portion of 559 nt was amplified by RT-PCR from 11 lung tissues (online Technical Appendix). In the phylogenetic analyses, all sequences from Germany formed 1 well-supported (PUZZLE [www.tree-puzzle.de]) and bootstrap support values >90%) monophyletic group consisting of 2 clusters. The first cluster contained S segment sequences from district Güstrow (trapping sites Pe1 and Pe3), Lüneburg (trapping site WG), Nordvorpommern (trapping site H), and the previously published DOBV sequence from an HFRS patient from northern Germany (H169; [8]; Figure 2, panel A). A second cluster was formed by S segment sequences originating from districts Ostrupritz-Ruppin (trapping sites Ka, To) and Demmin (trapping site K/A1). Notably, the A. flavicollis–derived sequences from sites Pe1, H, Ka, and K/A1 clustered together or were identical with A. agrarius–derived sequences from the same or neighboring trapping sites, suggesting multiple
spillover infections (Figure 2, panel A; online Technical Appendix).

The sequences from Germany share a common ancestor with the DOBV-Aa sequences originating from Slovakia and Russia. Together, they form a monophyletic group (DOBV-Aa lineage) that is clearly separated from A. flavicollis–borne (DOBV-Af) and A. ponticus–borne (DOBV-Ap) sequences and from A. agrarius–borne Saaremaa virus sequences. Subsequent analysis of nucleotide sequences of the entire nucleocapsid (N) protein– and glycoprotein precursor (GPC)–encoding regions confirmed these findings (Figure 2, panel B; online Technical Appendix). A pairwise comparison between nucleotide and amino acid sequences of the complete N and GPC open reading frames conﬁrmed these findings (Figure 2, panel B; online Technical Appendix). The highest identity values on the nucleotide and amino acid sequence level (91.2%–91.7% and 99%–99.7%) were found for an S segment sequence from Denmark (Lolland/1403; GenBank accession no. AJ616854; online Technical Appendix). The nucleotide and amino acid sequence divergence to other DOBV sequences was much higher, reaching 10.1%–14.3% (1%–3.3%) and 12.6%–20.7% (2.9%–9.4%), respectively.

Morphologic species determination for all DOBV-seroreactive and RT-PCR–positive rodents was conﬁrmed by a mitochondrial cytochrome b gene-speciﬁc PCR (9,10), sequence determination, and comparison with available GenBank sequences from A. agrarius and A. ﬂavicollis (online Technical Appendix).

Conclusions

Based on a large panel of the entire N- and GPC-encoding DOBV sequences, we report direct molecular evidence that DOBV in Germany is represented by a genetic lineage associated with A. agrarius (DOBV-Aa). In contrast, we found no evidence for the occurrence of DOBV-Af in A. ﬂavicollis or other Apodemus species from Germany. Consistent with the geographic distribution of A. agrarius (11) and the report of human DOBV disease exclusively in northern and northeastern Germany, this ﬁnding may conﬁrm DOBV-Aa as the sole causative agent of DOBV infections in Germany (4; Robert Koch-Institut, SurvStat, www.rki.de).

Previously A. agrarius–associated Saaremaa virus was experimentally shown to be able to infect A. agrarius and A. ﬂavicollis mice (12). We report multiple natural spillover infections of A. ﬂavicollis by a DOBV strain originally hosted by A. agrarius. The observed spillover infections represent a crucial prerequisite for genetic reassortment. This observation is in contrast to other reports from Slovenia and Slovakia where, although A. agrarius and A. ﬂavicollis are occurring sympatrically, A. ﬂavicollis...
is exclusively carrying the DOBV-Af and *A. agrarius* the DOBV-Aa lineage (4,13). In contrast to our observations, single DOBV-Af spillover infections of *A. sylvaticus* and *Mus musculus* have been reported previously (14).

The phylogenetic analyses demonstrated 2 well-separated clusters within the DOBV-Aa lineage. These rodent-derived DOBV sequences in Germany represent a major contribution to the DOBV genomics and phylogenetics. Future investigations should help to identify specific features of these DOBV-Aa strains resulting in its frequent spillover to *A. flavicollis* and to prove a putative adaptation of DOBV-Aa on *A. flavicollis* after spillover, as well as possible reassortment processes.

**Acknowledgments**

We kindly acknowledge the support of the various partners in the network “Rodent-borne pathogens,” additional collaborators from different parts of Germany, and Dörte Kaufmann, Daniel Balkema, and Heike Lerch.

