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1 **Application of Broad-Spectrum Resequencing Microarray for Genotyping**
2 **Rhabdoviruses**

3

4 Running title: Rhabdovirus Identification by Resequencing Microarray

5

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1

2 **ABSTRACT**

3 The rapid and accurate identification of pathogens is critical in the control of infectious
4 disease. To this end we analysed the capacity for viral detection and identification of a newly
5 described high density resequencing microarray (RMA), termed PathogenID, which was
6 designed for multiple pathogen detection using database similarity searching. We focused on
7 one of the largest and most diverse viral families described to date, the family *Rhabdoviridae*.
8 We demonstrate that this approach has the potential to identify both known and related
9 viruses for which precise sequence information is unavailable. In particular, we demonstrate
10 that a strategy based on consensus sequence determination for analysis of RMA output data
11 enabled successful detection of viruses exhibiting up to 26% nucleotide divergence with the
12 closest sequence tiled on the array. Using clinical specimens obtained from rabid patients and
13 animals, this method also shows a high species-level concordance with standard reference
14 assays, indicating that it is amenable for the development of diagnostic assays. Finally, twelve
15 animal rhabdoviruses which were currently unclassified, unassigned, or assigned as tentative
16 species within the family *Rhabdoviridae* were successfully detected. These new data allowed
17 an unprecedented phylogenetic analysis of 106 rhabdoviruses, and further suggests that the
18 principles and methodology developed here may be used for the broad-spectrum surveillance
19 and the broader-scale investigation of biodiversity in the viral world.

1 INTRODUCTION

2

3 The ability to simultaneously screen for a large panel of pathogens in clinical samples,
4 especially viruses, will represent a major development in the diagnosis of infectious diseases
5 and in surveillance programs for emerging pathogens. Currently, most diagnostic methods are
6 based on species-specific viral nucleic acid amplification. Although rapid and extremely
7 sensitive, these methods are suboptimal when testing for a large number of known pathogens,
8 when viral sequence divergence is high, when new but related viruses are anticipated, or when
9 no clear viral etiologic agent is suspected. To overcome these technical difficulties, newer
10 technologies have been employed, especially microarrays dedicated to pathogen detection.
11 Indeed, DNA-microarrays have been shown to be a powerful platform for the highly
12 multiplexed differential diagnosis of infectious diseases. For example, pathogen microarrays
13 can be simultaneously used to screen various viral or bacterial families, and have been
14 successfully used in the detection of microbial agents from different clinical samples (10-12,
15 19, 32, 35, 41, 42, 48).

16

17 The 'classical' DNA-microarrays developed so far are based on use of long oligonucleotide
18 pathogen specific probes (≥ 50 nt). Although powerful in terms of sensitivity, these diagnostic
19 tools have the disadvantage of decreased specificity, making it necessary to target multiple
20 markers, and rely on hybridization patterns for pathogen identifications, leading to
21 unquantifiable errors (4). Moreover, these methods lack comprehensive information about the
22 pathogen at the single nucleotide level, which could represent a major problem when the
23 sequences in question share a high degree of similarity (21). The microarray-based pathogen
24 resequencing assay represents a promising alternative tool to overcome these limitations. This
25 method identifies each specific pathogen and is capable of resequencing, or "fingerprinting",
26 multiple pathogens in a single test. Indeed, this technology uses tiled sets of 10^5 to 10^6 probes
27 of 25mers, which contain one perfectly matched and three mismatched probes per base for
28 both strands of the target genes (16). This technology also offers the potential for a single test
29 that detects and discriminates between a target pathogen and its closest phylogenetic near
30 neighbours, which expands the repertoire of identifiable organisms far beyond those that are
31 initially included on the array. Successful results have been obtained using this technology,
32 especially for the detection of broad-spectrum respiratory tract pathogens using respiratory
33 pathogen microarrays (2, 25, 26) or the detection of a broad range of biothreat agents (1, 23,
34 36, 45). The amplification step, which is more often limiting for this technology, has also

1 benefited from recent developments. Phi29 polymerase-based amplification methods provide
2 amplified DNA with minimal changes in sequence and relative abundance for many
3 biomedical applications (3, 31, 40). The amplification factor varied between 10^6 to 10^9 , and it
4 was also demonstrated that co-amplification occurred when viral RNA was mixed with
5 bacterial DNA (3). This whole transcriptome amplification (WTA) approach can also be
6 successfully applied to viral genomic RNA of all sizes. Amplifying viral RNA by WTA
7 provides considerably better sensitivity and accuracy of detection than random RT-PCR in the
8 context of resequencing microarrays (RMAs) (3).

9
10 The rhabdoviruses are single-stranded, negative-sense RNA genome viruses classified into six
11 genera, three of which – *Vesiculovirus*, *Lyssavirus* and *Ephemerovirus* – include arthropod-
12 borne agents that infect birds, reptiles and mammals, as well as a variety of non-vector-borne
13 mammalian or fish viruses (International Committee on Taxonomy of Viruses database,
14 ICTVdb) (reviewed in (7)). These rhabdoviruses are the etiological agents of human diseases,
15 such as rabies, that cause serious public health problems. Some rhabdoviruses also cause
16 important economic losses in livestock. The three others genera include *Nucleorhabdovirus*
17 and *Cytorhabdovirus* with are arthropod-borne viruses infecting plants, and *Novirhabdovirus*
18 which comprises fish viruses. Other than the well characterized rhabdoviruses that are known
19 to be important for agriculture and public health, there is also a constantly growing list of
20 rhabdoviruses, isolated from a variety of vertebrate and invertebrate hosts, that are partially
21 characterized and are still waiting for definitive genus or species assignment. Considering the
22 large spectrum of potential animal reservoirs of these viruses compared to the few identified
23 virus species, it is highly likely that the number of uncharacterised rhabdoviruses is immense.
24 Unclassified or unassigned viruses have been tentatively identified as members of the
25 *Rhabdoviridae* by electron microscopy, based on their bullet-shaped morphology – a
26 characteristic trait of members of this family – or using their antigenic relationships based on
27 serological tests (9, 38). Gene sequencing and phylogenetic relationships have then been
28 progressively applied to complete this initial virus taxonomy (6, 22, 27). Importantly, a
29 strongly conserved domain in the rhabdovirus genome, within the polymerase gene, is a
30 useful target for the exploration of the distant evolutionary relationships among these diverse
31 viruses (6). This region corresponds to block III of the viral polymerase, a region predicted to
32 be essential for RNA polymerase function as it is highly conserved among most of the RNA-
33 dependant RNA polymerases (14, 33, 46). A direct application using this sequence region was
34 recently described for lyssavirus RNA detection in human rabies diagnosis (13). Taking

1 advantage of these characteristics, this polymerase region was also used to design probes for
2 high-density RMAs, also called PathogenID arrays (Affymetrix), which are optimized for the
3 detection and sequence determination of several RNA viruses, particularly rhabdoviruses (1).

4

5 In the present study, PathogenID microarrays containing probes for the detection of up to 126
6 viruses were tested using a consensus sequence determination strategy for analysis of output
7 RMA data. We demonstrate that this approach has the potential to identify, in experimentally
8 infected and clinical specimens, known but also phylogenetically related rhabdoviruses for
9 which precise sequence information was not available.

1 MATERIALS AND METHODS

2

3 **Design of the PathogenID microarray for rhabdovirus detection**

4 Two generations of PathogenID arrays were used in this study: PathogenID v1.0 containing
5 probes for the detection of 42 viruses (including 3 prototype rhabdoviruses), 50 bacteria and
6 619 toxin or antibiotic resistance genes (previously described in (1)), and PathogenID v2.0
7 able to detect 126 viruses (including 30 different rhabdoviruses), 124 bacteria, 673 toxin or
8 antibiotic resistance genes and two human genes as controls. These arrays include prototype
9 sequences of all species (or genotypes) of the genus *Lyssavirus*, of the other major genera
10 defined in the family *Rhabdoviridae*, such as *Ephemerovirus* and *Vesiculovirus*, and of 13
11 rhabdoviruses awaiting classification or tentatively classified among minor groups such as Le
12 Dantec or Hark park groups (6). For all the selected probes tiled on the two versions of
13 PathogenID array, the same conserved region of the viral polymerase gene was used (block
14 III). However, the size of the target region tiled on the array was longer in the second version
15 (up to 937 nucleotides in length for some sequences compared to roughly 500 nucleotides in
16 the first version) (Tables 1 and 2).

17

18 **Virus strains and biological samples analyzed**

19 Detailed descriptions of all prototype and field virus strains used in this study and their
20 sources are listed in Tables 1 and 2. Briefly, a total of 16 and 31 different viruses were tested
21 using PathogenID v1.0 (15 lyssaviruses and one vesiculovirus) and PathogenID v2.0 (14
22 lyssaviruses, one vesiculovirus, 12 unassigned and four tentative species of animal
23 rhabdoviruses according to ICTVdb) respectively. Samples tested included *in vitro* infected
24 cells, synthetic nucleotide target (when the corresponding virus strain was not available),
25 brain biopsies obtained from experimentally infected mice, and biological specimens from
26 various animals (brains from bat, cat, dog and fox) and humans (brain, saliva and skin
27 biopsies).

28

29 **Extraction and amplification of viral RNA**

30 RNA extraction from biological samples was processed with TRI Reagent (Molecular
31 Research Center) according to the manufacturer's recommendations. After extraction, viral
32 RNA were reverse-transcribed, then amplified using the whole transcriptome amplification
33 (WTA) protocol (QuantiTect Whole Transcriptome Kit, Qiagen) as described previously (3).

34

1 **Microarrays assay**

2 All amplification products obtained from viral RNA were quantified by Quantit BR
3 (Invitrogen) according to manufacturer's instructions or by the NanoDrop ND-1000
4 spectrophotometer instrument (Thermo Scientific). A recommended amount of DNA target
5 was fragmented and labelled according to GeneChip Resequencing Assay Manual
6 (Affymetrix). The microarray hybridization process was carried out according to the protocol
7 recommended by the manufacturer (Affymetrix). All details and parameter settings for the
8 data analysis (essentially conversion of raw image files obtained from scanning the
9 microarrays into FASTA files containing the sequences of base calls made for each tiled
10 region of the microarray) have been described previously (1). The base call rate refers to the
11 percentage of base calls generated from the full-length tiled sequence.

12

13 **Data analysis**

14 In the first approach, resequencing data obtained by the PathogenID v1.0 microarray were
15 manually submitted to the nr/nt database of NCBI for BLASTN query. The default BLAST
16 options were modified. The word size was set to 7 nucleotides. The expected threshold was
17 increased from its default value of 10 up to 100,000 to reduce the filtering of short sequences
18 and sequences rich in undetermined calls, which can assist the correct taxonomic
19 identification. To avoid false negative results induced by high number of undetermined
20 nucleotides in the sequences, the 'low complexity level filter' (-F) was also turned off.
21 BLAST sorts the resulting hits according to their bit scores so that the sequence that is the
22 most similar to the entry sequence appears first. Identification of virus strains tested was
23 considered successful only when the best hit was unique and corresponded to the expected
24 species or isolate (according to the nucleotide sequences of these viruses already available in
25 the nr/nt database of NCBI).

