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Bat Rabies Surveillance in Europe

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Impacts

- Rabies in European bats is caused by at least four different lyssavirus species that seem to have an association with certain bat species, that is, the Serotine bat (EBLV-1), the Daubenton's and Pond bat (EBLV-2), Schreiber's long-fingered bat (WCBV) and the Natterer's bat (BBLV).
- Almost all bat rabies cases were detected in dead or clinically affected bats i.e. passive surveillance. This is a much more sensitive approach than active surveillance where oral swab samples taken from individuals randomly captured during planned surveys were screened for viral RNA and/or virus.
- The focus of bat rabies surveillance should be on testing of dead or moribund animals, and resulting positive FAT results should be further analysed to identify the lyssavirus species and all bats submitted for testing should be identified to species level.

Keywords:
Rabies; bats; lyssavirus; surveillance

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Summary

Rabies is the oldest known zoonotic disease and was also the first recognized bat associated infection in humans. To date, four different lyssavirus species are the causative agents of rabies in European bats: the European Bat Lyssaviruses type 1 and 2 (EBLV-1, EBLV-2), the recently discovered putative new lyssavirus species Bokeloh Bat Lyssavirus (BBLV) and the West Caucasian Bat Virus (WCBV). Unlike in the new world, bat rabies cases in Europe are comparatively less frequent, possibly as a result of varying intensity of surveillance. Thus, the objective was to provide an assessment of the bat rabies surveillance data in Europe, taking both reported data to the WHO Rabies Bulletin Europe and published results into account. In Europe, 959 bat rabies cases were reported to the RBE in the time period 1977–2010 with the vast majority characterized as EBLV-1, frequently isolated in the Netherlands, North Germany, Denmark, Poland and also in parts of France and Spain. Most EBLV-2 isolates originated from the United Kingdom (UK) and the Netherlands, and EBLV-2 was also detected in Germany, Finland and Switzerland. Thus far, only one isolate of BBLV was found in Germany. Published passive bat rabies surveillance comprised testing of 28 of the 52 different European bat species for rabies. EBLV-1 was isolated exclusively from Serotine bats (*Eptesicus serotinus* and *Eptesicus isabellinus*), while EBLV-2 was detected in 14 Daubenton's bats (*Myotis daubentonii*) and 5 Pond bats (*Myotis dasycneme*). A virus from a single Natterer's bat (*Myotis nattereri*) was characterized as BBLV. During active surveillance, only oral swabs from 2 Daubenton's bats (EBLV-2) and from several Eptesicus bats (EBLV-1) yielded virus positive RNA. Virus neutralizing antibodies against lyssaviruses were detected in various European bat species from different countries, and its value and implications are discussed.

Introduction

Bats have been shown to be reservoir of a plethora of viruses (Calisher et al., 2006) and are also associated with zoonotic viral diseases such as SARS, Nipah, Hendra and rabies. Rabies is a viral zoonotic disease inevitably fatal in humans and other mammals once clinical signs develop. It is caused by different lyssavirus species of the *Rhabdoviridae* family, order Mononegavirales (Table 1). Most known lyssavirus species have been isolated from bats except for Mokola virus and Ikoma lyssavirus (IKOV) for which the reservoirs are not yet known (World Health Organisation, 2005; Marston et al., 2012).

Based on their antigenic, genetic and immunological features lyssaviruses can be divided into two phylogroups. Phylogroup I comprises the majority of lyssavirus species, whereas Lagos Bat Virus (LBV), Mokola Virus (MOKV) and Shimoni Bat Virus (SHIBV) form phylogroup II (Badrane et al., 2001; Kuzmin et al., 2010). West Caucasian Bat Virus (WCBV) and IKOV may be representatives of a possible new phylogroup (Kuzmin et al., 2005; Marston et al., 2012).

Interestingly, rabies is the oldest known zoonotic disease and was also the first recognized bat associated disease. In the beginning of the 20th century, Carini (1911) suggested a link between rabies and vampire bat bites in South and Central America. However, it took a further three decades before rabies was also detected in non-haematophagous bat species. In 1953, the first rabies case in insectivorous bats was reported from Florida, USA (Venters et al., 1954), and only 1 year later, the first record of a rabid bat in Europe was reported from Hamburg in Germany (Mohr, 1957).

Four different lyssavirus species have been isolated from European bats: the European bat lyssaviruses type 1 and 2 (EBLV-1, EBLV-2) (Bourhy et al., 1992), the recently discovered putative new lyssavirus species Bokeloh Bat Lyssavirus (BBLV) (Freuling et al., 2011) and the West Caucasian Bat Virus (WCBV) (Kuzmin et al., 2005). The majority of isolates in Europe were characterized as EBLV-1 mostly associated with *Eptesicus serotinus* (Müller et al., 2007) and *Eptesicus isabellinus* (Vazquez-Moron et al., 2008a). EBLV-2 has only been isolated sporadically. Except for five cases in the Netherlands where EBLV-2 was isolated from Pond bats, *Myotis dasycneme* (van der Poel et al., 2005), all other bat isolates were collected from Daubenton's bats, *Myotis daubentonii* (Freuling et al., 2012). Both WCBV and BBLV have only been isolated once. Whilst WCBV was detected in a Schreiber's bent-winged bat (*Miniopterus schreibersii*) on the European side of the Caucasus Mountains, BBLV was isolated from a Natterer's bat (*Myotis nattereri*) in Germany (Freuling et al., 2011).

