Expanding Distribution of Lethal Amphibian Fungus *Batrachochytrium salamandrivorans* in Europe

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Emerging fungal diseases can drive amphibian species to local extinction. During 2010–2016, we examined 1,921 urodeles in 3 European countries. Presence of the chytrid fungus *Batrachochytrium salamandrivorans* at new locations and in urodeles of different species expands the known geographic and host range of the fungus and underpins its imminent threat to biodiversity.

Amphibians provide an iconic example of disease-driven global loss in biodiversity. The recently described chytrid fungus *Batrachochytrium salamandrivorans* (*Bsal*) is an emerging pathogen that is driving amphibian populations to local extinction (*1*,*2*). This highly pathogenic fungus causes a lethal skin disease that has so far been restricted to urodele amphibians (newts and salamanders); the fungus was most likely introduced from East Asia into Europe via the pet trade (*2*). In Europe, *Bsal* infection has led to dramatic declines of fire salamander (*Salamandra salamandra*) populations in the Netherlands and Belgium (*2*). Within 7 years after the supposed introduction of the fungus, a population in the Netherlands declined by 99.9% (*3*,*4*). In the United Kingdom and Germany, *Bsal* has been detected in captive salamanders and newts (*5*,*6*). Infection trials suggest that *Bsal* represents an unprecedented threat to diversity of Western Palearctic urodeles (*2*); nevertheless, reports of deaths among salamanders and newts in their naturalized ranges have been restricted to a few populations of a single salamander species in the southern Netherlands and adjacent Belgium (*1*,*3*). Using data from field surveillance, we examined the hosts and the geographic range of *Bsal* in Europe.

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DOI: http://dx.doi.org/10.3201/eid2207.160109

The Study

During 2010–2016, we collected samples of free-living populations of newts and salamanders from 48 sites in the Netherlands, Belgium, and adjacent regions of the Eifel region in Germany (near the border with the Netherlands and Belgium) (Figure; online Technical Appendix Table 1, http://wwwnc.cdc.gov/EID/article/22/7/16-0109-Techapp1.pdf). Site selection was based on reported amphibian deaths, apparent negative amphibian population trends, preventive *Bsal* surveillance in susceptible populations, or geographic proximity to known outbreak sites. Samples were also collected at 6 additional sites in Germany and 1 in the Netherlands, which were located >100 km from the nearest known outbreak (online Technical Appendix Table 2). Sampling was conducted by swabbing skin (*7*,*8*) of live animals and collecting skin samples from dead animals. All samples were kept frozen at -20°C until they were analyzed for the presence of *Bsal* DNA via real-time PCR, as described (*9*).

Across all 55 sites, we tested 1,019 fire salamanders (43 dead, 976 skin swab samples); at site 14, skin swab samples instead of tissue samples were collected from 16 dead salamanders. We also collected samples from 474 alpine newts (*Ichthyosaura alpestris*; 18 dead, 456 skin swab samples), 239 smooth newts (*Lissotriton vulgaris*; 2 dead, 237 skin swab samples), 80 palmate newts (*Lissotriton helveticus*; all skin swab samples), 79 crested newts (*Triturus cristatus*; all skin swab samples), and