26

27 In the second approach, an automatic bioinformatic-based analysis of RMA data provided by
28 PathogenID v2.0 was developed, including a consensus sequence determination strategy
29 completed with a systematic BLAST strategy. The general workflow of this strategy is
30 represented in Figure 2. A Perl script reads the input data, which consists of one FASTA file
31 per sample that contains all the sequences read by the GSEQ software from the hybridization.
32 A modified version of the filtering process described by Malanoski *et al.* (29) is applied to
33 the sequences. The retained sequences contain stretches of nucleotides that are ascertained
34 according to the following algorithm. Briefly, sequences that do not contain subsequences

1 fulfilling specific parameters (minimum m nucleotide length and maximum undetermined
2 nucleotides ' N ' content) defined by the user are discarded. These parameters differ from those
3 described in the original filtering process where m was fixed to 20 and ' N ' was a value
4 depending on m , leading to filter out all short subsequences even with a high base call rate.
5 For subsequence determination, the program starts from the first base call of the considered
6 sequence and searches for the first m -bases window area that scores the elongation threshold
7 defined by the user, which represents another difference with the filtering process described
8 by Malanoski *et al.* where this elongation threshold was fixed to 60% (29). The subsequence
9 is extended by one base ($m + 1$) if the percentage of ' N ' remains inferior to the elongation
10 threshold. When this threshold is exceeded, the elongation is stopped and the subsequence is
11 conserved. This process is reiterated until the end of the sequence is reached to generate as
12 many informative sequences as possible. All our analysis were performed using filtering
13 parameters as follow: $m = 12$, $N = 10$ and elongation threshold = 10%.

14
15 A systematic BLAST strategy to search for sequence homologues was then performed with
16 the filtered sequences containing subsequences. These sequences individually undergo a
17 BLAST analysis based on a local viral and bacterial database (sequences obtained after
18 filtering from the nr/nt database of NCBI, updated and used for BLAST queries on December,
19 2009), and the taxonomy of the best BLAST hits are retrieved (Fig. 2A). The default BLAST
20 options were modified as previously described. In the case where several hits obtain the
21 highest bit score, the script automatically retrieves the taxonomies of the 10 first BLAST hits.
22 The final taxonomic identification for each virus strain tested was done by the user as follows:
23 i) identification at the species or isolate level when an unique best hit corresponds to expected
24 the species or isolate, ii) identification at the genus level (if available) when multiple best
25 viral hits exist and correspond to different species within the same genus among the
26 *Rhabdoviridae*, iii) identification at the family level when multiple best viral hits exist and
27 correspond to different rhabdoviruses genera, or iv) negative or inaccurate identification when
28 BLAST query is not possible or when multiple best hits correspond to other viral families,
29 respectively.

30
31 For the consensus sequence determination strategy, resequencing data obtained from
32 rhabdoviral tiled sequences are filtered as previously described then submitted to a multiple
33 alignment with Clustal W (39) from which a consensus sequence is determined (Fig. 2B). For
34 each sequence in the alignment, if a called base has undetermined calls on both sides, it is

1 replaced by an undetermined call. If different calls appear in the sequences for a given
2 position, the majority base call is added to the consensus. The positions that contain an
3 undetermined call or a gap are not considered in the majority base call computation. If
4 multiple base calls tie for the majority, an undetermined call appears at this position in the
5 consensus sequence. This procedure generally increases the length and accuracy of the query
6 sequence for subsequent analysis. Homology searching of the consensus sequences is
7 performed with BLAST using the parameters previously described, and the taxonomy of the
8 best hit is retrieved as for the systematic homology searching approach. We tested if the
9 resulting consensus sequences had higher identification accuracy than any individual
10 sequence or could be used to design PCR primers for a characterization of a potential novel
11 isolate.

12

13 **Sequencing confirmation**

14 Conventional sequencing was undertaken after the PCR amplification of viral targets directly
15 from biological samples (after RNA extraction and reverse transcription) or from 10- to 100-
16 fold water-diluted WTA products. Primer design was first based on consensus sequences
17 obtained using the consensus sequence determination strategy previously described and/or on
18 rhabdovirus nucleotide sequences available on GenBank. Depending on the results obtained
19 and the virus strain tested, primer design, the set of primers used and PCR conditions for
20 partial polymerase gene amplification were then adjusted (list of primers and PCR conditions
21 available on request from the corresponding author). All PCR products were obtained using
22 the proofreading DNA polymerase ExtTaq (Takara). Sequence assembly and consensus
23 sequences were obtained using Sequencher 4.7 (Gene Codes). GenBank accession numbers
24 for the sequences newly acquired are designated GU815994-GU816024 and are indicated in
25 Tables 1 and 2.

26

27 **Phylogenetic analysis**

28 The data set of 15 newly sequenced rhabdoviruses from this study (including the Sandjimba
29 and Kolongo viruses previously only identified on partial nucleoprotein gene sequences, as
30 well as *Piry virus* for which the nucleotide sequences of different genes were available) was
31 compared with the corresponding block III polymerase amino acid sequences of 91 other
32 rhabdoviruses collected from GenBank (Table 6). DNA translation was performed with
33 BioEdit software (17) and sequence alignment was performed using Clustal W programme
34 (39) and then checked for accuracy by eye. This resulted in a final alignment of 106

1 sequences of 160 amino acid residues in length. Phylogenetic analysis of these sequences was
2 then undertaken using the Bayesian method available in the MrBayes package (18). This
3 analysis utilized the WAG model of amino acid replacement with a gamma distribution of
4 among-site rate variation. Chains were run for 10 million generations (with a 10% burnin) at
5 which point all parameter estimates had converged. The level of support for each node is
6 provided by Bayesian Posterior Probability (BPP) values.

1

2 **RESULTS**

3

4 **Identification of lyssaviruses based on two successive PathogenID microarray**
5 **generations using a systematic BLAST strategy**

6 To test whether PathogenID microarrays, and specifically the prototype tiled regions, could be
7 used for the identification of a broad number of viral variants without relying on
8 predetermined hybridization patterns, representative animal viruses from the family
9 *Rhabdoviridae* (including unassigned or tentatively classified rhabdoviruses according to
10 ICTVdb) were studied. The capability of these RMAs to identify and discriminate between
11 near phylogenetic neighbours was first tested using one sequence of the genus *Lyssavirus* (PV
12 strain, genotype or species 1) tiled on the first generation of PathogenID microarray (Table 1).
13 It was possible to use BLAST to successfully identify virus strains with approximately 18%
14 nucleotide divergence compared to the prototype (Fig. 1). The hybridization of 15 virus
15 strains representative of the genetic diversity found in this species indicated that a single tiled
16 sequence was able to detect all variant strains belonging to the same species.

17

18 In addition, we evaluated the spectrum of detection of the second generation of PathogenID
19 microarray, which included one prototype sequence representative of each of the seven
20 species described in the genus *Lyssavirus* (Table 2). All the tested isolates led to the correct
21 species identification using a systematic BLAST strategy when hybridizing target belonging
22 to the same species that is tiled on the array (Table 3). Moreover, all tested isolates of known
23 genotype were also recognized by heterospecific tiled sequences (Table 3). We also
24 investigated the capacity of this RMA to detect more distantly related viruses not yet
25 classified into a species. Isolates 0406SEN and WCBV, which have been proposed to
26 represent new species in the genus *Lyssavirus* (5, 15), were surprisingly recognized by almost
27 all of the seven species sequences tiled on the PathogenID v2.0 microarray (Table 3). This
28 recognition indicates that each sequence tiled on the array has the ability to identify strains
29 that are more than 18% divergent, and up to 25.9% in some cases (Table 3). This analysis also
30 reveals that information on a strain hybridized on PathogenID v2.0 can be obtained from
31 distinct species or isolates tiled on the array. Evaluation of the spectrum of detection of this
32 RMA was further extended to two other genera of the *Rhabdoviridae* - *Ephemerovirus* and
33 *Vesiculovirus* (Table 4). Here again a successful identification was achieved using
34 homospecific sequences tiled on the array confirming the reliability of the identification.

1

2 In both experiments (Tables 3 and 4), low base call rate values were obtained for several
3 combinations of hybridized and tiled sequences. These values were sufficient for a viral
4 identification by BLAST, despite the presence of sequence reads as short as 14 nucleotides.
5 This indicates that most of these short sequences corresponded to highly conserved sequence
6 domains. The accuracy of these short sequences was checked by comparison with those
7 obtained by classical sequencing (data not shown).

8

9 **Identification of lyssaviruses based on the consensus sequence determination strategy**

10 A bioinformatic workflow was developed to gather stretches of sequence reads obtained with
11 more or less distantly related sequences tiled on PathogenID v2.0. The aim of this strategy
12 was to enlarge the length of the determined sequence in order to improve the sensitivity of the
13 BLAST analysis compared to previously described methodologies (29). All sequence reads
14 obtained from prototype sequences of the genus *Lyssavirus* (at least 12 nucleotides long with
15 no more than one undetermined base, whether or not they initially led to a positive BLAST
16 identification), were used to generate a contiguous sequence. When overlapping fragments
17 were identified, a consensus sequence was generated to remove ambiguous or undetermined
18 base calls. The methodology used to obtain consensus sequences confirmed the species
19 identification after BLAST analysis in the case of the seven lyssavirus nucleotide sequences
20 used for hybridization (Table 5). Moreover, these consensus sequences were found to be more
21 powerful in identifying unclassified or new species of lyssaviruses not tiled on the RMA than
22 resequencing data collected individually from each tiled sequences, as shown for strains
23 0406SEN and WCBV. In both cases, an increase of the base call rate was observed using this
24 consensus sequence strategy, from 63.5% (best base call rate obtained from individual
25 prototype sequences) to 75.9% for 0406SEN strain and from 32.7% to 60.9% for WCBV
26 (Tables 3 and 5). Once again, this increase of nucleotide base determination was associated
27 with a relatively high accuracy (91.8% and 97.3% concordance between the consensus
28 sequences and the reference sequences of 0406SEN and WCBV isolates, respectively (Table
29 5). To further demonstrate the ability of this strategy to detect and identify novel virus
30 species, consensus sequences were generated based only on six of the seven prototype tiled
31 sequences (excluding the homospecific sequence of the same species tiled on the array). All
32 strains from the seven species tested were accurately and specifically identified using this
33 restricted approach (Table 5). These results indicate that the consensus sequences obtained

1 could improve the detection of novel domain(s) not identified using only the closest prototype
2 sequence tiled on the RMA.

3

4 **Assessment of clinical specimens**

5 A total of 17 brain biopsy samples originating from experimentally infected mice as well as
6 various clinical samples (n=8) obtained from the National Reference Centre for Rabies at
7 Institut Pasteur were tested for lyssavirus detection and identification using the two versions
8 of PathogenID microarray (Tables 1 and 2). These specimens were previously collected from
9 humans and animals with clinically documented encephalitis and suspected of rabies. They
10 were used to compare RMA results with conventional methods of diagnosis, including the
11 RT-hemi-nested PCR technique for the *intra-vitam* diagnosis of rabies in humans (13), the
12 fluorescent antibody test, the rabies tissue culture inoculation test, and the ELISA test for the
13 *post-mortem* diagnosis in humans and animals (8, 47). Among the eight clinical samples, most
14 were brain biopsies collected from different rabid mammals including bat (n=1), cat (n=1),
15 dog (n=1), fox (n=2) and in one case human (n=1). The two other samples comprised a saliva
16 specimen and a skin biopsy sample collected from two different rabid human patients (Tables
17 1 and 2). Except for the skin biopsy case which was not recognized, this comparison
18 demonstrated a complete concordance between our method and conventional methods for all
19 samples tested. Hence, the accuracy of the sequences provided with PathogenID microarray
20 was close to that obtained using classical sequencing (data not shown). The failure to detect
21 lyssaviruses in the skin biopsy samples was probably due to insufficient sensitivity of the
22 current RMA method, as viral RNA was only weakly detected after RT-hnPCR.