The relevance of bat rabies for public health in Europe is illustrated by the fact that both EBLV-1 and EBLV-2 have caused human deaths, although the number is small (Johnson et al., 2010). Spill-over infections to other mammals have only been described for EBLV-1 in a stone marten (*Martes foina*), (Müller et al., 2004), sheep (Tjornehoj et al., 2006) and cats (Dacheux et al., 2009).

Recently, guidelines on passive and active bat rabies surveillance were established by a European research consortium Med-Vet-Net (Med-Vet-Net Working Group, 2005). Passive surveillance was defined as the testing of sick or dead bats of all indigenous bat species for lyssavirus infections using standard antigen detection, that is,

Table 1. Current diversity of lyssavirus species (World Health Organisation, 2005, Kuzmin et al. 2010, Freuling et al., 2011; Marston et al., 2012)

Lyssavirus	Geographical distribution	Potential vector(s)/ reservoirs	Phylogroup
Classical rabies virus (RABV)	Worldwide	Bats in America	I
Lagos bat virus (LBV)	Africa	Frugivorous bats (Megachiroptera)	II
Mokola virus (MOKV)	Africa	Unknown	II
Duvenhage virus (DUVV)	Southern Africa	Insectivorous bats	I
European bat lyssavirus type 1 (EBLV 1)	Europe	Insectivorous bats (*Eptesicus serotinus, E. isabellinus*)	I
European bat lyssavirus type 2 (EBLV 2)	Europe	Insectivorous bats (*Myotis daubentonii, Myotis dasycneme*)	I
Australian bat lyssavirus (ABLV)	Australia	Frugivorous bats/insectivorous bats (Megachiroptera/Microchiroptera)	I
Aravan virus (ARAV)	Central Asia	Insectivorous bats (*Myotis blythii*)	I
Irkut virus (IRKV)	East Siberia	Insectivorous bats (*Murina leucogaster*)	I
Khujand virus (KHUV)	Central Asia	Insectivorous bats (*Myotis mystacinus*)	I
West Caucasian bat virus (WCBV)	Caucasian region	Insectivorous bats (*Miniopterus schreibersii*)	III?
Bokeloh bat lyssavirus (BBLV)	Europe	Insectivorous bats (*Myotis nattereri*)	I
Shimoni bat virus (SHIBV)	East Africa	Insectivorous bats (*Hipposideros commersoni*)	II
Ikoma lyssavirus (IKOV)	Tanzania	?(Civettictis civetta)	III?

the fluorescent antibody test (FAT). It comprises both dead bats submitted primarily by bat biologist networks, and also bats submitted by the public due to exposure concerns. Active surveillance is the monitoring of bat populations for lyssavirus infections by screening oral swab (detection of lyssavirus-specific RNA using different RT-PCRs or virus isolation) and/or sera (detection of virus neutralizing antibodies using virus neutralization assays, for example, modified RFFIT) (Med-Vet-Net Working Group, 2005). This scheme was adopted by the UN EUROBATS Resolution 5.2, Bats and Rabies in Europe (Anon, 2006) and by a recent expert opinion on request of the European Food Safety Authority (Cliquet et al., 2010). However, it is not clear yet whether these guidelines had an impact on European bat rabies surveillance. The objective of this study was to assess the current state of bat rabies surveillance in Europe using all available data sources, that is, officially reported or published bat rabies surveillance data, including bat species identification.

Material and Methods

Data on bat rabies surveillance were retrieved from the WHO Rabies Bulletin Europe (RBE), established and maintained by the WHO Collaboration Centre for Rabies Surveillance and Research, at the Friedrich-Loeffler-Institute, Germany. This European database collects data on all rabies cases in Europe since 1977 (Meslin, 2007). The veterinary authorities of participating European countries are encouraged to regularly submit data on officially confirmed rabies cases per species. All data are summarized at a certain regional level depending on the administrative unit and per quarter of the year. Rabies positive cases in bats were analysed cumulative for the years 1977–2010, and additionally attributed to quarters. For rabies surveillance, the number of negative bats tested and reported to the RBE for the years 2006–2010 were analysed. In addition, a more specific questionnaire on bat rabies surveillance was developed. The contributors to the RBE were asked to provide data on the number of animals tested per bat species and the number of positive cases thereof, cumulative for the same time period. Furthermore, publications from 1995 to 2011 on bat rabies surveillance in Europe were also analysed for data on both passive and active bat lyssavirus surveillance.

Results

In Europe, 959 cases of bat rabies were reported in the time period 1977–2010 (Fig. 1, 2). The majority of them were characterized as EBLV-1, frequently isolated in the Netherlands, North Germany, Denmark, Poland and also in parts of France and Spain (Fig. 1a). Few cases were reported from Romania, Hungary, Slovenia and Russia. Most EBLV-2 isolates originated from the UK and the Netherlands, and EBLV-2 was also detected in Germany, Finland and Switzerland. Thus far, only one isolate of BBLV was found in Germany (Fig. 1b).