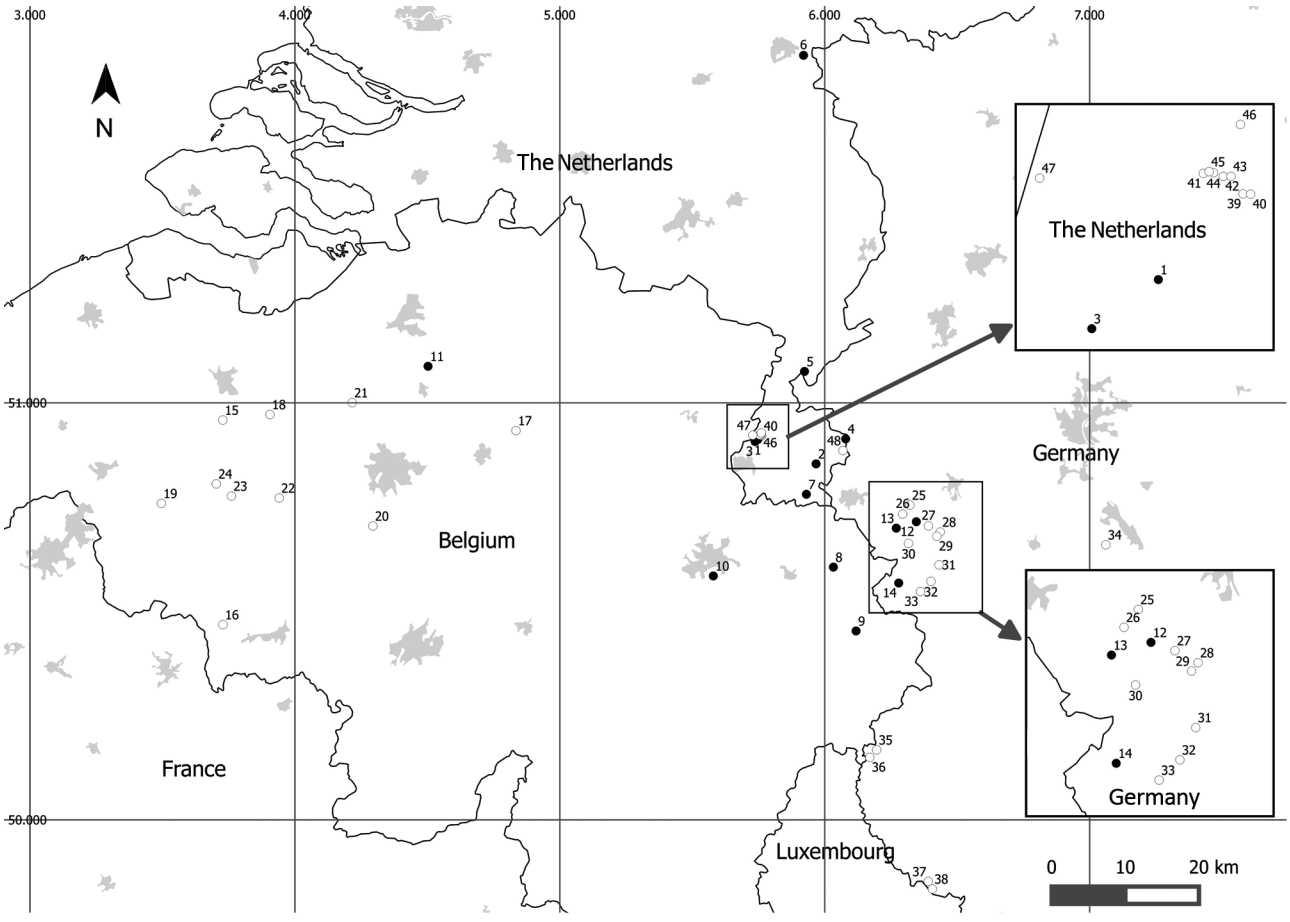

Figure. Study sites for collection of amphibians in Western Europe, 2010–2016. Numbers correspond to field sites at which amphibians were collected and examined for *Batrachochytrium salamandrivorans* (*Bsal*) (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/7/16-0109-Techapp1.pdf). Solid circles, *Bsal* detected; open circles, *Bsal* not detected. Larger cities are indicated in light gray. Note that there are additional sites where the fungus remained undetected (not shown).

30 Italian crested newts (*Triturus carnifex*; all skin swab samples). To obtain a Bayesian 95% credible interval for prevalence (online Technical Appendix), we used the computational methods of Lötters et al. (*10*). We ran 3 parallel Markov chains with 20,000 iterations each and discarded the first 5,000 iterations as burn-in; chains were not thinned.