23

24 In sum, these results demonstrated that the newly developed amplification process by WTA
25 coupled to hybridization to the PathogenID microarray allowed the detection of a large range
26 of viral variants from various complex biological samples, including clinical samples (Tables
27 1 and 2).

28

29 **Application of the RMA strategy to characterize new rhabdoviruses**

30 Broad spectrum detection was demonstrated using the consensus sequences-based analysis
31 strategy amongst viruses of the family *Rhabdoviridae*, and the more distantly related viruses
32 examined included many viruses that are not yet classified as species. Accordingly, 17
33 different rhabdoviruses were tested, using brain samples from experimentally infected mice
34 (n=16) or infected cell suspension. These viruses included four strains belonging to the genus

1 *Vesiculovirus*, with *Vesicular stomatitis Indiana virus* (VSIV), Boteke (BOTK), Jurona
2 (JURV) and Porton's (PORV) viruses, currently classified as tentative species for the three
3 latter; two strains belonging to the genus *Ephemerovirus* with Kimberley (KIMV) and
4 kotonkan (KOTV) viruses, corresponding to a tentative and unassigned species respectively;
5 and 11 presently unassigned rhabdoviruses, namely Kamese (KAMV), Mossuril (MOSV),
6 Sandjimba (SAJV), Keuraliba (KEUV), Nkolbisson (NKOV), Garba (GARV), Nasoule
7 (NASV), Ouango (OUAV), Bimbo (BBOV), Bangoran (BGNV) and Gossas (GOSV) viruses
8 (virus taxonomy according to ICTVdb) (Table 2).

9

10 In the first step, successful detection and identification of these viruses using the PathogenID
11 v2.0 microarray was obtained for 12 out of 17 (70.5%) viruses; an accurate taxonomic
12 positioning – that is, within the family *Rhabdoviridae*– was also achieved, and for some the
13 corresponding genus (when available) was also matched accurately (data not shown). In the
14 second step, specific and consensus primers were designed based on the stretches of
15 sequences identified by the microarray using the consensus sequence determination strategy,
16 then subsequently used for PCR and classical sequencing of the amplified target nucleotide
17 sequences. For four (GARV, NASV, OUAV and BBOV) of the five rhabdoviruses that failed
18 to be detected by the microarray, a region of 1000 nucleotides of the polymerase gene
19 encompassing that tiled on the array was successfully amplified by PCR and sequenced using
20 the primers described above. The only exception was the GOSV isolate which remained
21 undetected either using the microarray or by PCR. Further, two other rhabdoviruses not
22 previously tested with the PathogenID v2.0 microarray – Kolongo virus (KOLV, unclassified
23 species) and *Piry virus* (PIRYV, vesiculovirus) – were also amplified and sequenced using
24 these primers.

25

26 All the newly sequenced nucleotide regions of the polymerase gene were further
27 translated into protein sequences and aligned with 88 sequences of animal or plant
28 rhabdoviruses obtained from GenBank, producing a total data set of 106 sequences, 160
29 amino acid residues in length. A Bayesian phylogenetic analysis of these sequences
30 tentatively distinguished 15 groups of viruses based on their strongly supported monophyly
31 (Table 6 and Fig. 3). The members of the six genera – *Ephemerovirus*, *Lyssavirus*,
32 *Vesiculovirus*, *Cytorhabdovirus*, *Nucleorhabdovirus*, and *Novirhabdovirus* – fall into well-
33 supported monophyletic groups (BPP value ≥ 0.97) (Fig. 3). Interestingly, this analysis
34 suggested the existence of at least nine more groups of currently unclassified rhabdoviruses,

1 which reflect important biological characteristics of the viruses in question. Five of these
2 groups have been proposed previously and were further supported by our analysis (data
3 available at the CRORA database website:
4 <http://www.pasteur.fr/recherche/banques/CRORA/>) (6, 27), reviewed in (7). The first group,
5 tentatively named Hart Park group, contains the previously described Parry Creek (PCR_V),
6 Wongabel (WON_V), Flanders (FLAN_V) and Ngaingan (NGAV) viruses added to the newly
7 identified BGN_V, KAM_V, MOS_V and POR_V viruses. This group has a large distribution that
8 encompasses Africa, Australia, Malaysia and the USA. These viruses have a wide host range,
9 as they have been found to infect dipterans, birds and mammals. The second group is the
10 Almpiwar group containing four members – two strains of Charleville (CHV_V) virus with
11 CHV_V_Ch9824 and CHV_V_Ch9847 – and the Almpiwar (ALM_V) and Humpty Doo
12 (HDOO_V) viruses. Viruses of this group were isolated from Australia and are associated with
13 infection in dipterans, lizards but also in birds and mammals including humans. Another
14 group, herein referred to as the Le Dantec group, was also seen to form a distinct cluster with
15 Le Dantec virus (LD_V), Fukuoka virus (FUK_V) and the two newly molecularly identified
16 KEU_V and NKO_V viruses. Members were isolated in Japan and in Africa where they were
17 shown to infect dipterans and mammals including humans. The fourth group has been
18 tentatively named the Tibrogargan group and includes the Tupaia (TUP_V) and Tibrogargan
19 (TIB_V) viruses. These viruses were isolated in South-East Asia, Australia and New Guinea
20 from dipterans and mammals. Finally, we observed the Sigma group as previously described
21 (27). It includes *Drosophila affinis* (DAff_{SV}), *Drosophila obscura* (DObs_{SV}) and two strains
22 of *Drosophila melanogaster* (SIGMAV_AP30 and SIGMAV_HAP23) sigma viruses,
23 infecting *Drosophila* which were found in USA and in Europe.

24 In addition, four other tentative groups of viruses are newly described in this study.
25 The Sandjimba group includes the first molecularly classified BBO_V, BTK_V, NAS_V, GAR_V
26 and OUAV viruses and the previously described Oak-Vale (OVR_V), SJAV and KOL_V
27 viruses (identification based only on a limited region of the nucleoprotein gene for the two
28 latter). These viruses were isolated from birds and dipterans from the Central African
29 Republic and Australia (data available at <http://www.pasteur.fr/recherche/banques/CRORA/>)
30 (6, 9). Interestingly, all the African members of this group clustered closely, whereas the sole
31 Australian virus was more divergent, suggesting a potential geographical segregation. Second,
32 the Sinistar group includes the *Siniperca chuatsi* rhabdovirus (SCR_V) isolated from mandarin
33 fish in China (37) and the starry flounder rhabdovirus (SFR_V) identified from starry flounder
34 in USA (30). These two viruses appear to be more closely related to Le Dantec group than to

1 viruses in the genus *Vesiculovirus* in which several other fish rhabdoviruses are classified.
2 The third one is the Moussa group including two isolates of Moussa virus (MOUV_D24 and
3 MOUV_C23) collected from mosquitoes in Ivory Coast (34). Finally, a phylogenetic analysis
4 suggests the presence of another group within the plant rhabdoviruses: the Taastrup group
5 which comprises the single isolate Taastrup virus (TV) isolated from leafhoppers
6 (*Psammotettix alienus*) originally collected in France (28). All these groups were strongly
7 supported by the Bayesian analysis (BPP \geq 0.98), with the exception of the Sigma group
8 which exhibits a BPP of 0.88.

9 In addition, classification of some uncharacterised rhabdoviruses from our
10 phylogenetic analysis diverged from that previously suggested by serology (according to
11 ICTVdb), and will probably need further investigation to determine their precise taxonomic
12 position within the family *Rhabdoviridae* (Table 4) (9, 38). In particular, the PORV and
13 BTKV viruses previously identified as vesiculoviruses were included within the Hart Park
14 and Sandjimba groups, respectively, and NKOV was classified into the Le Dantec group
15 instead of the Kern Canyon group. Moreover, in contrast to a previous phylogenetic study
16 (22), TUPV was found to be more closely related to TIBV than to any other isolates of the
17 Sandjimba group. Finally, our study confirmed the previous serologically-based classification
18 of JURV and the recently identified *Scophthalmus maximus* rhabdovirus (SMRV) within the
19 *Vesiculovirus* genus (38, 49).

20

21

1 DISCUSSION

2

3 We have analysed the capacity of viral detection and identification of two versions of a newly
4 described RMA, termed PathogenID, which was designed specifically for multiple pathogen
5 detection using database similarity searching (1). To evaluate this microarray we focused on
6 one of the largest and most diverse viral families described to date, the *Rhabdoviridae*
7 (ITCvdb, reviewed in (7)). All virus strains tested (except WCBV) were extracted from
8 biological samples and amplified using a non-specific and unbiased WTA step as previously
9 described (3). Rhabdovirus targeted sequences were selected among blocks of conservation
10 within the polymerase gene (6). This region was chosen so as to encompass a sufficient
11 number of homologous but also polymorphic sites. The key advantage of this RMA strategy
12 is that it does not require a specific match between the tested samples and tiled sequences;
13 indeed, mismatches add value as they allow a precise typing of the unknown genetic re-
14 sequenced element. In our case, the conserved nature of the target region in the polymerase
15 gene (block III) and the capability of detection of the RMA allows a precise taxonomic
16 identification (i.e. family, genus, species) and also provides key information on phylogenetic
17 relationships for some unclassified, unassigned or tentative species of rhabdoviruses. For
18 example, results obtained by the PathogenID v1.0 microarray evaluation demonstrated that
19 most of the intra-species nucleotide diversity found in the genus *Lyssavirus* can be covered by
20 a single prototype sequence tiled on the microarray. Using the second version of PathogenID,
21 which included one prototype sequence of each of the seven species recognized thus far
22 within the genus *Lyssavirus*, we extended the spectrum of detection of the RMA to potentially
23 all known or unknown lyssaviruses (i.e. positive detection of virus isolates presenting up to
24 25.9% nucleotide divergence with the considered tiled sequence) which is higher than
25 previous reports (24-26, 43, 44).

26

27 This study also indicates that accurate viral identification may still be possible even when
28 only shorter sequences are obtained from individual tiled prototype sequences. Indeed, taken
29 individually, these short-length stretches of nucleotide sequence could not give positive
30 results during the initial BLAST query. However, when used in the consensus sequence
31 determination strategy employed here, they improved the identification of virus strains
32 distantly related with that tiled on the RMA. For example, we were able to test and detect
33 rhabdoviruses based on sequence data obtained with tiled sequences that originated from
34 other viral genera.

1
2 The strategy developed here also allowed the potential detection of genetically diverse
3 rhabdoviruses, previously identified or unknown, using a limited number of sequences tiled
4 on the microarray. Using the PathogenID v2.0 microarray we were able to identify 30
5 rhabdoviruses in total. This included 12 viruses currently unclassified, unassigned or assigned
6 as tentative species within the family *Rhabdoviridae* (according to ICTVdb). Moreover, the
7 consensus sequence based-analysis of RMA results was shown to be accurate when compared
8 to sequences obtained through classical sequencing (Table 5 and data not shown). Sequence
9 data provided by the PathogenID v2.0 microarray were also extremely helpful in the design of
10 specific primers to further sequence the targeted region of the viral polymerase gene of some
11 other rhabdoviruses. Finally, this approach allowed us to undertake the largest phylogenetic
12 analysis of the family *Rhabdoviridae* (Table 6 and Fig. 3), even though it is important to note
13 that the list of viruses and potential taxa described here is still incomplete and more viruses
14 will clearly be characterized in the near future. Despite these phylogenetic divisions, all the
15 viruses included in these proposed groups are closely related to vesiculoviruses and
16 ephemeroviruses and were found to infect a large spectrum of animals, included dipterans and
17 mammals (and previously referred to as the dimarhabdovirus supergroup (6) but also lizards
18 (Almpiwar group), birds (especially with Sandjimba group but also with Hart Park group) and
19 fish (Sinistar group) (Table 6).