From 1977 to 1984, only two bat rabies cases were reported to the RBE. Since 1985, the number of reported rabies cases in bats increased with the highest numbers in 1986 (n = 122) and 1987 (n = 140), respectively. From 2006 to 2010, the number of positive bats ranged between 26 and 36 (Fig. 2a). Of all reported bat rabies cases in Europe (1977–2010) the majority (n = 418) were detected in the third quarter, while within the fourth quarter only 92 bats tested rabies positive. The number of bat rabies cases reported in first quarter amount to 270, while in second quarter 167 bat rabies cases were reported (Fig. 2

Fig. 1. Geographical origin of reported bat rabies cases in Europe (1977–2010): (a) all cases except confirmed cases of EBLV-2, (b) EBLV-2 cases (dots) and BBLV (star).

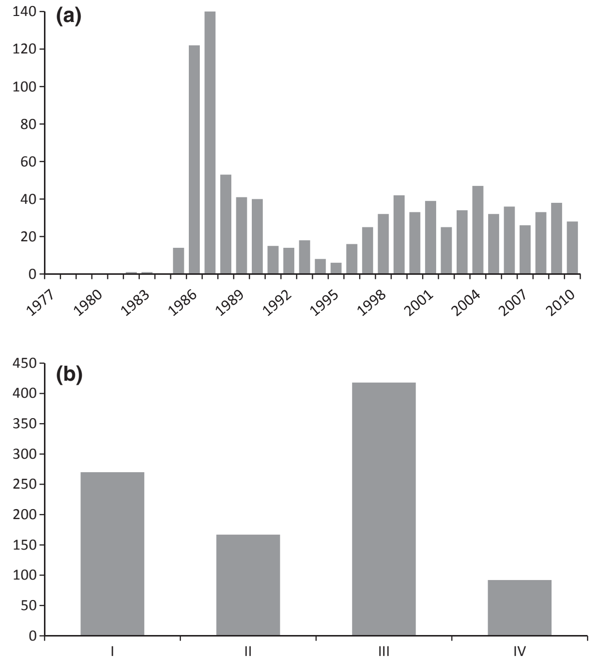

Fig. 2. Number of reported bat rabies case in Europe (1977–2010): (a) annually, (b) per quarter.

Table 2. Number of rabies negative and positive tested bats in several countries (2006–2010)

Country	Tested negative	Rabies cases
Albania	148	0
Austria	541	0
Belgium	157	0
Bulgaria	1	0
Croatia	81	0
Czech Republic	75	0
Denmark	90	13
Estonia	5	0
Finland	33	1
France	982	30
Germany	405	35
Greece	5	0
Hungary	41	2
Italy	26	0
Lithuania	6	0
Moldova	18	0
Poland	413	18
Romania	Unknown	1
Russian Federation	Unknown	2
Serbia	311	0
Slovak Republic	24	0
Slovenia	611	0
Spain	189	7
Switzerland + Liechtenstein	101	0
The Netherlands	619	45
Turkey	1	0
Ukraine	27	3
United Kingdom	5163	5
$\sum$	10073	162

b). In 12 cases, no information on the isolation date was submitted to the RBE.

Of 46 European countries, 29 submitted data on bat rabies surveillance to the RBE for the time period 2006–2010 (Table 2). Most countries reported several hundred bats being tested, while others only investigated single animals. In the UK, for instance, in this time, period a total of 5163 grounded bats were investigated. Bat rabies cases ($n = 162$) were reported from 12 European countries, with the majority from the Netherlands ($n = 45$), Germany ($n = 35$), France ($n = 30$), Poland ($n = 18$) and Denmark ($n = 13$). In Finland, Hungary, Romania, the Russian Federation and the Ukraine only few cases of positive bats were notified (Table 2).

The response to the questionnaire was limited, only 11 countries replied and 6 questionnaires concerning data on individual bat species were analysed (Table S1). While the proportion of unknown bat specimens that were not identified to the species level was fairly high in some countries, in Switzerland and Serbia, all tested bats were assigned to their species level. The most frequently tested bat species is the Common pipistrelle bat (*Pipistrellus pipistrellus*). From all assumed reservoir species, *Myotis daubentonii* ($n = 57$) and *Miniopterus schreibersii* ($n = 36$) were most frequently analysed in those countries; however, without a positive rabies result. Of 28 Serotine bats tested, 11 rabies cases were reported from Hungary, Spain and Poland. Furthermore, rabies was found in 6 of the 14 investigated *Eptesicus isabellinus* bats. Whilst in Poland 8 bat rabies cases could not be attributed to a certain bat species, other cases were formally attributed to *Eptesicus serotinus* ($n = 8$), *Myotis myotis* ($n = 1$) and *Plecotus auritus* ($n = 1$).