This work was supported by the Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz, grant number 07HS027 (to R.G.U.); by the Deutsche Forschungsgemeinschaft, grant no. KR 1293/9-1 (to D.K.); by the Slovak Scientific Grant Agency VEGA, grant number 2/0189/09 (to B.K.), by the Förderverein of the Friedrich-Loeffler-Institut (to M.S.), and by the Paul und Ursula Klein-Stiftung (to J.S.C.).
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References

1. Avsic-Zupanc T, Petrovec M, Furlan P, Kaps R, Elgh F, Lundkvist A. Hemorrhagic fever with renal syndrome in the Dolenjska region of Slovenia—a 10-year survey. Clin Infect Dis. 1999;28:860–5. DOI: 10.1086/515185

2. Krüger DH, Ulrich R, Lundkvist Å. Hantavirus infections and their prevention. Microbes Infect. 2001;3:1129–44. DOI: 10.1016/S1286-4579(01)01474-5

3. Klempa B, Tkachenko EA, Dzagurova TK, Yunicheva YV, Morozov VG, Okulova NM, et al. Hemorrhagic fever with renal syndrome caused by 2 lineages of Dobrava hantavirus, Russia. Emerg Infect Dis. 2008;14:617–25. DOI: 10.3201/cid.1404.071310

4. Sibold C, Ulrich R, Labuda M, Lundkvist Å, Martens H, Schütt M, et al. Dobrava hantavirus causes hemorrhagic fever with renal syndrome in central Europe and is carried by two different Apodemus mice species. J Med Virol. 2001;63:158–67. DOI: 10.1002/1096-9071(20000201)63:2<158::AID-JMV1011>3.0.CO;2-

5. Klempa B, Stanko M, Labuda M, Ulrich R, Meisel H, Krüger DH. Central European Dobrava hantavirus isolate from striped field mouse, Apodemus agrarius. J Clin Microbiol. 2005;43:2756–63. DOI: 10.1128/JCM.43.6.2756-2763.2005

6. Sjölander KB, Golovljova I, Vasilenko V, Plyusnin A, Lundkvist A. Serological divergence of Dobrava and Saaremaa hantaviruses: evidence for two distinct serotypes. Epidemiol Infect. 2002;128:99–103.

7. Meisel H, Lundkvist Å, Gantzer K, Bär W, Sibold C, Krüger DH. First case of infection with hantavirus Dobrava in Germany. Eur J Clin Microbiol Infect Dis. 1998;17:884–5. DOI: 10.1007/ s100960050214

8. Klempa B, Schütt M, Auste B, Ulrich R, Meisel H, Krüger DH. First molecular identification of human Dobrava virus infection in Central Europe. J Clin Microbiol. 2004;42:1322–5. DOI: 10.1128/ JCM.42.3.1322-1325.2004

9. Essbauer S, Schmidt J, Conraths FJ, Friedrich R, Koch J, Hautmann W, et al. A new Puumala hantavirus subtype in rodents associated with an outbreak of nephroptasia epidemica in South-East Germany in 2004. Epidemiol Infect. 2006;134:1333–44. DOI: 10.1017/ S0950268806006170

10. Kocher TD, Thomas WK, Meyer A, Edwards SV, Pääbo S, Villablan CA, et al. Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. Proc Natl Acad Sci U S A. 1989;86:6196–200. DOI: 10.1073/pnas.86.16.6196

11. Mitchell-Jones AJ, Amori G, Bogdanowicz W, Krystufek B, Reijnders PJH, Spitznerberger F, et al. The atlas of European mammals. London: Academic Press; 1999.

12. Klingström J, Heyman P, Estudina S, Sjölander KB, De Jaegere F, Henntenon H, et al. Rodent host specificity of European hantaviruses: evidence of Puumala virus interspecific spillover. J Med Virol. 2002;68:581–8. DOI: 10.1002/jmv.10232

13. Avsic-Zupanc T, Nemirov K, Petrovec M, Trilar T, Poljak M, Vaheki A, et al. Genetic analysis of wild-type Dobrava hantavirus in Slovenia: co-existence of two distinct genetic lineages within the same natural focus. J Gen Virol. 2000;81:1747–55.