20
21 Although promising, inadequate sequence selection for the design of the RMA, and
22 consequently a lack of coverage of the viral sequence space, represents an important
23 limitation. A proper selection of blocks of conserved sequence across taxonomic subdivisions
24 in the viral world could be similarly defined and targeted by the RMA assay, and in doing so
25 improve the detection power of this tool and therein greatly aid viral identification among the
26 *Rhabdoviridae*, or even in other viral families. The results presented here validated the
27 usefulness of the design methodology. It emphasizes the gain in identification using a
28 consensus sequence strategy determination compared to systematic BLAST strategy (29).
29 Indeed, this strategy allows us to use and accurately analyze the RMA output data, even if
30 only short subsequences with a high base call rate are obtained. It provides an informative
31 alternative to current molecular methods, such as classical or multiplex PCR, for the rapid
32 identification of viral pathogens. It is currently being applied to assist in a new generation of
33 RMA aimed at the detection and identification of genetically diverse and unknown viral
34 pathogens, and more broadly of *any* virus present in a clinical specimen. In contrast to

1 conventional microarrays, it is not limited by the requirement for prior knowledge on the
2 identities of viruses present in biological samples, and it is not restricted to the detection of a
3 limited number of candidate viruses. As such, this strategy has a great potential for being
4 implemented as a high throughput platform to identify more divergent viral organisms. This
5 technology could be especially useful in clinical diagnosis or in surveillance programs for
6 detecting uncharacterized viral pathogens or highly variable virus strains among a same
7 taxonomic genus or family, which is frequently the case for RNA viruses (2). The potential
8 applications of such a methodology therefore appear to be numerous: differential diagnostics
9 for illnesses with multiple potential causes (for example, central nervous diseases like
10 encephalitis and meningitis), tracking of emergent pathogens, the distinction of biological
11 threats from harmless phylogenetic neighbours, and the broader-scale investigation of
12 biodiversity in the viral world.

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11 their excellent work realized for sequencing of the different rhabdoviruses.

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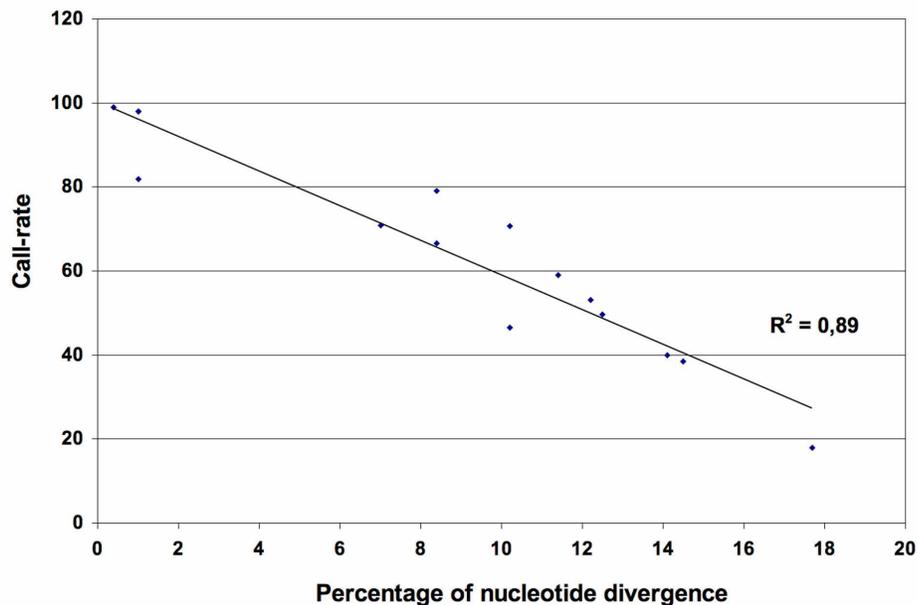
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- 26

1 **FIGURES**

2
3
4



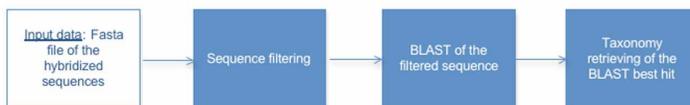
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9 **Figure 1: Spectrum of detection of the PathogenID v1.0 microarray among the genus**
10 ***Lyssavirus* according to the natural nucleotide variation of the virus strains tested.**

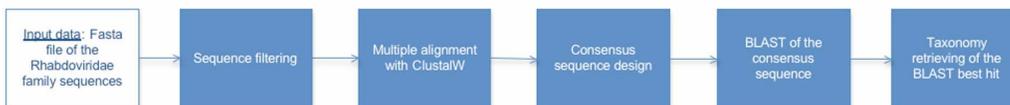
11 For each lyssavirus strain tested (n=15, indicated with black diamonds), results are indicated
12 by the percentage nucleotide divergence (compared to the single Lyssavirus prototype
13 sequence tiled on the microarray, x-axis) according to the percentage of nucleotide bases
14 determined (call-rate, y-axis). The linear correlation curve between these two values is
15 presented, demonstrating a high correlation between these two parameters (correlation
16 coefficient value of 0.89). All these 15 virus strains belonged to the same species as the tiled
17 prototype sequence (genotype 1) and were accurately identified after BLAST analysis (at the
18 species level). Other species (or genotypes) of lyssaviruses were not successfully detected
19 with PathogenID v1.0 microarray (nucleotide divergence over 20%, data not shown).

20 For further details concerning the lyssavirus strains used, see Table 1.

A: Systematic blast strategy



B: Consensus sequence determination strategy



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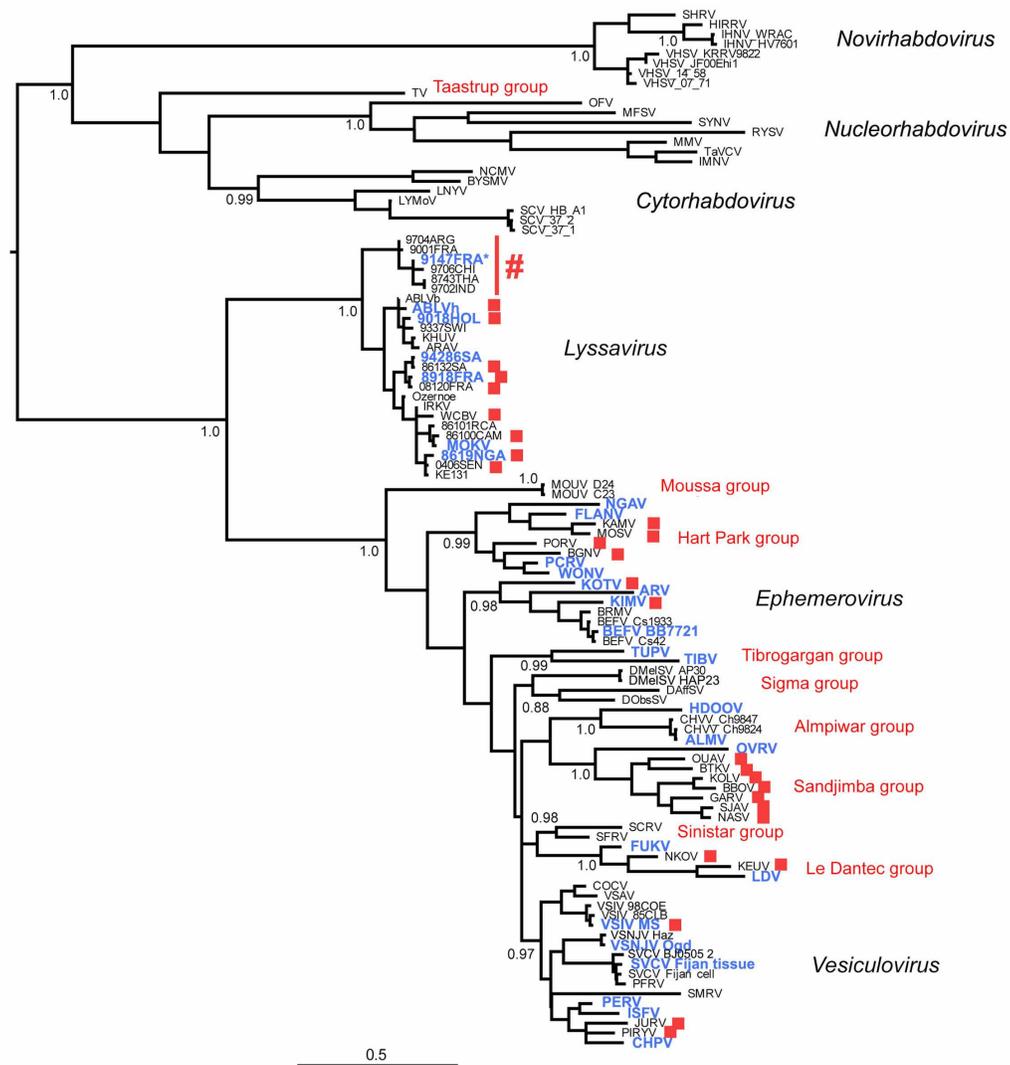
Figure 2: Descriptive workflow of the automatic Perl bioinformatic-based analysis of the PathogenID v2.0 data.

A: Systematic BLAST strategy

This strategy consists of filtering the sequences obtained from the output data of the RMA with filter parameters defined by the user (see Methods section for further details), followed by the systematic researching of homologues using a local BLAST viral and bacterial database, and finally retrieve taxonomy of the best BLAST hits.

B: Consensus sequence determination strategy

A consensus sequence is generated using a multiple alignment with Clustal W based on the sequences obtained from prototype rhabdovirus sequences tiled on the microarray. With this process, the length and the accuracy of the query sequence can be increased. Homology searching of the consensus sequences is performed with BLAST using the previously described parameters and database. The taxonomy of the best BLAST hit is retrieved as for the systematic homology searching approach.



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Figure 3: Phylogenetic relationships of the *Rhabdoviridae* based on a 160 amino acid alignment of the polymerase gene.

1 Phylogenetic analysis of 106 amino-acid sequences of the block III of the polymerase (160
2 amino acid residues in length) of rhabdoviruses using a Bayesian method. This analysis
3 utilized the WAG model of amino acid replacement with a gamma distribution of among-site
4 rate variation. Chains were run for 10 million generations (with a 10% burnin) at which point
5 all parameter estimates had converged. The level of support for each node is provided by
6 Bayesian Posterior Probability (BPP) values. The genera (black font) and groups (red font) of
7 the *Rhabdoviridae* are indicated, along with their associated BPP values. All horizontal
8 branch lengths are drawn to a scale of amino acid replacements per residue. The tree is mid-
9 point rooted for purposes of clarity only. Sequence tiled on the array or their closely related
10 sequence (* 9147FRA instead of PV) are indicated in blue font. Sequences corresponding to
11 lyssavirus species 1 and positively detected by PathogenID v1.0 are indicated by a red line
12 (#). Sequences detected by PathogenID v2.0 are indicated by red squares.
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TABLES

Table 1 :
Description of virus species belonging to the *Rhabdoviridae* used for selection of tiled sequences and for validation of the PathogenID v1.0 microarray.