During the past two decades, published studies on passive bat rabies surveillance originated from Finland, France, Germany, Serbia, Slovenia, Spain, UK, Sweden, Switzerland, the Netherlands and United Kingdom covering different time periods (Table 3). Overall these studies comprised testing of 28 of the 52 different European bat species for rabies. EBLV-1 was isolated from 287 Serotine bats (*Eptesicus serotinus*); EBLV-2 was detected in 14 Daubenton's bats (*Myotis daubentonii*) and 5 Pond bats (*Myotis dasycneme*). A virus from a single Natterer's bat was characterized as BBLV (Table 3). Rabies cases in other bat species were not reported. Notably, only viral RNA (EBLV-1) was detected in the brains of 3 *Myotis myotis*, 1 *Miniopterus schreibersii*, 1 *Myotis nattereri* and 1 *Plecotus*

Table 3. Passive surveillance for bat rabies in Europe

Bat species	Region	No. of bats	FAT pos.	Lyssavirus	Reference
Unknown/unspecified	Finland	21	0		Jakava-Viljanen et al., 2010b
	France	947	0		Picard-Meyer et al., 2006
	Serbia	82	0		Vranješ et al., 2010
	Slovenia	50	0		Presetnik et al., 2010
	Swiss	14	0		Megali et al., 2010
	The Netherlands	9	0		van der Poel et al., 2005
	UK	47	0		Harris et al., 2006
Barbastella barbastellus	Russia	3	0		Botvinkin et al., 2003; *
	Swiss	3	0		Megali et al., 2010
	UK	6	0		Harris et al., 2006
Eptesicus isabellinus	Spain	25	5		Echevarria et al., 2001
Eptesicus nilssonii	Sweden	24	0		SVA 2009/2010
	Swiss	13	0		Megali et al., 2010
	The Netherlands	1	0		van der Poel et al., 2005
Eptesicus serotinus	France	Unknown	21	EBLV-1	Picard-Meyer et al., 2006
	France	Unknown	15	EBLV-1	Picard-Meyer et al., 2010a
	Spain	1	0		Serra-Cobo et al., 2002
	Swiss	21	0		Megali et al., 2010
	The Netherlands	1219	251	EBLV-1	van der Poel et al., 2005
	UK	82	0		Harris et al., 2006
H. savii	Swiss	1	0		Megali et al., 2010
Miniopterus schreibersii	Russia	24	1	WCBV	Botvinkin et al., 2003; *
	Spain	9	0		Serra-Cobo et al., 2002
M. bechsteinii	Swiss	5	0		Megali et al., 2010
	UK	2	0		Harris et al., 2006
M. brandtii/mystacinus	UK	168	0		Harris et al., 2006
M. brandtii	Sweden	6	0		SVA 2009/2010
Myotis blythii	Russia	10	0		Botvinkin et al., 2003; *
	Swiss	2	0		Megali et al., 2010
Myotis daubentonii	Germany	45	1	EBLV-2	Freuling et al., 2008
	Russia	43	0		Botvinkin et al., 2003; *
	Sweden	1	0		SVA 2009
	Swiss	64	3	EBLV-2	Megali et al., 2010
	The Netherlands	111	0		van der Poel et al., 2005
	UK	112	4	EBLV-2	Harris et al., 2006
Myotis dasycneme	The Netherlands	129	5	EBLV-2	van der Poel et al., 2005
Myotis emarginatus	Russia	9	0		Botvinkin et al., 2003; *
	Spain	2	0		Serra-Cobo et al., 2002
Myotis spp.	Sweden	1	0		SVA 2009
	Swiss	1	0		Megali et al., 2010
Myotis myotis	Spain	15	0		Serra-Cobo et al., 2002
	Spain	17	0		Amengual et al., 2007
	Swiss	41	0		Megali et al., 2010
	UK	1	0		Harris et al., 2006
Myotis mystacinus	Sweden	6	0		SVA 2009/2010
	Swiss	45	0		Megali et al., 2010
	The Netherlands	18	0		van der Poel et al., 2005
Myotis nattereri	Germany	65	1		Freuling et al., 2011
	Spain	2	0		Serra-Cobo et al., 2002
	Sweden	2	0		SVA 2009/2010
	Swiss	4	0		Megali et al., 2010
	The Netherlands	9	0		van der Poel et al., 2005
	UK	139	0		Harris et al., 2006

Table 3. (Continued)

Bat species	Region	No. of bats	FAT pos.	Lyssavirus	Reference
Nyctalus noctula	Russia	28	0		Botvinkin et al., 2003; *
	Sweden	1	0		SVA 2010
	Swiss	68	0		Megali et al., 2010
	The Netherlands	61	0		van der Poel et al., 2005
	UK	56	0		Harris et al., 2006
N. leisleri	Swiss	21	0		Megali et al., 2010
	The Netherlands	3	0		van der Poel et al., 2005
	UK	9	0		Harris et al., 2006
Pipistrellus kuhlii	Russia	9	0		Botvinkin et al., 2003; *
	Swiss	57	0		Megali et al., 2010
Pipistrellus nathusii	Swiss	112	0		Megali et al., 2010
	The Netherlands	256	0		van der Poel et al., 2005
	UK	33	0		Harris et al., 2006
Pipistrellus pipistrellus	Russia	2	0		Botvinkin et al., 2003; *
	Spain	50	0		Serra-Cobo et al., 2002
	Swiss	224	0		Megali et al., 2010
	The Netherlands	1837	0		van der Poel et al., 2005
P. pygmaeus	Sweden	9	0		SVA 2009/2010
	Swiss	11	0		Megali et al., 2010
P. pipistrellus/pygmaeus	UK	3439	0		Harris et al., 2006
P. savii	Swiss	2	0		Megali et al., 2010
Plecotus spp.	Swiss	3	0		Megali et al., 2010
Plecotus auritus	Sweden	20	0		SVA 2009/2010
	Swiss	63	0		Megali et al., 2010
	The Netherlands	214	0		van der Poel et al., 2005
	UK	731	0		Harris et al., 2006
Plecotus austriacus	Spain	4	0		Serra-Cobo et al., 2002
	Sweden	4	0		SVA 2009
	Swiss	5	0		Megali et al., 2010
	UK	9	0		Harris et al., 2006
Pl. macrobullaris	Swiss	2	0		Megali et al., 2010
Rhinolophus ferrumeqinum	Russia	6	0		Botvinkin et al., 2003; *
	Spain	7	0		Serra-Cobo et al., 2002
	UK	5	0		Harris et al., 2006
Rhinolophus hipposideros	Spain	1	0		Serra-Cobo et al., 2002
	UK	32	0		Harris et al., 2006
Verspertilio murinus	Sweden	4	0		SVA 2010
	Swiss	31	0		Megali et al., 2010
	The Netherlands	6	0		van der Poel et al., 2005