14. Weidmann M, Schmidt P, Vackova M, Krivacek K, Munchinger P, Hufert FT. Identification of genetic evidence for Dobrava virus spillover in rodents by nested reverse transcription (RT)-PCR and TaqMan RT-PCR. J Clin Microbiol. 2005;43:808–12. DOI: 10.1128/ JCM.43.2.808-812.2005

15. Martin DP, Williamson C, Posada D. RDP2: recombination detection and analysis from sequence alignments. Bioinformatics. 2005;21:260–2. DOI: 10.1093/bioinformatics/bth490

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### Technical Appendix

| Federal state and district | Trapping site | Rodent species (sex) | Rodent no. | DOBV (Slo)-IgG-ELISA† | DOBV-Aa (SK) | DOBV-Af (Slo) | Nested RT-PCR L segment | Obtained sequences |
|----------------------------|---------------|----------------------|------------|------------------------|--------------|---------------|--------------------------|------------------|
| Lower Saxony              |               |                      |            |                        |              |               |                          |                  |
| Lüneburg                  |              | Aa (F)               | GER/08/    | ++                     | 80           | 20            | Pos                      | ND GQ260183      |
|                            |              |                      | 124/Aa     |                        |              |               |                          |                  |
|                            |              | Aa (M)               | GER/08/    | +++                    | 40           | 40            | Pos                      | GQ205407, GQ205412 |
|                            |              |                      | 118/Aa     |                        |              |               |                          |                  |
|                            |              | Aa (F)               | GER/08/    | +++                    | ND           | ND            | pos                      | GQ205407, GQ205412 |
|                            |              |                      | 125/Aa     |                        |              |               |                          |                  |
| Mecklenburg-Western Pomerania |        | Aa (M)               | GER/07/    | +++                    | 40           | 20            | pos                      | GQ205404, GQ205407 |
| Demmin                    |              |                      | 1064/Aa    |                        |              |               |                          |                  |
|                            |              | Af (M)               | GER/07/    | ++                     | 640          | 80            | pos                      | Partial (nt 219-1675); identical to GQ205404 |
|                            |              |                      | 1058/Af    |                        |              |               |                          |                  |
|                            |              | Aa (F)               | GER/07/    | ++                     | ND           | ND            | pos                      | Partial (nt 219-1675); identical to GQ205404 |
|                            |              |                      | 1081/Aa    |                        |              |               |                          |                  |
|                            |              | Aa (M)               | GER/07/    | neg                    | ND           | ND            | pos                      | ND                |
|                            |              |                      | 1029/Aa    |                        |              |               |                          |                  |
|                            |              | Af (M)               | GER/07/    | neg                    | ND           | ND            | pos                      | ND                |
|                            |              |                      | 1027/Aa    |                        |              |               |                          |                  |
|                            |              | Aa (F)               | GER/07/    | ++                     | 80           | 40            | pos                      | ND                |
|                            |              |                      | 992/Af     |                        |              |               |                          |                  |
|                            |              | Af (F)               | GER/07/    | ++                     | ND           | ND            | pos                      | ND                |
|                            |              |                      | 83/Aa      |                        |              |               |                          |                  |
|                            |              | Aa (F)               | GER/08/    | +                      | ND           | ND            | pos                      | ND                |
|                            |              |                      | 79/Aa      |                        |              |               |                          |                  |
|                            |              | Aa (M)               | GER/08/    | +                      | ND           | ND            | pos                      | ND                |
|                            |              |                      | 80/Aa      |                        |              |               |                          |                  |
|                            |              | Af (M)               | GER/08/    | +                      | ND           | ND            | pos                      | ND                |
|                            |              |                      | 84/Aa      |                        |              |               |                          |                  |
| Nordvor-pommern            | H            | Af (M)               | GER/08/    | +                      | 10           | <10           | pos                      | GQ205408, GQ205413 |
|                            |              |                      | 131/Af     |                        |              |               |                          |                  |
|                            |              | Aa (M)               | GER/08/    | neg                    | ND           | ND            | pos                      | ND                |
|                            |              |                      | 82/Aa      |                        |              |               |                          |                  |
|                            |              | Af (M)               | GER/08/    | neg                    | ND           | ND            | pos                      | ND                |