Bsal was found at 14 of the 55 sites; infected amphibians were fire salamanders, alpine newts, and smooth newts. Our results demonstrate that the range of *Bsal* distribution may be up to ≈10,000 km^2 (measured as the surface of a minimum convex polygon encompassing the outermost points) across Germany, Belgium, and the Netherlands (Figure). The presence of *Bsal* in wild alpine newts and smooth newts shows distinct expansion of the known host range in the wild (online Technical Appendix Table 1). Furthermore, we document that *Bsal* is present in natural fire salamander populations in Germany (confined to the Eifel region). At some sites, because of our sample sizes, the upper limit of the 95% credible interval for *Bsal* prevalence was as high as 0.7; therefore, we may have failed to detect *Bsal* at these sites (online Technical Appendix Table 1). In addition, the fungus may have been present at several sites before first detection. For example, *Bsal* was detected at site 4, where population-monitoring efforts in the years before detection (2000–2013) showed declines in 4 newt species (http://www.ravon.nl/EID_SI_Spitzen_et_al_2016). However, because no samples were collected before 2015, we have no evidence for a causal relationship between the presence of *Bsal* and the declines. We have also recorded the presence of *Bsal* in populations with no evidence of population change so far, such as the incidental findings of dead *Bsal*-positive newts in fyke nets at sites 5 and 11, and the incidental findings of dead *Bsal*-positive fire salamanders at sites 12 and 14. Clinical signs of mycosis, such as lethargy and skin shedding (*1*), were observed at some *Bsal*-positive sites (1, 2, 7, 8, 14) but not at others.

Conclusions

Our study provides evidence that *Bsal* among wild amphibians in Europe is more widely distributed and affects a wider host range than previously known, which can either indicate recent spread of the fungus or point to historically infected sites that hitherto remained undetected. The presence of *Bsal* in wild populations can easily remain unnoticed because the lesions develop only near the final stage of the disease (*1*). This information is crucial for the design of field surveys for *Bsal* surveillance. Our data might be used to inform a management strategy and to implement the recommendation of the Bern Convention (*11*) to halt the spread of *Bsal* in Europe. Research to search for molecular evidence that the outbreak locations are connected is under way. Chytrid disease dynamics are affected by multiple factors (e.g., temperature regimes [*1*]), and yet undetermined environmental determinants might be essential for disease outbreaks (*12*). Untangling these factors, as well as the modes of *Bsal* spread and its geographic distribution, are points for further research to fully map the problem and identify populations and species at risk.

Acknowledgments

We thank A. Geiger, K. Fischer, W. Bosman, M. Haffmann, J. Janse, J. Munro, L. Paulssen, E. Schotmans, and S. van Baren for their invaluable help and support to this project. We also thank all volunteers from Hyla, Natagora and Reptile, Amphibian & Fish Conservation Netherlands for collecting amphibians.

This study was supported by the Deutsche Bundesstiftung Umwelt, Dutch Ministry of Economic Affairs, Flemish Agency for Nature and Forests, Land Nordrhein Westfalen, Province of Limburg, StädteRegion Aachen, Stiftung Artenschutz, Trier University Research Assistantship, Verband der Zoologischen Gärten, Zoo Landau, and a PhD fellowship of the Research Foundation–Flanders.

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Article DOI: http://dx.doi.org/10.3201/eid2207.160109

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Technical Appendix

Technical Appendix Table 1. Field sites where *Bsal* was detected, sampled species, numbers of *Bsal*-positive and total sampled specimens*