*Wild bats were caught and subject of rabies diagnosis (FAT) after necropsy.

austriacus (Serra-Cobo et al., 2002; Amengual et al., 2007).

In Europe, wild-caught bats from selected colonies of 19 different species were the subject of active surveillance. Most studies were performed in Spain, and the most frequently sampled species was *Myotis myotis*, followed by *Myotis daubentonii* and *Eptesicus serotinus*. Eleven species tested serologically positive for virus neutralizing antibodies (VNAs, Table 4). Eptesicus bats (*E. serotinus or E. isabellinus*) had measurable levels of VNAs in Spain, Germany and France. A single Serotine bat from the UK also had VNAs, whereas all tested Serotines from Switzerland were negative. VNAs were identified in Daubenton's bats from UK, Switzerland and Sweden. Both *Miniopterus schreibersii* (France, Spain) and *Myotis nattereri* (Belgium, UK) also tested positive for VNAs (Table 4).

Individual sera from *Myotis myotis* from Spain, France and Belgium were positive for VNAs, while all sera from Switzerland were negative. Especially, the results of one survey in Spain indicated very high seroprevalences in this bat species (Amengual et al., 2007).

None of the sera from *Eptesicus nilssonii, Myotis emarginatus, Nyctalus noctula, Pipistrellus kuhlii, Pipistrellus*

Table 4. Sero-surveillance and detection of lyssavirus neutralizing antibodies in European bats

Bat species	Region	*N*	VNA pos.	Seroprevalence (%)	Reference
Unspecified	Slovenia	216	0	0	Hostnik et al., 2010
B. barbastellus	France	2	1	9.5–90.5	Picard-Meyer et al., 2011
E. isabellinus	Spain	626	51	9	Vazquez-Moron et al., 2008b
E. serotinus	Germany	98	5	5	unpublished data
	France	28	7	11.4–45.2	Picard-Meyer et al., 2011
	Spain (Balearic)	99	0	0	Serra-Cobo et al., 2002
	Spain	175		0-74	Perez-Jorda et al., 1995
	Switzerland	17	0	0	Megali et al., 2010
	UK (England)	299	1	0.001–1.6	Harris et al., 2009
E. nilssonii	Sweden	47	0	0	SVA, 2009
M. blythii	France	5	1	1.0–70.1	Picard-Meyer et al., 2011
	Spain (Balearic)	23	0	0	Serra-Cobo et al., 2002
M. capaccinii	Spain (Balearic)	3	0	0	Serra-Cobo et al., 2002
M. daubentonii	Switzerland	124	3	2.4	Megali et al., 2010
	Sweden	77	8	10,39	SVA, 2009
	UK (England)	329	7–13	1.0–4.1	Harris et al., 2006
	UK (Scotland)	198 (88)	6–18	0.05–3.8	Brookes et al., 2005
	UK (Scotland)	>900	~27		SNH, 2009
M. emarginatus	Spain (Balearic)	16	0	0	Serra-Cobo et al., 2002
M. myotis	Belgium	8	2	25	Klein et al., 2007
	France	37	5	5.1–29.6	Picard-Meyer et al., 2011
	Spain (Balearic)	371	167		Amengual et al., 2007
	Spain (Balearic)	295	66	18	Serra-Cobo et al., 2002
	Switzerland	47	0	0	Megali et al., 2010
M. myotis/blythii	France	11	2	3.2–52.2	Picard-Meyer et al., 2011
M. nattereri	Belgium	8	2	25	Klein et al., 2007
	Spain (Balearic)	1	0	0	Serra-Cobo et al., 2002
	UK (Scotland)	20	0	0	Brookes et al., 2005
	UK (Scotland)	Unknown	2		SNH, 2009
Mi. schreibersii	France	17	2	2.1–37.7	Picard-Meyer et al., 2011
	Spain (Balearic)	168	6		Serra-Cobo et al., 2002
N. noctula	Switzerland	14	0	0	Megali et al., 2010
P. kuhlii	Spain (Balearic)	1	0	0	Serra-Cobo et al., 2002
P. pipistrellus	Spain (Balearic)	234	0	0	Serra-Cobo et al., 2002
	UK (Scotland)	6	0	0	Brookes et al., 2005
Plecotus spp.	Belgium	8	1	13	Klein et al., 2007
Pl. austriacus	Spain (Balearic)	12	0	0	Serra-Cobo et al., 2002
R. ferrumeqinum	France	10	1	0.5–45.9	Picard-Meyer et al., 2011
	Spain (Balearic)	58	2		Serra-Cobo et al., 2002
R. euryale	Spain (Balearic)	16	0	0	Serra-Cobo et al., 2002
R. hipposideros	Spain (Balearic)	16	0	0	Serra-Cobo et al., 2002
Tadarida teniotis	Spain (Balearic)	34	2		Serra-Cobo et al., 2002