Genus and specie name ^a (abbreviation)	Reference no.	Host species/ vector	Origin	Year of first isolation	Tiled region ^b	Length (bp)	Biological samples tested	GenBank accession no.
<i>Origin of tiled sequences</i>								
<i>Lyssavirus</i>								
<i>Rabies virus</i> (RABV)	PV	Vaccine	--	--	7452-7953	502	--	NC_001542
<i>Vesiculovirus</i>								
<i>Vesicular stomatitis Indiana virus</i> (VSIV)	VSVLMS	--	--	--	7453-7953	497	--	K02378
<i>Ephemerovirus</i>								
<i>Bovine ephemeral fever virus</i> (BEFV)	BB7721	<i>Bos taurus</i>	Australia	1968	7454-7952	498	--	NC_002526
<i>Tested rhabdovirus species</i>								
<i>Lyssavirus</i>								
Genotype 1								
<i>Rabies virus</i> (RABV)	8764THA	Human	Thailand	1983	--	--	Human brain	EU293111
<i>Rabies virus</i> (RABV)	9147FRA	Red fox	France	1991	--	--	Fox brain	EU293115
<i>Rabies virus</i> (RABV)	93128MAR	Fixed strain	Morocco	?	--	--	Mouse brain	GU815994
<i>Rabies virus</i> (RABV)	9811CHI	Dog	China	1998	--	--	Mouse brain	GU815995

<i>Rabies virus</i> (RABV)	0435AFG	Dog	Afghanistan	2004	--	--	Mouse brain	GU815996
<i>Rabies virus</i> (RABV)	9001FRA	Dog bitten by a bat	French Guyana	1990	--	--	Mouse brain	EU293113
<i>Rabies virus</i> (RABV)	9026CI	Dog	Ivory Coast	1990	--	--	Mouse brain	GU815997
<i>Rabies virus</i> (RABV)	9105USA	Fox	USA	1991	--	--	Fox brain	GU815998
<i>Rabies virus</i> (RABV)	9233GAB	Dog	Gabon	1992	--	--	Dog brain	GU815999
<i>Rabies virus</i> (RABV)	93127FRA	Fixed strain	France	?	--	--	Mouse brain	GU816000
<i>Rabies virus</i> (RABV)	9503TCH	Fixed strain (Vnukovo, SAD)	Tchekosllovakia	?	--	--	Mouse brain	GU816001
<i>Rabies virus</i> (RABV)	9737POL	Raccoon dog	Poland	1997	--	--	Mouse brain	GU816002
<i>Rabies virus</i> (RABV)	Challenge Virus Strain (CVS_IP13)	Fixed strain	--	--	--	--	Mouse brain	GU816003
<i>Rabies virus</i> (RABV)	ERA	Fixed strain	--	--	--	--	Mouse brain	GU816005
<i>Rabies virus</i> (RABV)	LEP	Fixed strain	--	--	--	--	Chicken embryo fibroblasts	GU816004

Vesiculovirus

<i>Vesicular stomatitis Indiana virus</i> (VSIV)	Orsay (0503FRA)	Fixed strain	--	--	--	--	BSR cells ^c	GU816006
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^a Classification and names of viruses correspond to approved virus taxonomy according to the International Committee on Taxonomy of Viruses database (ICTVdb). Names of viruses in italic correspond to validated virus species.

^b Position according to the reference Pasteur virus genome (NC_001542), after alignment of all the tiled sequences with the reference sequence.

^c A clone of baby hamster kidney cell line (BHK-21).

Table 2 : Description of virus species belonging to the *Rhabdoviridae* used for selection of tiled sequences and for validation of the PathogenID v2.0 microarray.

Genus or group ^a	Species and name ^a	UA/TS/UC ^b	Reference no.	Host species/vector	Tiled region ^c	Length (bp)	Biological samples tested	Origin of samples	Year of first isolation	GenBank accession no.
<i>Origin of tiled sequences</i>										
<i>Lyssavirus</i>										
	Genotype 1 <i>Rabies virus</i> (RABV)		PV	Vaccine	7040-7977	937	--	--	--	NC_001542
	Genotype 2 <i>Lagos bat virus</i> (LBV)		8619NGA	Bat : <i>Eidolon helvum</i>	7040-7977	937	--	Nigeria	1956	EU293110
	Genotype 3 <i>Mokola virus</i> (MOKV)		MOKV	Cat	7040-7977	937	--	Zimbabwe	1981	NC_006429
	Genotype 4 <i>Duvenhage virus</i> (DUVV)		94286SA	Bat : <i>Miniopterus</i> species	7040-7977	937	--	South Africa	1981	EU293120
	Genotype 5 <i>European</i>		8918FRA	Bat : <i>Eptesicus</i>	7040-	937	--	France	1989	EU293112

<i>bat lyssavirus 1</i> (EBLV-1)		<i>serotinus</i>	7977					
Genotype 6 <i>European bat lyssavirus 2</i> (EBLV-2)	9018HOL	Bat : <i>Myotis dasycneme</i>	7040-7977	937	--	The Netherlands	1986	EU293114
Genotype 7 <i>Australian bat lyssavirus</i> (ABLV)	ABLh	Human	7040-7977	937	--	Australia	1986	AF418014

Vesiculovirus

<i>Chandipura virus</i> (CHPV)	I 653514	Human	7040-7981	935	--	India	1965	AJ810083
<i>Isfahan virus</i> (ISFV)	91026-167	<i>Phlebotomus papatasi</i>	7040-7981	935	--	Iran	1975	AJ810084
<i>Vesicular stomatitis New Jersey virus</i> (VSNJV)	VSV NJ-O	<i>Bos taurus</i> , equine / <i>Culex nigripalpus</i> , <i>Culicoides</i> species, <i>Mansonia indubitans</i>	7040-7981	935	--	USA	1949	AY074804
<i>Vesicular stomatitis</i>	VSVLMS	--	7040-7981	935	--	--	--	K02378

<i>Indiana virus (VSIV)</i>									
Perinet virus (PERV)	TS	Ar Mg 802	<i>Anopheles coustani, Culex antennatus, Culex gr. pipiens, Mansonia uniformis, Phlebotomus berentensis</i>	7089-7502	405	--	Madagascar	1978	AY854652
Spring viremia of carp virus (SVCV)	TS	VR-1390	<i>Cyprinus carpio</i>	7040-7981	935	--	Yougoslavia	1971	U18101

Ephemerovirus

<i>Adelaide River virus (ARV)</i>		DPP 61	<i>Bos taurus</i>	7089-7502	408	--	Australia	1981	AY854635
<i>Bovine ephemeral fever virus (BEFV)</i>		BB7721	<i>Bos taurus</i>	7089-7502	408	--	?	1968	AY854642
<i>Kimberley virus (KIMV)</i>	TS	CS 368	<i>Bos taurus</i>	7089-7502	408	--	Australia	1980	AY854637
<i>Kotonkan virus^d (KOTV)</i>	UA	Ib Ar23380	<i>Culicoides</i> species	7089-7502	408	--	Nigeria	1967	AY854638

*Other
dymarhabodviruses^d*

***Almpiwar
group***

Almpiwar virus (ALMV)	UA	MRM4059	<i>Ablepharus boutonii virgatus</i>	7089-7502	411	--	Australia	1966	AY854645
Humpty doo virus (HDOOV)	UA	CS 79	<i>Lasiohelea</i> species	7089-7502	411	--	Australia	1975	AY854643
Oak-Vale virus (OVRV)	UA	CS 1342	<i>Culex</i> species	7089-7502	408	--	Australia	1981	AY854670

***Hart Park
group***

Flanders virus (FLANV)	UA	61-7484	<i>Culiseta melanura</i> , <i>Culex</i> species / <i>Seiurus aurocapillus</i>	7089-7502	410	--	USA	1961	AF523199
Ngaingan virus (NGAV)	UA	NRM14556	<i>Culicoides brevitarsis</i> / wallabies, kangaroos, cattle	7089-7502	408	--	Australia	1970	AY854649
Parry Creek virus (PCRIV)	UA	OR 189	<i>Culex annulirostris</i>	7089-7502	408	--	Australia	1972	AY854647
Wongabel	UA	CS 264	<i>Culicoides</i>	7089-	408	--	Australia	1979	AY854648

virus (WONV)			<i>austropalpalis</i>	7502						
<i>Le Dantec and Kern Canyon group</i>										
Fukuoka virus (FUKV)	UA	FUK-11	<i>Culicoides punctatus</i>	7089-7502	408	--	Japan	1982	AY854651	
Le Dantec virus (LDV)	UA	DakHD 763	Human	7089-7502	408	--	Senegal	1965	AY854650	
<i>Tibrogargan group</i>										
Tibrogargan virus (TIBV)	UA	CS 132	<i>Culicoides brevitarsis</i> , water buffaloes, cattle	7089-7502	408	--	Australia	1976	AY854646	
<i>Other animal rhabdoviruses</i>										
Tupaia rhabdovirus (TUPV)	UA	TRV 1591	<i>Tupaia belangeri</i>	7089-7502	408	--	Thailand	?	NC_007020	
Sigma virus (SIGMAV)	UA	234HRC	<i>Drosophila melanogaster</i>	6220-6642	408	--	?	?	X91062	
Sea trout rhabdovirus (STRV)	UC	28/97	<i>Salmo trutta trutta</i>	7108-7576	415		Sweden	1996	AF434992	

Tested rhabdovirus species

Lyssavirus

Genotype 1

<i>Rabies virus</i> (RABV)	93127FR A	Fixed strain	--	--	Mouse brain	France	?	GU816000
<i>Rabies virus</i> (RABV)	8764THA	Human	--	--	Human brain	Thailand	1983	EU293111
<i>Rabies virus</i> (RABV)	08339FR A	Human (probably contaminated by a bat)	--	--	Human saliva	France (French Guyana)	2008	GU816007
<i>Rabies virus^h</i> (RABV)	07029SEN	Human	--	--	Skin biopsy	Senegal	2006	--

Genotype 2

<i>Lagos bat virus</i> (LBV)	8619NGA	Bat : <i>Eidolon helvum</i>	--	--	Mouse brain	Nigeria	1956	EU293110
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Genotype 3

<i>Mokola virus</i> (MOKV)	86100CA M	Shrew	--	--	Mouse brain	Cameroon	1981	NC_006429
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Genotype 4

<i>Duvenhage virus</i> (DUVV)	86132SA	Human	--	--	Mouse brain	South Africa	1971	EU293119
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Genotype 5

European bat lyssavirus 1

subtype a (EBLV-1a)

European bat lyssavirus 1

subtype a (EBLV-1b)

European bat lyssavirus 1

subtype b (EBLV-1b)

Genotype 6

European bat lyssavirus 2

(EBLV-2)

Genotype 7

Australian bat lyssavirus

(ABLV)

Genotype 8

(tentative species)

07240FR A	Cat (contaminated by a bat)	--	--	Cat brain	France	2007	EU626552
08341FR A	Bat : <i>Eptesicus serotinus</i>	--	--	Bat brain	France	2008	GU816009
8918FRA	Bat : <i>Eptesicus serotinus</i>	--	--	Mouse brain	France	1989	EU293112
9018HOL	Bat : <i>Myotis dasycneme</i>	--	--	Mouse brain	Holland	1986	EU293114
9810AUS	Bat	--	--	Mouse brain	Australia	?	GU816008