Site no., location, and amphibian collected	No. *Bsal*-positive/total tested (year)	Observed prevalence (Bayesian 95% credible intervals)	Remarks
The Netherlands			
1, Bunderbos, deciduous forest			
Fire salamander	3/3 (2010)	1.00 (0.42–1.00)	Past mass deaths; 99.9% population decline (1997–2014)
	1/1 (2011)	1.00	
	1/1 (2012)	1.00	
	0/3 (2014)	0 (0–0.61)	
	2/14 (2015)	0.14 (0.04–0.40	
	0/1 (2016)	0	
Alpine newt	1/1 (2013)	1.00	Possibly declining (monitoring started in 2013)†
	1/39 (2014)	0.03 (0.01–0.13)	
	1/10 (2015)	0.10 (0.02–0.43)	
	0/6 (2016)	0 (0–0.43)	
2, Putberg, deciduous forest			
Smooth newt	0/2 (2014)	0 (0–0.70)	Possibly declining†
Alpine newt	0/10 (2014)	0 (0–0.31)	Possibly declining†
	1/1‡ (2014)	1.00	
	1/1‡ (2015)	1.00	
3, Meerssen, garden pond			
Fire salamander	0/1 (2015)	0	No evidence of decline§
Smooth newt	4/43 (2015)	0.09 (0.04–0.21)	No evidence of decline§
Alpine newt	0/9 (2015)	0 (0–0.30)	No evidence of decline§
4, Wormdal, clusters of natural ponds in nature conservation area¶			
Smooth newt	1/22 (2015)	0.05 (0.01–0.21)	87% decline(2000–2013)†#
Alpine newt	0/12 (2015)	0 (0–0.26)	96% decline (2000–2013)†#
5, Pepinusbeekdal, extensive agriculture			
Smooth newt	1/2‡ (2014)	0.50 (0.09–0.88)	No evidence of decline†
6, Berg en Dal, garden pond			
Alpine newt	12/12‡ (2015)	1.00 (0.74–1.00)	Yearly mass deaths; species still present§
7, Vijlenerbosch, deciduous forest			
Alpine newt	0/1 (2013)	0	No evidence of decline§
	0/30 (2014)	0 (0–0.11)	
	1/18 (2015)	0.05 (0.02–0.24)	
Smooth newt	0/8 (2014)	0 (0–0.31)	No evidence of decline§
	0/11 (2015)	0 (0–0.26)	
Palmate newt	0/1 (2014)	0	No evidence of decline§
	0/9 (2015)	0 (0–0.30)	
Belgium			
8, Eupen, deciduous forest			
Fire salamander	1/2 (2013)	0.50 (0.09–0.88)	Deaths, probably fire salamanders severely declining, no monitoring trend available
9, Robertville, deciduous forest			
Fire salamander	16/30 (2014)	0.53 (0.36–0.69)	Deaths, severe decline, monitoring ongoing
10, Liège, deciduous forest			

Site no., location, and amphibian collected	No. *Bsal*-positive/total tested (year)	Observed prevalence (Bayesian 95% credible intervals)	Remarks
Fire salamander	5/5 (2014)	1.00 (0.55–.00)	Deaths
11, Duffel, garden pond			
Alpine newt	2/30‡ (2015)	0.07 (0.02–0.22)	2 dead in fyke; no evidence of decline
Smooth newt	0/16 (2015)	0 (0–0.20)	No deaths; no evidence of decline
Germany			
12, Weisse Wehe, deciduous forest			
Fire salamander	4/11‡ (2015)	0.36 (0.15–0.65)	No evidence of decline†
13, Solchbachtal, mixed forest			
Fire salamander	0/2 (2014)	0 (0–0.70)	Decreased newts and salamanders§
	1/51 (2015)	0.02 (0.01–0.10)	
Palmate newt	0/19(2014)	0 (0–0.18)	Decreased newts and salamanders§
Alpine newt	0/5(2014)	0 (0–0.44)	Decreased newts and salamanders§
14, Belgenbachtal, mixed forest			
Fire salamander	21/22‡ (2015)	0.96 (0.79–0.99)	Remarkable deaths (16 dead), noted only since Nov 2015†

**Bsal, Batrachochytrium salamandrivorans.* Data provide an overview of novel information and previously published data. Site numbers correspond to those on map (Figure).
†Population monitored.
‡Includes individual(s) found dead by chance.
§Anecdotal reports.
¶At this site, crested newts and smooth newts decreased with similar percentages over the same period (−96%; −94%, respectively).
#http://www.ravon.nl/EID_SI_Spitzen_et_al_2016.