pipistrellus, Plecotus austriacus, Rhinolophus euryale and Rhinolophus hipposideros had measurable VNAs.

Analyses of oro-pharyngeal swab samples were published for bats from France, Spain, UK, Switzerland, Slovenia and Serbia (Table 5). A total of 3880 oral swabs from 23 different European bat species were screened for viral RNA mostly using RT-PCR methods. Only in two bat species, that is, Serotine bats and Daubenton's bats viral RNA was found. Positive results (EBLV-1) from Eptesicus species were reported from Spain (*Eptesicus isabellinus*). Oral swabs from 2 Daubenton's bats from Scotland (2008) and Switzerland (2009) yielded EBLV-2 specific RNA. Viable virus could not be isolated from any oral swab sample.

Discussion

The understanding of lyssavirus diversity and abundance has increased over the past decades. The collection of rabies data for the RBE, including those on positive bats, started in 1977, before the discovery of the different lyssaviruses in Europe. The first human case of EBLV-2 origin which occurred in 1985 (Lumio et al., 1986), initiated subsequent surveillance activities particularly in Germany,

Table 5. Active surveillance for bat rabies in Europe using oral swab samples for detection of lyssavirus-specific RNA or virus. Number of positive and no. of tested () samples per bat species is shown

Bat species	Picard-Meyer et al., 2011; France	Picard-Meyer et al., 2011; France	Vazquez-Moron et al., 2008b; Spain	Echevarria et al., 2001; Spain	SNH, 2009, UK - Scotland	Harris et al., 2006; UK	Brookes et al., 2005; UK - Scotland	Megali et al., 2010; Switzerland	Presetnik et al., 2010; Slovenia	Hostnik et al., 2010; Slovenia	Vranies et al., 2010; Serbia
B. barbastellus	0 (6)	0 (7)									
E. isabellinus			34 (1226)	15 (71)							
E. serotinus	0 (18)	0 (72)				0 (327)		0 (23)	0 (145)	0 (61)	0 (8)
Mi. schreibersii	0 (4)	0 (18)									0 (31)
M. alcathoe		0 (1)									
M. bechsteinii	0 (7)	0 (5)									
M. blythii		0 (5)									0 (41)
M. capaccinii										0 (1)	0 (33)
M. daubentonii	0 (15)	0 (22)			1 (>900)	0 (416)	0 (198)	1 (148)	0 (61)	0 (21)	0 (44)
M. emarginatus	0 (27)	0 (10)							0 (50)		
M. myotis	0 (22)	0 (36)						0 (51)	0 (57)	0 (55)	0 (33)
M. myotis/blythii		0 (11)									
M. mystacinus	0 (3)	0 (3)							0 (15)	0 (5)	
M. nattereri	0 (10)	0 (7)					0 (20)			0 (1)	
N. noctula								0 (15)	0 (57)	0 (35)	0 (24)
P. kuhlii	0 (1)								0 (19)	0 (3)	
P. nathusii										0 (2)	
P. pipistrellus	0 (11)	0 (15)									
P. pygmaeus										0 (13)	
Pl. auritus	0 (8)	0 (7)									
Pl. austriacus	0 (15)	0 (15)									
R. euryale	0 (4)	0 (5)									
R. ferrumeqinum	0 (11)	0 (21)									
R. hipposideros	0 (11)	0 (11)							0 (63)	0 (63)	
Methods	RTCIT	hnRT-PCR	RT-PCR	RT-PCR		RT-PCR	RT-PCR/RTCIT	nRT-PCR	RT-PCR	RT-PCR	RT-PCR, MIT

the Netherlands, Denmark and the UK (King et al., 2004). This may be one explanation for the increased number of bat rabies cases reported in Europe in 1986 and 1987. Following this peak, the number of positive bats per year ranged between 6 and 53 with an average number of 29 (Fig. 2a). The effect of disease awareness on number of submitted animals was also evident in the UK (Harris et al., 2006), where increase was observed after the tragic rabies death of a Scottish biologist after EBLV-2 infection (Fooks et al., 2003).

The peak of positives in the third quarter (Fig. 2b), was also observed in individual European countries, for example, Germany (Müller et al., 2007) and the Netherlands (van der Poel et al., 2005). It is likely an effect of the higher activities of bat handlers, for example, the control of maternity colonies and banding of juveniles etc., and thus, a higher likelihood of contact with diseased animals. However, it may also reflect a disease-specific seasonal pattern. For instance, in Colorado, United States, it was shown that the rabies infections clearly followed the cyclic behaviour of bats (George et al., 2011).