Dakar bat lyssavirus (DBLV)	UC	0406SEN (AnD 42443)	Bat : <i>Eidolon helvum</i>	--	--	Mouse brain	Senegal	1985	EU293108
Not assigned West Caucasian Bat Virus (WCBV)	UC	--	Bat : <i>Myotis schreibersi</i>	--	--	Plasmid ^e	Russia	2002	EF614258

Vesiculovirus

<i>Vesicular stomatitis Indiana virus</i> (VSIV)		Orsay (0503FRA)	?	--	--	BSR cells ^f	?	?	GU816006
Boteke virus (BTKV)	TS	DakArB 1077 (0417RCA)	<i>Coquillettidia maculipennis</i>	--	--	Mouse brain	Central African Republic	1968	GU816014
Jurona virus (JURV)	TS	BeAr 40578 (0414BRE)	<i>Haemagogus spegazzinii</i>	--	--	Mouse brain	Brazil	1962	GU816024
Porton's virus (PORV)	TS	1643 (0416MAL)	<i>Mansonia uniformis</i>	--	--	Mouse brain	Malaysia (Sarawak)	?	GU816013

Ephemerovirus

Kotonkan virus ^d	UA	Ib Ar23380	<i>Culicoides</i> species	--	--	Mouse brain	Nigeria	1967	AY854638
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(KOTV)		(9145NIG)								
Kimberley virus (KIMV)	TS	CS 368	<i>Bos taurus</i>	--	--	Mouse brain	Australia	1980	AY854637	
Other animal rhabdoviruses										
Hart Park group										
Kamese virus (KAMV)	UA	MP 6186 (08343RC A)	<i>Aedes africanus</i> , <i>Culex</i> species	--	--	Mouse brain	Central African Republic	1967	GU816011	
Mossuril virus (MOSV)	UA	SA Ar 1995 (0418SA)	<i>Aedes abnormalis</i> , <i>Culex</i> species / <i>Andropadus virens</i> , <i>Coliuspasser macrourus</i>	--	--	Mouse brain	Central African Republic, Gambia	1959	GU816012	
Kolongo and Sandjimba group										
Sandjimba virus (SJAV)	UA	DakAnB 373d (07244RC A)	<i>Acrocephalus schoenobaenus</i>	--	--	Mouse brain	Central African Republic	1970	GU816019	
Le Dantec										

**and Kern
Canyon
group**

Keuraliba virus (KEUV)	UA	DakAnD 5314 (9715SEN , 0420SEN)	<i>Tatera kempi</i> , <i>Taterillus</i> species	--	--	Mouse brain	Senegal	1968	GU816021
Nkolbisson virus (NKOV)	UA	Ar Y 31/65 (0425CAM)	<i>Aedes</i> species, <i>Eretmapodites</i> species, <i>Culex telesilla</i>	--	--	Mouse brain	Ivory Coast, Cameroon	1965	GU816022

Ungrouped

Garba virus [§] (GARV)	UA	DakAnB 439a (0422RCA)	<i>Corythornis cristata</i> , <i>Nectarina pulchellabelan geri</i>	--	--	Mouse brain	Central African Republic	1970	GU816018
Nasoule virus [§] (NASV)	UA	DakAnB 4289a (0410RCA)	<i>Andropadus virens</i>	--	--	Mouse brain	Central African Republic	1973	GU816017
Ouango virus [§] (OUAV)	UA	DakAnB 1582a (9718RCA)	<i>Ploceus melanocephalus</i>	--	--	Mouse brain	Central African Republic	1970	GU816015
Bimbo virus [§] (BBOV)	UA	DakAnB 1054d (9716RCA)	<i>Euplectes afra</i>	--	--	Mouse brain	Central African Republic	1970	GU816016
Bangoran	UA	DakArB	<i>Turdus</i>	--	--	Mouse	Central	1969	GU816010

virus (BGNV)		2053 (0424RCA)	<i>libonyanus / Culex perfuscus</i>			brain	African Republic		
Gossas virus ^h (GOSV)	UA	DakAnD 401 (08344SE N)	<i>Tadarida species</i>	--	--	Mouse brain	Senegal	1964	NA ⁱ

^a Unless stated, the classification and names of viruses correspond to approved virus taxonomy according to the International Committee on Taxonomy of Viruses database (ICTVdb). Names in *italic* correspond to validated virus species.

^b UA : unassigned, TS : tentative species, UC : unclassified (not found in the ICTVdb).

^c Position according to the reference Pasteur virus genome (NC_001542), after alignment of all the tiled sequences with the reference sequence (except for tiled sequences from ungrouped rhabdovirus TUPV and SIGMAV which were aligned independently with the reference sequence).

^d Taxonomical classification according to (6).

^e A 977 nucleotides fragment of the polymerase gene (from 7020 to 7997 according to the reference Pasteur virus genome (NC_001542)) was synthesized in vitro then cloned into pCR2.1 plasmid (Operon).

^f A clone of baby hamster kidney cell line (BHK-21).

^g Not detected using PathogenID v2.0 microarray but amplified by PCR or nested PCR using consensus or specific primers.

^h Not detected using PathogenID v2.0 microarray neither amplified by PCR or nested PCR using consensus or specific primers.

ⁱ NA : Not applicable.

Table 3: Level of taxonomic identification of virus species among the genus *Lyssavirus* based on lyssavirus sequences tiled on the PathogenID_v2.0 microarray.

Strains of lyssavirus tested			Results from lyssavirus sequences tiled						
Genotype (abbreviation)	Isolate		1 (RABV)	2 (LBV)	3 (MOKV)	4 (DUVV)	5 (EBLV-1)	6 (EBLV-2)	7 (ABLV)
			PV	8619NGA	MOKV	94286SA	8918FRA	9018HOL	ABLV
1 (RABV)	93127FRA	Base call rate ^a	95.0	3.8	4.6	6.2	8.0	9.0	6.7
		Identification ^b	A	C	B	A	A	A	B
		Divergence ^c	0.2	25.6	24.8	22.8	23.2	21.0	22.0
	8764THA	Base call rate	32.6	5.4	5.7	7.0	6.0	3.3	9.0
		Identification	A	A	B	C	A	A	B
		Divergence	13.7	24.3	24.9	22.7	22.9	21.2	20.7
2 (LBV)	8619NIG	Base call rate	2.7	96.6	11.1	6.9	7.1	5.3	6.7
		Identification	Neg	A	A	A	Neg	Neg	B
		Divergence	25.8	0.0	22.3	22.8	25.2	23.8	23.3
3 (MOKV)	86100CAM	Base call rate	2.7	7.2	56.3	8.1	7.0	7.7	3.6
		Identification	A	A	A	A	A	A	B
		Divergence	24.9	22.5	10.2	22.0	23.6	22.2	24.5
4 (DUVV)	86132SA	Base call rate	3.9	1.1	1.4	97.3	0.6	2.5	5.6
		Identification	A	Neg	Neg	A	A	A	Neg
		Divergence	23.2	22.8	22.2	6.0	20.6	21.6	21.9
5 (EBLV-1)	8918FRA	Base call rate	8.1	6.9	15.2	13.3	93.8	7.8	4.8
		Identification	B	A	A	A	A	A	Neg

		Divergence	23.8	25.5	23.8	20.7	0.6 ^d	23.4	22.1
6 (EBLV-2)	9018HOL	Base call rate	5.7	2.1	3.5	6.5	4.1	98.4	8.7
		Identification	Neg	Neg	B	A	B	A	A
		Divergence	21.2	23.8	23.5	21.7	23.3	0.0	22.3
7 (ABLV)	9810AUS	Base call rate	8.4	8.3	1.4	12.9	3.8	11.3	94.9
		Identification	B	A	Neg	A	B	B	A
		Divergence	22.5	23.7	24.4	21.6	22.1	22.4	1.6
8 ^e (DBLV)	0406SEN	Base call rate	19.3	63.5	29.4	16.3	22.8	18.3	19.5
		Identification	A	A	A	A	A	A	A
		Divergence	25.0	20.1	21.5	23.4	23.5	22.8	23.8
Non classified	WCBV	Base call rate	25.3	28.3	32.7	26.9	26.5	23.8	24.5
		Identification	C	A	A	A	A	A	A
		Divergence	25.7	23.8	24.2	24.9	24.6	24.8	25.9

^a Percentage of the base calls generated from full-length tiled sequences.

^b Taxonomic identification according to:

A = identification at the species or isolate level when a unique best hit corresponds to expected species or isolate

B = identification at the genus level when multiple best viral hits exist and correspond to the genus *Lyssavirus*

C = identification at the family level when multiple best viral hits exist and correspond to genera of the family *Rhabdoviridae*

Neg = negative or inaccurate identification when BLAST query is not possible or when multiple best hits and some or all of them correspond to other viral families, respectively

^c Percentage of nucleotide divergence (based on a 937 nucleotide region of the polymerase gene, position 7040-7977 according to the reference Pasteur virus genome (NC_001542).

^d The tiled sequence of 8918FRA correspond to a preliminary result of sequencing and the complete genome of this virus strain was obtained latter (EU293112), which may explain the nucleotide differences (n=7) between those two sequences.

^e Tentative genotype.

In grey: results obtained using the sequence belonging to the same species tiled on the array (homonymous sequence).

Table 4: Level of taxonomic identification of virus species among the genera *Vesiculovirus* and *Ephemerovirus* based on vesiculovirus and ephemerovirus sequences tiled on the PathogenID_v2.0 microarray.

Strains of rhabdovirus tested			Results from specific rhabdovirus sequences tiled										
Genus	Isolate (reference)		<i>Vesiculovirus</i>						<i>Ephemerovirus</i>				<i>Lyssavirus</i>
			CHPV	ISFV	PERV	SVCV	VSIV	VSJNV	ARV	BEFV	KIMV	KOTV	RABV (PV)
<i>Vesiculovirus</i>	VSIV (0503FRA)	Base call rate ^a	1.2	4.1	1.0	1.0	98.6	2.9	0	0	0	0	0
		Score ^b	Neg	Neg	Neg	Neg	A	A	Neg	Neg	Neg	Neg	Neg
<i>Ephemerovirus</i>	KIMV ^c (CS 368)	Base call rate	1.9	1.1	0	0	0.3	0.3	9.4	7.3	70.6	9.1	1.4
		Score	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	A	Neg	Neg
	KOTV ^d (Ib Ar23380, 9145NIG)	Base call rate	6.6	3.8	5.7	3.2	3.7	7.2	8.8	5.2	3.4	100	2.1
		Score	Neg	Neg	Neg	Neg	Neg	C	Neg	Neg	Neg	A	Neg
<i>Lyssavirus</i>	RABV (93127FRA)	Base call rate	0.3	1.2	2.6	1.5	0	0	0.1	0	0.1	2.3	95.0
		Score	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	A

^a Percentage of the base calls generated from full-length tiled sequences.

^b Taxonomic identification according to:

A = identification at the species or isolate level when an unique best hit corresponds to expected species or isolate

C = identification at the family level when multiple best viral hits exist and correspond to genera of the family *Rhabdoviridae*

Neg = negative or inaccurate identification when BLAST query is not possible or when multiple best hits exist and some or all of them correspond to other viral families.

In grey: results obtained using the sequence belonging to the same species or isolate tiled on the array (homonymous sequence).

^c TS : tentative species, according to the International Committee on Taxonomy of Viruses database (ICTVdb).

^d Taxonomical classification according to (6).

Table 5: Identification of virus species among the genus *Lyssavirus* based on lyssavirus sequences tiled on the PathogenID_v2.0 microarray and using the consensus sequence determination strategy.