Page 1 of 4
| Location            | Gender | Species | Code   | Reactivity | OD | Titer | Status | Accession Numbers |
|---------------------|--------|---------|--------|------------|----|-------|--------|------------------|
| Güstrow             | Pe3    | Aa      | GER/07/293/Aa | +++ | 640 | 80 | pos | GQ205401 complete GQ205409 complete GQ260171 |
|                     |        |         | GER/07/634/Aa | +   | ND  | ND | neg | ND ND ND GQ260174 |
|                     |        |         | GER/07/372/Aa | +   | ND  | ND | neg | ND ND ND GQ260172 |
|                     | Pe1    | Af      | GER/07/607/Af | ++ | <10 | <10 | pos | GQ205402 complete GQ205410 complete GQ260186 |
|                     |        |         | GER/07/424/Aa | ++ | ND  | ND | pos | GQ205403 complete ND ND GQ260173 |
|                     |        | Aa      | GER/05/239/Aa | +++ | ND  | ND | pos | ND GQ260167 |
|                     |        |         | GER/05/44/Aa  | +   | ND  | ND | neg | ND ND ND GQ260169 |
|                     |        |         | GER/06/49/Aa  | +   | ND  | ND | neg | ND ND ND GQ260170 |
|                     |        | Aa      | GER/05/477/Aa | +   | ND  | ND | pos | ND GQ260168 |
| Ostprignitz-Ruppin  | To     | Aa      | GER/05/239/Aa | +++ | ND  | ND | pos | GQ205405 complete ND ND GQ260167 |
|                     |        |         | GER/05/44/Aa  | +   | ND  | ND | neg | ND ND ND GQ260169 |
|                     |        | Aa      | GER/06/49/Aa  | +   | ND  | ND | neg | ND ND ND GQ260170 |
|                     |        | Af      | GER/05/477/Aa | +   | ND  | ND | pos | ND GQ260168 |

*FRNT, focus reduction neutralization test; DOBV, Dobrava-Belgrade virus; Aa, Apodemus agrarius; Af, A. flavicollis; Slo, strain Slovenia; SK, strain Slovakia; cyt b, cytochrome b; ND, not done; nt, nucleotide; neg, negative; pos, positive.
†Optical density values: +++ >2.0; ++, 1.9-1.0; +, 0.9-0.2; < lower cut-off (in average 0.041).
‡Endpoint titers.
Table 2. Pairwise nucleotide and amino acid sequence divergence between the entire N- and GPC-encoding DOBV S- and M-segment sequences originating from Germany to those from other regions in Europe*