Technical Appendix Table 2. Field sites studied where *Bsal* was not detected, number of sampled species and specimens*

Site no., location, and amphibian collected	Number of specimens tested (year)	Observed prevalence (Bayesian 95% credible intervals)	Remarks
Belgium			
15, Nerenbos, deciduous forest			
Fire salamander	30 (2015)	0 (0–0.11)	No evidence of decline†
16, Heilig Geestgoed, deciduous forest			
Fire salamander	30 (2015)	0 (0–0.11)	No evidence of decline†
17, Kasteel van Horst, deciduous forest			
Fire salamander	30 (2015)	0 (0–0.11)	No evidence of decline†
18, Smetledebos, deciduous forest			
Fire salamander	30 (2015)	0 (0–0.11)	No evidence of decline†
19, Kluisbos, deciduous forest			
Fire salamander	30 (2015)	0 (0–0.11)	No evidence of decline†
20, Hallerbos, deciduous forest			
Fire salamander	30 (2015)	0 (0–0.11)	No evidence of decline†
21, Buggenhoutbos, deciduous forest			
Fire salamander	30 (2015)	0 (0–0.11)	No evidence of decline†
22, Raspaillebos, deciduous forest			
Fire salamander	30 (2015)	0 (0–0.11)	No evidence of decline†
23, Haeyesbos, deciduous forest			
Fire salamander	30 (2015)	0 (0–0.11)	No evidence of decline†
24, t Burreken, deciduous forest			
Fire salamander	30 (2015)	0 (0–0.11)	No evidence of decline†
Germany			
25, Lamersiefen, deciduous forest			
Fire salamander	17 (2014)	0 (0–0.19)	No evidence of decline†
	32 (2015)	0 (0–0.11)	
26, Fischbach, deciduous forest			
Fire salamander	36 (2014)	0 (0–0.09)	No evidence of decline; 3 dead-found specimens Tested negative for *Bsal* via histology (2014)†
	51 (2015)	0 (0–0.07)	
Alpine newt	1 (2015)	0	Live-studied specimen by chance; no evidence of decline