Strains of lyssavirus tested			Strategy of analysis		
Genotype (Abbreviation)	Strain tested		Use of the prototype sequence	Use of the consensus sequence (based all tiled sequences)	Used of consensus sequence (excluding the prototype sequence)
1 (RABV)	93127FRA	Base call rate ^a	95.0	96.3	32.7
		BLAST score ^b	791	801	38
		Accuracy ^c	100	99.9	95.9
	8764THA	Base call rate	32.6	47.4	26.7
		BLAST score	46	64	31
		Accuracy	94.8	99.1	98.4
2 (LBV)	8619NIG	Base call rate	96.6	96.4	28.1
		BLAST score	816	814	39
		Accuracy	99.9	99.9	97.7
3 (MOKV)	86100CAM	Base call rate	56.3	67.4	28.4
		BLAST score	66	112	64
		Accuracy	98.2	99.8	98.5
4 (DUVV)	86132SA	Base call rate	97.3	97.3	18.1
		BLAST score	843	833	20
		Accuracy	99.9	99.8	96.4
5 (EBLV-1)	8918FRA	Base call rate	93.8	96.0	41.1
		BLAST score	757	807	83
		Accuracy	100	100	97.9
6 (EBLV-2)	9018HOL	Base call rate	98.4	98.8	26.8

		BLAST score	871	879	44
		Accuracy	100	99.9	99.6
7 (ABLV)	ABLV	Base call rate	94.9	95.6	29.7
		BLAST score	749	741	40
		Accuracy	100	99.9	94.5
8 ^e (DBLV)	0406SEN	Base call rate	NA	75.9	NA
		BLAST score	NA	82	NA
		Accuracy	NA	91.8	NA
?	WCBV	Base call rate	NA	60.9	NA
		BLAST score	NA	56	NA
		Accuracy	NA	97.3	NA

^a Percentage of the base calls generated from full-length tiled sequences.

^b BLAST score (bit score), obtained after BLAST query on a local viral and bacterial database using the consensus sequence determination strategy with $m = 12$ (minimum nucleotide length), $N = 10$ (maximum undetermined nucleotides content). Default BLAST parameters, except for the minimum length word length (7 nucleotides), the expect threshold (increased from the default of 10 to 100,000), and the 'low complexity level filter' (-F, turned off). All the BLAST scores indicate a correct identification at the species or isolate level (i.e., unique best hit corresponds to the expected species or isolate).

^c Percentage of correct nucleotide identification, compared to the sequence obtained after classical sequencing of the corresponding lyssavirus species tested.

^d NA: Not applicable.

^e Tentative species.

Table 6 : Description and final classification of rhabdovirus species used for phylogenetic analysis.

Genus and name ^a	UA/TS/UC ^b	Abbreviation	Reference no. (strain)	Principal host species/vector ^c	Origin of samples	Year of first isolation	GenBank accession no.
<i>Lyssavirus (Genotype)</i>							
<i>Rabies virus</i> (Gt 1)		RABV	9001FRA	Dog bitten by a bat	French Guyana	1990	EU293113
<i>Rabies virus</i> (Gt 1)		RABV	9147FRA	Fox	France	1991	EU293115
<i>Rabies virus</i> (Gt 1)		RABV	8743THA	Human	Thailand	1983	EU293121
<i>Rabies virus</i> (Gt 1)		RABV	9704ARG	Bat : <i>Tadarida brasiliensis</i>	Argentina	1997	EU293116
<i>Rabies virus</i> (Gt 1)		RABV	9706CHI	Vaccine AG	China		AY854663
<i>Rabies virus</i> (Gt 1)		RABV	9702IND	Human	India	1997	AY854665
<i>Lagos bat virus</i> (Gt 2)		LBV	8619NGA	Bat : <i>Eidolon helvum</i>	Nigeria	1956	EU293110
<i>Mokola virus</i> (Gt 3)		MOKV	MOKV	Cat	Zimbabwe	1981	NC_006429
<i>Mokola virus</i> (Gt 3)		MOKV	86100CAM	Shrew	Cameroon	1974	EU293117
<i>Mokola virus</i> (Gt 3)		MOKV	86101RCA	Rodent	Republic of Central Africa	1981	EU293118
<i>Duvenhage virus</i> (Gt 4)		DUVV	94286SA	Bat : <i>Miniopterus</i> species	South Africa	1981	EU293120
<i>Duvenhage virus</i> (Gt 4)		DUVV	86132SA	Human	South Africa	1971	EU293119
<i>European bat lyssavirus 1</i> (Gt 5)		EBLV-1	8918FRA	Bat : <i>Eptesicus serotinus</i>	France	1989	EU293112
<i>European bat lyssavirus 1</i> (Gt 5)		EBLV-1	08120FRA	Bat : <i>Eptesicus serotinus</i>	France	2008	EU626551
<i>European bat lyssavirus 2</i> (Gt 6)		EBLV-2	9018HOL	Bat : <i>Myotis dasycneme</i>	The Netherlands	1986	EU293114
<i>European bat lyssavirus 2</i> (Gt 6)		EBLV-2	9337SWI	Bat : <i>Myotis</i>	Switzerland	1993	AY854657

<i>Australian bat lyssavirus</i> (Gt 7)		ABLV	ABLh	<i>daubentonii</i> Human	Australia	1986	AF418014
<i>Australian bat lyssavirus</i> (Gt 7)		ABLV	ABLb	Bat : <i>Pteropus</i> species	Australia	1996	NC_003243
Dakar bat lyssavirus (proposed Gt 8)	UC	DBLV	0406SEN (AnD 42443)	Bat : <i>Eidolon helvum</i>	Senegal	1985	EU293108
Dakar bat lyssavirus (proposed Gt 8)	UC	DBLV	KE131	Bat : <i>Eidolon helvum</i>	Kenya	2007	EU259198
Irkut virus (proposed Gt 9)	UC	IRKV		Bat : <i>Murina leucogaster</i>	Russia	2002	EF614260
Ozernoe virus (proposed Gt 9)	UC	IRKV		Human	Russia	2007	FJ905105
Aravan virus	UC	ARAV		Bat : <i>Myotis blythi</i>	Kyrgyzstan	1991	EF614259
Khujand virus	UC	KHUV		Bat : <i>Myotis mystacinus</i>	Tajikistan	2001	EF614261
West Caucasian bat virus	UC	WCBV		Bat : <i>Miniopterus schreibersii</i>	Russia	2002	EF614258

Vesiculovirus

<i>Chandipura virus</i>		CHPV	I 653514	Human ; Domestic animals ^d ; Hedgehog : <i>Atelerix</i> species ; Dipteran : <i>Phlebotomus</i> species	India	1965	AJ810083
<i>Cocal virus</i>		COCV	TRVL 40233	Livestock : equine, bovine ; Mites : <i>Gigantolaelaps</i> species	Trinidad and Tobago; Trinidad	1961	EU373657
<i>Isfahan virus</i>		ISFV	91026-167	Dipteran : <i>Phlebotomus papatasi</i>	Iran	1975	AJ810084
<i>Piry virus</i>		PIRYV	BeAn 24232 (0413BRE)	Human ; Opossum : <i>Philander opossum</i>	Brazil	1960	GU816023

<i>Vesicular stomatitis New Jersey virus</i>	VSNJV	VSV NJ-O	Livestock : Several species including <i>Bos taurus</i> , equine ; Dipterans : Several species including <i>Culex nigripalpus</i> , <i>Culicoides</i> species, <i>Mansonia indubitans</i>	Utah, USA	1949	AY074804
<i>Vesicular stomatitis New Jersey virus</i>	VSNJV	VSV NJ-H	Livestock : Several species including <i>Sus scrofa</i> ; Dipterans : Several species including <i>Culex nigripalpus</i> , <i>Culicoides</i> species, <i>Mansonia indubitans</i>	Georgia, USA	1952	AY074803
<i>Vesicular stomatitis Indiana virus</i>	VSIV	Mudd-Summers (MS)	Bovine : <i>Bos taurus</i>	Indiana, USA	1925	EU849003
<i>Vesicular stomatitis Indiana virus</i>	VSIV	85CLB	Bovine	Colombia	1985	AF473865
<i>Vesicular stomatitis Indiana virus</i>	VSIV	98COE	Equine	Colorado, USA	1998	AF473864
<i>Vesicular stomatitis Alagoas virus</i>	VSAV	Indiana 3	Livestock : equine (mule), <i>Bos taurus</i> ; Dipterans : <i>Phlebotomus</i> species	Brazil	1964	EU373658
Jurona virus ^e	TS	JURV	Dipteran : <i>Haemagogus spegazzinii</i>	Brazil	1962	GU816024

Perinet virus	TS	PERV	Ar Mg 802	Dipterans : <i>Anopheles coustani</i> , <i>Culex antennatus</i> , <i>Culex gr. pipiens</i> , <i>Mansonia uniformis</i> , <i>Phlebotomus berentensis</i>	Madagascar	1978	AY854652
Pike fry rhabdovirus	TS	PFRV	F4	Fish : <i>Esox lucius</i>	The Netherlands	1972	FJ872827
<i>Scophthalmus maximus</i> rhabdovirus	UC	SMRV	QZ-2005	Fish : <i>Scophthalmus maximus</i>	China	?	AY895167
Spring viremia of carp virus	TS	SVCV	Fijan_cell (VR-1390, isolated from fat head minnow cells)	Fish : <i>Cyprinus carpio</i>	Yougoslavia	1971	AJ318079
Spring viremia of carp virus	TS	SVCV	Fijan_tissue (VR-1390, isolated from tissues of diseased common carp)	Fish : <i>Cyprinus carpio</i>	Yougoslavia	1971	U18101
Spring viremia of carp virus	TS	SVCV	BJ0505-2	Fish : <i>Cyprinus carpio</i>	China	2005	EU177782
<i>Ephemerovirus</i>							
<i>Adelaide River virus</i>		ARV	DPP 61	Bovine : <i>Bos taurus</i>	Australia	1981	AY854635
<i>Berrimah virus</i>		BRMV	DPP 63	Bovine : <i>Bos taurus</i>	Australia	1981	AY854636
<i>Bovine ephemeral fever virus</i>		BEFV	Cs 1933	Bovine : <i>Bos taurus</i>	Australia	1973	AY854641
<i>Bovine ephemeral fever virus</i>		BEFV	Cs 42	Dipteran : <i>Anopheles bancrofti</i>	Australia	1975	AY854639
<i>Bovine ephemeral fever virus</i>		BEFV	BB7721	Bovine : <i>Bos taurus</i>	Australia	1968	NC_002526
Kimberley virus	TS	KIMV	CS 368	Bovine : <i>Bos taurus</i>	Australia	1980	AY854637
Kotonkan virus	UA	KOTV	IbAr 23380	Dipteran : <i>Culicoides</i>	Nigeria	1967	AY854638

				species			
Almpiwar group							
Almpiwar virus	UA	ALMV	MRM 4059	Mammals ^d : bovine, equine, ovine, kangaroo, bandicoot, human ^d ; Birds ^d ; Lizard: <i>Ablepharus boutonii virgatus</i> and other shink ^d	Australia	1966	AY854645
Charleville virus	UA	CHVV	Ch 9824	Human ^d ; Dipteran: <i>Phlebotomus</i> and <i>Lasiohelea</i> species	Australia	1969	AY854644
Charleville virus	UA	CHVV	Ch 9847	Human ^d ; Dipteran: <i>Phlebotomus</i> and <i>Lasiohelea</i> species	Australia	1969	AY854672
Humpty Doo virus	UA	HDOOV	CS 79	Dipteran: <i>Lasiohelea</i> species, <i>Culicoides marki</i>	Australia	1975	AY854643
Hart Park group							
Bangoran virus ^e	UA	BGNV	DakArB 2053 (0424RCA)	Bird: <i>Turdus libonyanus</i> ; Dipteran: <i>Culex perfuscus</i>	Central African Republic	1969	GU816010
Flanders virus	UA	FLANV	61-7484	Birds: <i>Seiurus aurocapillus</i> , <i>Agelaius phoeniceus</i> ; Dipterans: <i>Culiseta</i>	New York, USA	1961	AF523199