Bat rabies cases in Europe seem to concentrate in the lowlands of the Netherlands, Germany and Denmark. While a higher number of cases were also reported from Poland, bat rabies seems to be sporadic in Eastern European countries (Fig. 1). However, the data analysed indicate that the level of surveillance is very heterogeneous (Table 2). While some countries only test individual bats, in the UK for instance on average more than 1000 bats are submitted annually, through close collaboration between public and animal health authorities with other stakeholder groups. As stated in the EUROBATS agreement, the collaboration between bat biologists and rabies scientist is a prerequisite. Thus, in some European countries where the number of bat preservationists is small and where they are not well organized, any attempt to establish bat rabies surveillance is also hampered. Also, the remoteness in some parts of Europe may prevent the establishment of effective bat rabies surveillance *per se*. Therefore, the reasons for countries that have not submitted any data are elusive. Possibly, in some countries which are historically rabies-free the intensity of classical rabies surveillance may be low and may not cover bat rabies. In fact, Norway does not have bat rabies surveillance and Portugal intends to start bat rabies surveillance in the future. Also, the awareness for bat rabies may not be sufficient to encourage people to submit dead grounded or sick animals.

To understand the epidemiology of the diverse bat lyssavirus infections, it is important to have comprehensive data on bat samples, for example, geographical origin, age, sex and species. The latter is most important as about 52 different bat species occur in Europe (http://www.eurobats.org) covering a diversity of biological niches.

Furthermore, it seems that some bat lyssaviruses have a certain reservoir host association. Unfortunately, data submission to the RBE database only requests information on the mammal order, that is, bats (*Chiroptera*), as historically, the focus of this database was on classical rabies. Therefore, as part of this study, the exact bat species tested were requested from the contributors to the RBE. Since only few countries completed the respective questionnaire, it is difficult to assess whether species identification is regularly performed in the non-responding countries.

Based on the information provided, it was shown that the Common pipistrelle bat (*Pipistrellus pipistrellus*) is the most frequently tested species. The overall proportions of bats that were tested but were not specified or the results were not submitted was 46.8%. The value of knowing the bat species is illustrated by the fact that one Greater mouse-eared bat (*Myotis myotis)* and one Brown long-eared bat (*Plecotus auritus)* tested rabies positive in Poland. These species had only been associated with bat rabies in Germany before. Single rabies cases were described in a Greater mouse-eared bat (Hentschke and Hellmann, 1975) and Brown long-eared bat (Müller et al., 2007), respectively.

Based on published data on passive surveillance, lyssavirus infections were detected in *Eptesicus serotinus, Myotis daubentonii* and *Myotis dasycneme* using standard FAT. Furthermore, one Natterer's bat (*Myotis nattereri*) also tested positive by FAT, and the isolated virus was characterized as a novel member of the lyssavirus genus (Freuling et al., 2011). According to previous publications, bat rabies cases in Europe were also found in *Nyctalus noctula, Pipistrellus nathusii, Pipistrellus pipistrellus, Rhinolophus ferrumeqinum and Verspertilio murinus* (King et al., 2004; Müller et al., 2007). Surprisingly, viral RNA (EBLV-1) was detected in *Miniopterus schreibersii* and *Myotis nattereri* from Spain (Serra-Cobo et al., 2002), whereas those species have also been associated with WCBV and BBLV, respectively (Table 1). While the first EBLV-2 case in Germany was detected in the frame of routine rabies diagnosis, that is, a suspect animal was submitted to the laboratory (Freuling et al., 2008), a second case was only found during a retrospective passive surveillance. In fact, the bat was stored for a year in a freezer, until it was subject of rabies testing (Freuling et al., 2012). Interestingly, some of the European bat species have never been investigated for rabies (Table 3), thus their role in the epidemiology of lyssaviruses remains elusive. The same holds true for those species where only few individuals were tested. Identifying bats to species level can be challenging particularly as submitted samples may be in poor condition, therefore confounding reports. Standard techniques include the use of morphological keys, genetic speciation and the use of independent experts, which may lead to conflicting results. The abundance, density and availability of samples from certain bat species may also hamper higher surveillance efforts in individual countries. Therefore, comparison of incidence and or prevalence from country to country using these data is challenging, as the sampling effort and sample selection criteria and testing techniques are not uniform. Providing consistent effort is maintained, however, comparison is more valid from year to year.

All bat species in Europe are strictly protected under the Flora, Fauna, Habitat Guidelines of the European Union (92/43/EEC) and the Agreement on the Conservation of Populations of European Bats (http://www.eurobats.org). Therefore, invasive sampling of bats similar to classical rabies in wildlife is now prohibited. In addition to the sampling of dead found bats, several studies tried to investigate lyssavirus infections in bat colonies by testing oral swabs and sera. The selection of sampling sites may be based on previous bat rabies cases notified (Echevarria et al., 2001; Picard-Meyer et al., 2010b) or randomly selected sites. Except for a study in Spain (Echevarria et al., 2001) and single RNA-positive Daubenton's bats, the outcome of these published investigations are thus far a disillusion as all other attempts to detect viable virus or viral RNA failed (Table 5), and interpretation is confounded by differences in sensitivity of assays used. This low level of detection of virus in oral swabs to some extent corroborates experimental studies in bats, where only very few animals were found to shed virus in the saliva (Franka et al., 2008; Johnson et al., 2008; Freuling et al., 2009b). One problem associated with this fact is that the transmission of virus among bat conspecifics still remains poorly understood (Vos et al., 2007; Freuling et al., 2009a). Even the discovery of EBLV-2 in Finland during active surveillance was based on a clinically suspect animal only (Jakava-Viljanen et al., 2010a).