Site no., location, and amphibian collected	Number of specimens tested (year)	Observed prevalence (Bayesian 95% credible intervals)	Remarks
Palmate newt	1 (2015)	0	Live-studied specimen by chance; no evidence of decline
27, Kallerbach, deciduous forest			
Fire salamander	24(2015)	0 (0–0.15)	No evidence of decline†
28, Rosbach, deciduous forest			
Fire salamander	47 (2015)	0 (0–0.07)	No evidence of decline†
29, Zweifallshammer, deciduous forest			
Fire salamander	41 (2015)	0 (0–0.08)	No evidence of decline†
30, Peterbach, mixed forest			
Palmate newt	12 (2014)	0 (0–0.26)	No evidence of decline
Alpine newt	4 (2014)	0 (0–0.52)	No evidence of decline
31, Haftenbach, deciduous forest			
Fire salamander	46 (2015)	0 (0–0.08)	No evidence of decline†
32, Sauerbach, deciduous forest			
Fire salamander	22 (2015)	0 (0–0.15)	No evidence of decline†
Alpine newt	1 (2015)	0,00	No evidence of decline
33, Härtgessief, deciduous forest			
Fire salamander	15 (2014)	0 (0–0.19)	Strong evidence of decline†
34, Kottenforst, deciduous forest			
Fire salamander	51 (2015)	(0–0.07)	No evidence of decline
35, Großkampenberg, mixed forest			
Alpine newt	4 (2015)	0 (0–0.52)	No evidence of decline
Palmate newt	1 (2015)	0	No evidence of decline
36, Lützkampen -mixed forest			
Alpine newt	8 (2015)	0 (0–0.31)	No evidence of decline
37, Ferschweiler- mixed forest			
Alpine newt	2 (2015)	0 (0–0.70)	No evidence of decline
Palmate newt	8 (2015)	0 (0–0.31)	No evidence of decline
38, Ernzen, mixed forest			
Fire salamander	4 (2015)	0 (0–0.52)	No evidence of decline†
The Netherlands			
39, Moerveld surroundings (A), Bunderbos vicinity			
Alpine newt	13 (2015)	0 (0–0.22)	No evidence of decline‡
40, Moerveld surroundings (B), Bunderbos vicinity			
Alpine newt	34 (2015)	0 (0–0.11)	No evidence of decline‡
41, Snijdersbergweg 21, garden pond			
Alpine newt	60 (2015)	0 (0–0.06)	No evidence of decline‡
42, Mevr van der Meijstraat 12, garden pond			
Alpine newt	19 (2015)	0 (0–0.18)	No evidence of decline‡
43, Mevr van der Meijstraat 20, garden pond			
Alpine newt	17 (2015)	0 (0–0.19)	No evidence of decline‡
44, Snijdersbergweg 20, 2 garden ponds			
Alpine newt	30 (2015)	0 (0–0.11)	No evidence of decline‡
45, Snijdersbergweg 23b, garden pond			
Alpine newt	15 (2015)	0 (0–0.19)	No evidence of decline‡
46, Broekhoven, garden pond			
Fire salamander	2 (2015)	0 (0–0.70)	No evidence of decline‡
47, Meerssen, deciduous forest			
Fire salamander	57 (2013)	0 (0–0.06)	No deaths; no evidence of decline†
	43 (2014)	0 (0–0.08)	
	29 (2015)	0 (0–0.11)	
	2 (2016)	0 (0–0.70)	
48, Carisberg, deciduous forest			
Alpine newt	8 (2014)	0 (0–0.31)	No information available
Palmate newt	23 (2014)	0 (0–0.14)	No information available
Smooth newt	2 (2014)	0 (0–0.70)	No information available
Additional far-out sites (Germany)			
N.S., Solling, deciduous forest			
Fire salamander	23 (2015)	0 (0–0.14)	No evidence of decline‡
N.S., Ilsenburg, deciduous forest			
Fire salamander	8 (2015)	0 (0–0.31)	No evidence of decline‡
N.S., Lelm, deciduous forest			
Alpine newt	57 (2015)	0 (0–0.06)	No evidence of decline‡
Palmate newt	6 (2015)	0 (0–0.43)	No evidence of decline‡

Site no., location, and amphibian collected	Number of specimens tested (year)	Observed prevalence (Bayesian 95% credible intervals)	Remarks
Smooth newt	16 (2015)	0 (0–0.20)	No evidence of decline‡
Crested newt	29 (2015)	0 (0–0.11)	No evidence of decline‡
N.S., Kleiwiesen, exposed ponds surrounded by deciduous forest			
Alpine newt	27 (2015)	0 (0–0.13)	No evidence of decline‡
Smooth newt	117 (2015)	0 (0–0.03)	No evidence of decline‡
Crested newt	27 (2015)	0 (0–0.13)	No evidence of decline‡
N.S., Waldecker Schlossgrund, deciduous forest			
Fire salamander	22 (2015)	0 (0–0.15)	No evidence of decline‡
N.S., Closewitz, exposed ponds surrounded by deciduous forest			
Crested newt	23 (2015)	0 (0–0.14)	No evidence of decline‡
Additional far-out site (the Netherlands)			
N.S., Veluwe, deciduous forest			
Italian crested newt	0 (2015)	0 (0–0.11)	No evidence of decline‡

**Bsal, Batrachochytrium salamandrivorans*; N.S., not shown on map (Figure). Data provide an overview of novel information and previously published data. Site numbers correspond to those on map (Figure).
†Population monitored.
‡Anecdotal report.