Kamese virus ^e	UA	KAMV	MP 6186 (08343RCA)	<i>melanura</i> , <i>Culex</i> species Dipterans : <i>Aedes</i> <i>africanus</i> , <i>Culex</i> species including <i>Culex</i> <i>annulioris</i>	Uganda, Republic of Central Africa Mozambique, Republic of Central Africa, South Africa, Bostwana, Guinea	1967	GU816011
Mossuril virus ^e	UA	MOSV	SA Ar 1995 (0418SA)	Birds : <i>Andropadus</i> <i>virens</i> , <i>Coliuspasser</i> <i>macrourus</i> ; Dipterans : <i>Aedes</i> <i>abnormalis</i> , <i>Culex</i> species including <i>Culex</i> <i>sitiens</i>	Africa, South Africa, Bostwana, Guinea	1959	GU816012
Ngaingan virus	UA	NGAV	MRM 14556	Mammals ^d : wallabies, kangaroo, bovine ; Dipteran : <i>Culicoides</i> <i>brevitarsis</i>	Australia	1970	AY854649
Parry Creek virus	UA	PCRV	OR 189	Dipteran : <i>Culex</i> <i>annulirostris</i>	Australia	1972	AY854647
Porton's virus ^e	TS (VSV)	PORV	1643 (0416MAL)	Dipteran : <i>Mansonia</i> <i>uniformis</i>	Malaysia (Sarawak)	?	GU816013
Wongabel virus	UA	WONV	CS 264	Sea birds ^d ; Dipteran : <i>Culicoides</i> <i>austropalpalis</i>	Australia	1979	AY854648
Le Dantec group							
Fukuoka virus	UA (Kern Canyon Group)	FUKV	FUK-11	Bovine ; Dipteran : <i>Culicoides</i> <i>punctatus</i> , <i>Culex</i> <i>tritaeniorhynchus</i>	Japan	1982	AY854651
Keuraliba virus ^e	UA	KEUV	DakAnD 5314	Rodents : <i>Tatera</i>	Senegal	1968	GU816021

Le Dantec virus	UA	LDV	(9715SEN, 0420SEN) DakHD 763	species including <i>Tatera kempi</i> , <i>Taterillus</i> species Human	Senegal	1965	AY854650
Nkolbisson virus ^e	UA (Kern Canyon Group)	NKOV	Ar YM 31/65 (0425CAM)	Dipteran : <i>Aedes</i> species including <i>Eretmapodites leucopus</i> , <i>Culex telesilla</i>	Ivory Coast, Cameroon	1965	GU816022
Moussa group							
Moussa virus	UC	MOUSV	C23	Dipteran : <i>Culex decens</i>	Ivory Coast	2004	FJ985748
Moussa virus	UC	MOUSV	D24	Dipteran : <i>Culex</i> species	Ivory Coast	2004	FJ985749
Sandjimba group							
Bimbo virus ^e	UA	BBOV	DakAnB 1054d (9716RCA)	Bird : <i>Euplectes afra</i>	Central African Republic	1970	GU816016
Boteke virus ^e	TS (VSV)	BTKV	DakArB 1077 (0417RCA)	Dipteran : <i>Coquillettidia maculipennis</i>	Central African Republic	1968	GU816014
Garba virus ^e	UA	GARV	DakAnB 439a (0422RCA)	Birds : <i>Corythornis cristata</i> , <i>Nectarina pulchella</i>	Central African Republic	1970	GU816018
Kolongo virus	UA	KOLV	DakAnB 1094d (9717RCA)	Birds : <i>Euplectes afra</i> , <i>Ploceus cucullatus</i>	Central African Republic	1970	GU816020
Nasoule virus ^e	UA	NASV	DakAnB 4289a (0410RCA)	Bird : <i>Andropadus virens</i>	Central African	1973	GU816017

Oak-Vale virus	UA	OVRV	CS 1342	Ferral pigs ^d ; Dipteran : <i>Aedes vigilax</i> , <i>Culex</i> species including <i>Culex edwardsi</i>)	Australia	1981	AY854670
Ouango virus ^e	UA	OUAV	DakAnB 1582a (9718RCA)	Bird : <i>Ploceus melanocephalus</i>	Central African Republic	1970	GU816015
Sandjimba virus	UA	SJAV	DakAnB 373d (07244RCA)	Bird : <i>Acrocephalus schoenobaenus</i>	Central African Republic	1970	GU816019
Sigma group							
<i>Drosophila affinis</i> sigma virus	UC	DAffSV	10	Dipteran : <i>Drosophila affinis</i>	New Connecticut, USA	2007	GQ410980
<i>Drosophila melanogaster</i> sigma virus	UA	SIGMA V (DMelS V)	AP30	Dipteran : <i>Drosophila melanogaster</i>	Florida, USA	2005	NC_013135
<i>Drosophila melanogaster</i> sigma virus	UA	SIGMA V (DMelS V)	HAP23	Dipteran : <i>Drosophila melanogaster</i>	France	?	GQ375258
<i>Drosophila obscura</i> sigma virus	UC	DObsSV	10A	Dipteran : <i>Drosophila obscura</i>	UK	2007	GQ410979
Sinistar group							
<i>Siniperca chuatsi</i> rhabdovirus	UC	SCRV		Fish : <i>Siniperca chuatsi</i>	China	?	NC_008514
Starry flounder rhabdovirus	UC	SFRV		Fish : <i>Platichthys</i>	Washington,	2000	AY450644

				<i>stellatus</i>	USA		
Tibrogargan group							
Tibrogargan virus	UA	TIBV	CS 132	Bovine ^d : water buffaloes, cattle ; Dipteran : <i>Culicoides brevitarsis</i>	Australia, New Guinea	1976	AY854646
Tupaia virus	TS (VSV)	TUPV	TRV 1591	Tree shrew : <i>Tupaia belangeri</i>	Thailand	?	NC_007020
Novirhabdovirus							
<i>Hirame rhabdovirus</i>		HIRRV	CA 9703	Fish : including Cultured korean flounders , <i>Paralichthys olivaceus</i> , <i>Plecoglossus altivelis</i> , <i>Milio macrocephalus</i> , <i>Sebastes inermis</i>	Korea	1997	NC_005093
<i>Infectious hematopoietic necrosis virus</i>		IHNV	HV7601				AB231660
<i>Infectious hematopoietic necrosis virus</i>		IHNV	WRAC strain	Fish : including salmonid, <i>Oncorhynchus tshawytscha</i>	Idaho, USA		NC_001652
<i>Snakehead rhabdovirus</i>		SHRV		Fish : including <i>Ophicephalus striatus</i> , <i>Clarias bratachus</i> , <i>Oxyeleotus marmoratus</i>	Thailand		NC_000903
<i>Viral hemorrhagic septicemia virus</i>		VHSV	KRRV9822	Fish : Japanese flounder	Japan		AB179621
<i>Viral hemorrhagic septicemia virus</i>		VHSV	07-71	Fish : <i>Oncorhynchus mykiss</i>	France		AJ233396

<i>Viral hemorrhagic septicemia virus</i>		VHSV	JF00Eh1	Fish : <i>Paralichthys olivaceus</i>	Japan	2000	AB490792
<i>Viral hemorrhagic septicemia virus</i>		VHSV	14-58	Fish : <i>Oncorhynchus mykiss</i>	France		AF143863
<i>Nucleorhabdovirus</i>							
<i>Maize mosaic virus</i>		MMV		Plant (host) : <i>Graminae</i> including <i>Zea mays</i> ; Hemipterans (vector) : <i>Delphacidae</i>	USA		NC_005975
<i>Rice yellow stunt virus</i>		RYSV		Plant (host) : <i>Oryza sativa</i> ; Homoptera (vector) : <i>Cicadellidae</i>			NC_003746
<i>Sonchus yellow net virus</i>		SYNV		Plant (host) : <i>Asteraceae</i> including <i>Sonchus oleraceus</i> ; Hemipterans (vector) : <i>Aphididae</i>			NC_001615
Iranian maize mosaic nucleorhabdovirus	UC	IMMNV		Plant (host) : <i>Graminae</i> including <i>Zea mays</i> ; Hemipterans (vector) : <i>Delphacidae</i>	Iran		NC_011542
Maize fine streak virus	UC	MFSV		Plant (host) : <i>Graminae</i> including <i>Zea mays</i> ; Homoptera (vector) : <i>Cicadellidae</i>	Georgia, USA	1999	NC_005974
Orchid fleck virus ^f	UC	OFV	So	Plant (host) : <i>Orchidaceae</i> including <i>Cymbidium species</i> ; Acaris (vector) : <i>Brevipalpus</i>	Japan		NC_009609

Taro vein chlorosis virus	UC	TaVCV		<i>californicus</i> Plant (host) : <i>Colocasia esculenta</i>	Fiji Islands	NC_006942
Cytorhabdovirus						
<i>Barley yellow striate mosaic</i>		BYSMV	Zanjan-1	Plant (host) : <i>Graminae</i> including <i>Triticum</i> species ; Hemipterans (vector) : <i>Delphacidae</i>	Iran	FJ665628
<i>Lettuce necrotic yellows virus</i>		LNYSV	318	Plant (host) : several plant families and species including <i>Allium sativum</i> , Lactuca sativa ; Hemipterans (vector) : <i>Aphididae</i>	Australia	NC_007642
<i>Northern cereal mosaic virus</i>		NCMV		Plant (host) : <i>Graminae</i> including <i>Hordeum</i> vulgare ; Hemipterans (vector) : <i>Delphacidae</i>	Japan	NC_002251
<i>Strawberry crinkle virus</i>		SCV	HB-A1	Plant (host) : Fragaria species ; Hemipterans (vector) : <i>Aphididae</i>		AY331389
<i>Strawberry crinkle virus</i>		SCV	37-2	Plant (host) : Fragaria species ; Hemipterans (vector) : <i>Aphididae</i>		AY331388
<i>Strawberry crinkle virus</i>		SCV	37-1	Plant (host) : Fragaria species ;		AY331387

Lettuce yellow mottle virus	UC	LYMoV	Hemipterans (vector) : <i>Aphididae</i> Plant (host) : <i>Lactuca sativa</i>	France	1998	NC_011532
<i>Taastrup group</i>						
Taastrup virus	UC	TV	Hemipteran (potential vector) : <i>Psammotettix alienus</i>	France	1996	AY423355

^a Names of viruses in italic correspond to approved virus species by the eighth report of the International Committee on Taxonomy of Viruses database (ICTVdb).

^b UA : unassigned, TS : tentative and UC : unclassified (not found in the ICTV) species.

^c In bold : identification of the host species where the first viral isolation was obtained when data are available.

^d Serological detection only.

^e First identification based on nucleic acid determination and classification based on phylogenic analysis (this study).

^f Also tentatively classified into the new genus *Dichorhabdovirus* according to its unusual bipartite genome (20).