Virus neutralizing antibodies against lyssaviruses were detected in various European bat species from different countries (Table 4), of which *Tadarida teniotis* and *Barbastella barbastellus* have never been associated with bat rabies through passive surveillance. Eptesicus bats from several countries showed measurable levels of VNAs. However, in an experimental study, none of the *Eptesicus serotinus* infected with EBLV-1 developed VNAs (Freuling et al., 2009b), while in *Eptesicus fuscus* VNAs were detected in survivors and deceased animals (Franka et al., 2008). Surprisingly, blood samples from a high number of investigated Greater mouse-eared bats (*Myotis myotis)* in Spain were VNAs positive (Serra-Cobo et al., 2002; Amengual et al., 2007). In contrast, data of passive rabies surveillance provide currently only two rabies cases in *Myotis myotis* from Germany and Poland. Although none of the Daubenton's bats experimentally infected with EBLV-2 had VNAs, free living Daubenton's bat from the UK, Switzerland and Sweden showed VNAs (Table 4). Generally, seropositivity in bats cannot be attributed to specific lyssaviruses because of the cross-reactivity of antibodies to lyssaviruses within phylogroups (Horton et al., 2010). While in the Americas where only one virus species (RABV) is responsible for rabies, four different lyssavirus species may be circulating in bats in Europe. Thus, serological tests in the New World can use the homologous test virus, whereas in Europe several modifications of non-standardized serological test procedures exist that make the results of serological tests hardly comparable as discussed previously (Freuling et al., 2009a). The lack of standardized positive control sera for each assay and differences in interpretation of serological thresholds can lead to vastly different estimates of seroprevalence. For example, a minor change in detection threshold for specific neutralization can double seroprevalence estimates. Interestingly, the first bat rabies case in Europe in 1954 also led to active surveillance activities. A total of 295 sera from *Myotis myotis* bats were tested for VNAs, and the investigators carefully discussed their apparently positive results (Dennig, 1958).

So, what is the message of positive serological results in bats? For example, VNAs in the Natterer's bat as observed in Belgium and Scotland may be the result of EBLV-1 infection, or, based on the recent discovery of BBLV, more likely associated with this novel lyssavirus. It also remains elusive, whether the detection of VNAs in *Myotis blythii* is attributed to EBLV-1, 2 or BBLV infection or whether this indicates the circulation of Aravan virus, which was isolated from this bat species in Central Asia (Kuzmin et al., 2003). Likewise, the discovery of VNAs in *Miniopterus schreibersii* from France and Spain questions the association of this species with WCBV, for which no cross-reactivity exists.

Conclusions

The level of bat rabies surveillance in Europe is still very heterogeneous, despite previous recommendations (Med-Vet-Net, EUROBATS). In the United States, comparatively higher numbers of bats are submitted annually (Blanton et al., 2011). Although federal and state protections also exist for threatened and endangered species in the United States, higher population densities might cause more human encounters. Also, given the fact that bat rabies was found in all parts of the United States, awareness is likely to be higher than in Europe. Another factor that could contribute to higher submission rates is the encouragement to submit bats from the public if a person believes they may have been bitten. A similar approach is not used in Europe. Here, the EUROBATS agreement specifically stipulates that only dead bats should be submitted and tested for rabies, and currently passive surveillance is considered priority where resources are limited. The growing body of evidence also provided by active surveillance adds useful information regarding the dynamics of infection in natural populations, which will also help to inform about public and animal health risks. Standardized serological and virus detection techniques are needed, to aid comparison of those results. EUROBATS also emphasizes that this surveillance should be in close cooperation with bat biologists. Therefore, to establish and increase bat rabies surveillance a network of bat biologists needs to be present and should be encouraged where missing. Also, as with any other disease surveillance vigilance and awareness needs to be at a high level to guarantee a submission of any dead found or grounded bats. The focus of bat rabies surveillance should be on testing of dead or moribund animals. Resulting positive FAT results should be further analysed to identify the lyssavirus species and all bats submitted for testing should be identified to species level, either by morphological or by genetic features. International harmonization of identification techniques would also allow more robust comparison between countries.

The current data reporting for rabies in Europe, the WHO RBE is focused on classical rabies. Future developments should include the submission of additional data to bat rabies surveillance, for example, the virus species, bat species, geographical origin etc.

Given the discovery of a novel lyssavirus in central Europe (Freuling et al., 2011) bat rabies surveillance should be maintained at a high level where already existing or should be established following recommendations to better assess the veterinary and public health impact of bat lyssaviruses in Europe.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Results of the questionnaire on passive surveillance.

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