| Segment and strain | Species | Country (site) | % identity with strain |
|--------------------|---------|----------------|------------------------|
|                    |         |                | 1  2  3  4  5  6  7  8  9  10 11 12 13 |
| S segment ORF      |         |                |                        |
| 1. GER/07/293      | Aa      | GER/Pe3        | – 98.5 91.2 98.2 95.1 89.1 87.4 87.2 87.1 87.3 89.0 85.9 91.7 |
| 2. GER/07/607      | Af      | GER/Pe1        | 99.7 – 91.8 98.0 95.3 88.7 86.9 87.1 87 87.1 89.3 85.7 91.2 |
| 3. GER/05/477      | Af      | GER/Ka         | 98.8 99.0 – 91.2 91.9 88.9 87.5 86.5 86.8 86.0 89.9 86.7 91.4 |
| 4. GER/08/118      | Aa      | GER/WG         | 99.3 99.5 98.6 – 94.7 88.8 87.1 87.1 87 87.1 89.3 85.7 91.2 |
| 5. GER/08/131      | Af      | GER/H          | 99.5 99.7 98.8 99.3 – 89.3 87.4 88 88 88.1 89.6 87.5 91.4 |
| 6. SK/Aa           | Aa      | SVK            | 98.8 99.0 98.6 98.6 98.8 – 86.4 86.4 85.2 84.6 91.3 86.5 89.4 |
| 7. Saa160V         | Aa      | EST            | 96.9 97.2 97.6 96.7 97.4 96.7 – 87.7 87.6 87.4 87.1 84.8 89.7 |
| 8. Slo/Af          | Af      | SVN            | 97.6 97.9 97.4 97.4 97.6 97.4 – 96 95.1 87.5 88.2 87.5 |
| 9. AP/Af19         | Af      | GRC            | 98.3 98.6 98.1 98.1 98.3 98.1 97.2 99.3 – 96.4 87.1 87.6 87.1 |
| 10. Es400/Af       | Af      | SVK            | 98.1 98.3 97.9 97.9 98.1 97.9 96.9 99 99.7 – 87.1 87.4 86.6 |
| 11. Lipetsk/Aa     | Aa      | RUS            | 98.1 98.3 97.9 98.3 98.1 98.8 96.0 96.7 97.4 97.2 – 86.8 89.3 |
| 12. Sochi/Ap       | Ap      | RUS            | 97.6 97.9 97.4 97.4 98.1 97.4 96.2 97.6 97.9 97.6 96.7 – 86.8 |
| 13. Lolland/Aa1403 | Aa      | DNK            | 99.3 99.5 99.0 99.0 99.7 99.0 97.6 97.9 98.6 98.3 98.3 98.3 – |
| M segment ORF      |         |                |                        |
| 1. GER/07/293      | Aa      | GER/Pe3        | – 97.9 91.7 97.5 93.2 87.0 86.9 82.8 82.1 82.6 86.7 79.3 – |
| 2. GER/07/607      | Af      | GER/Pe1        | 99.1 – 91.9 97.9 93.5 87.4 87.2 83.4 82.4 83.2 87.1 79.5 – |
| 3. GER/05/477      | Af      | GER/Ka         | 98.3 98.6 – 92.0 91.7 86.5 87.0 83.3 82.9 83.3 87.2 80.1 – |
| 4. GER/08/118      | Aa      | GER/WG         | 98.7 99.2 98.4 – 93.0 86.9 87.4 83.3 82.5 83.0 86.9 79.7 – |
| 5. GER/08/131      | Af      | GER/H          | 98.2 98.6 98.8 98.4 – 86.6 87.0 83.1 82.3 83.5 87.3 80 – |
| 6. SK/Aa           | Aa      | SVK            | 96.0 96.4 96.2 96.2 96.4 – 87.1 82.5 82.5 82.5 87.6 79.5 – |
| 7. Saa160V         | Aa      | EST            | 95.7 96.0 95.7 95.8 95.9 95.8 – 82.2 82.4 82.1 86.4 79.1 – |
| 8. Slo/Af          | Af      | SVN            | 93.6 94.0 94.1 93.8 94.0 93.6 94.1 – 93.7 92.8 83.5 80.4 – |
| 9. AP/Af19         | Af      | GRC            | 93.7 94.1 94.2 93.9 94.0 93.6 94.1 – 98.6 – 93.2 83.2 80.4 |
| 10. Es400/Af       | Af      | SVK            | 94.0 94.4 94.5 94.1 94.3 94.0 94.3 98.9 99.0 – 83.6 80.8 – |
| 11. Lipetsk/Aa     | Aa      | RUS            | 96.7 97.1 96.7 97.0 96.6 97.0 96.2 94.0 94.1 94.4 – 80.6 – |
| 12. Sochi/Ap       | Ap      | RUS            | 90.6 91.0 90.9 90.7 90.8 90.5 90.3 93.3 93.3 93.8 91.4 – |

*Values above the diagonal are nucleotide sequence differences and values below the diagonal are amino acid differences. ORF, open reading frame.
Figure. Maximum-likelihood (ML) phylogenetic tree of Dobrava-Belgrade virus (DOBV) based on complete glycoprotein precursor coding nucleotide sequences (M-segment open reading frame) of 3,405 nt. The ML tree (Tamura-Nei evolutionary model) was calculated using TREE-PUZZLE package (www.tree-puzzle.de). Scale bar indicates an evolutionary distance of 0.1 substitutions per position in the sequence. Values above the branches represent PUZZLE support values. Values below the branches are the bootstrap values of the corresponding neighbor-joining tree (Tamura-Nei evolutionary model) calculated with the PAUP* software from 10,000 bootstrap pseudoreplicates. Phylogenetic trees with all evolutionary models available in TREE-PUZZLE were constructed and compared using statistical tests implemented in TREE-PUZZLE (Kishino-Hasegawa test, Shimodaira-Hasegawa test, and Expected Likelihood Weight). In most cases, they were not significantly different and therefore only trees with Tamura-Nei evolutionary model, which showed highest PUZZLE and bootstrap support values, are shown. Different DOBV lineages are indicated by gray boxes. HTNV, *Hantaan virus*; SEOV, *Seoul virus*; THAIV, *Thailand virus*; Saa, Saaremaa virus; Aa, *Apodemus agrarius*; Ap, *A. ponticus*; Af, *A. flavicollis*. WG, district Lüneburg, Lower Saxony (LS); Pe1 and Pe3, district Güstrow; H, district Nordvorpommern, K/A1, district Demmin, all Mecklenburg-Western Pomerania (MWP); To and Ka, district Ostprignitz-Ruppin, Brandenburg (BB). By automated screening for recombination between the M-segment sequences using program RDP3 (15) no putative recombinant regions could be detected by ≥3 programs and subsequently verified by phylogenetic trees.