

NATIONAL REFERENCE CENTRE FOR *BORDETELLA PERTUSSIS*

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Evolution

The incidence of *B. pertussis* infection typically shows a cyclical pattern, where yearly averages tend to drop and rise again every 3 to 5 years (Clark, 2014). Additionally, yearly peaks appear during the summer months. This was the typical evolution of case numbers in Belgium up until a couple of years ago, with a peak in 2016 where the NRC confirmed 1486 cases of *B. pertussis*. A persistent but not abnormal decrease followed during the period 2017-2019. Moreover, in 2019 a modification was implemented in the pricing of the diagnostic *B. pertussis* PCR (art. 24bis). This change was intended to encourage more selective testing, resulting in a decrease in the number of requested analyses.

In 2020 an extreme decrease in the number of pertussis cases was caused by the distancing measures related to the COVID-19 pandemic, in Belgium and worldwide. From half 2020 to half 2022 barely any cases were confirmed by the NRC. Only in the second half of 2022 a mild increase was observed.

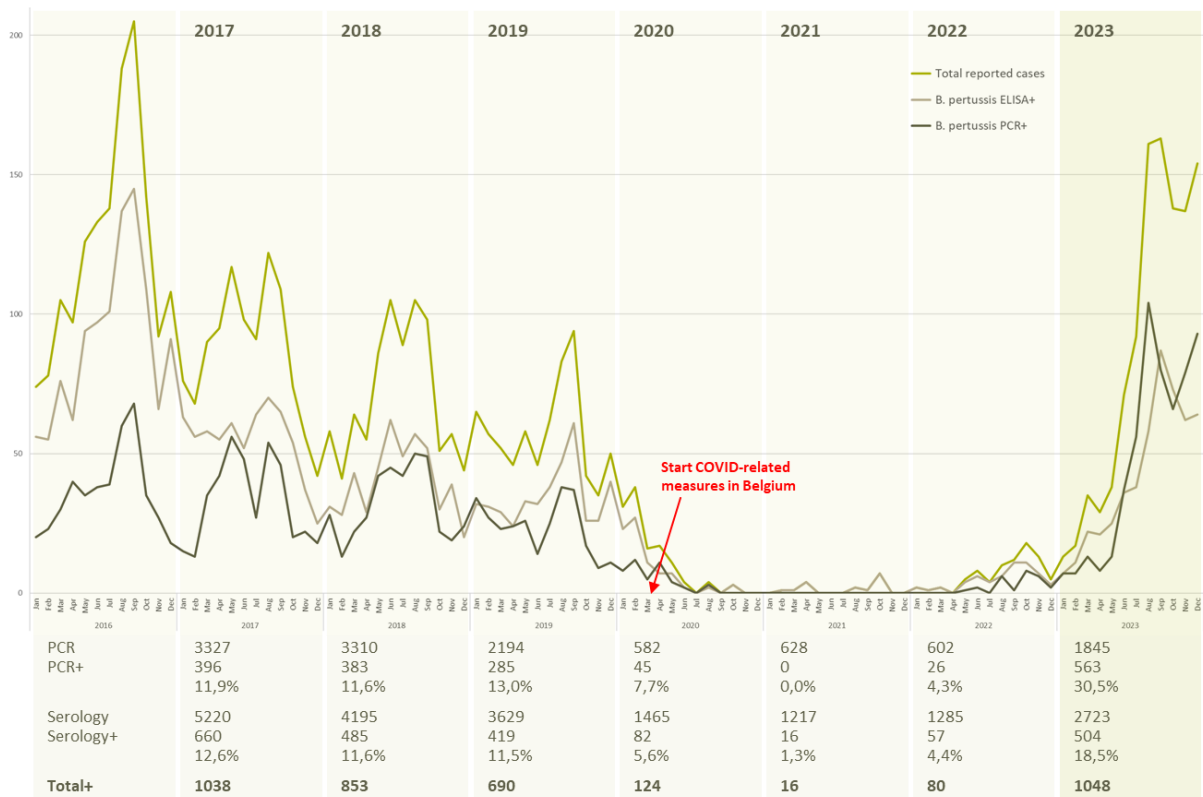


Figure 1: The graph shows the evolution of the total number of laboratory-confirmed pertussis cases. The figures below show the number of tested patients, and the number and percentage of pertussis-positive results, for both PCR-based and serology-based detection.

In 2023 both the total number of requested analyses and the number and percentage of pertussis positive cases rose very quickly. The NRC confirmed a total of 1018 cases of *B. pertussis*.

In 2022 and 2023 we also observe an unusual rise in the number of PCR results positive for *B. parapertussis*. In 2023, 221 cases of *B. parapertussis* were counted by the NRC. However, these consist of mostly (over 90 %) weak positive results, remaining negative in culture. It is possible these are false-positive results caused by contamination of sampling material. On October 19, 2021, a communication was released stating the possibility of a contamination of Copan eSwab® sampling material, often used for respiratory sampling in Belgium. This may cause false positive results in qPCR for *B. parapertussis* (NOT *B. pertussis*) (Copan, 19/10/2021).

After PCR detection of *Bordetella* sp. in a sample, culture and isolation of the strain is attempted. Sensitivity hereof is, however, low. In 2022, 3 *B. pertussis* strains were isolated and stored (out of 26 PCR-positive samples). In 2023 there were 90 (out of 563).

Epidemiology

Usually, a difference is observed in the age distribution of pertussis cases confirmed by qPCR and those confirmed by serology. The mean and range of patient ages for each diagnostic method is shown in table 1. Age distribution is shown in figure 2.

	qPCR	Serology	Total
Mean age	6 y	13 y	9 y
Age range	17 d – 95 y	3 m – 84 y	17 d – 95 y

Table 1: Mean age and range of patients with NRC-confirmed pertussis infection in 2023. Abbreviations: y (years), m (months), d (days).

Patients diagnosed by serology are, on average, older. In 2019 the mean age of these patients was 44 years. However, in 2023 the mean age is remarkably low at 13 years. There were more serological tests especially in children older than 1 year of age and adolescents.

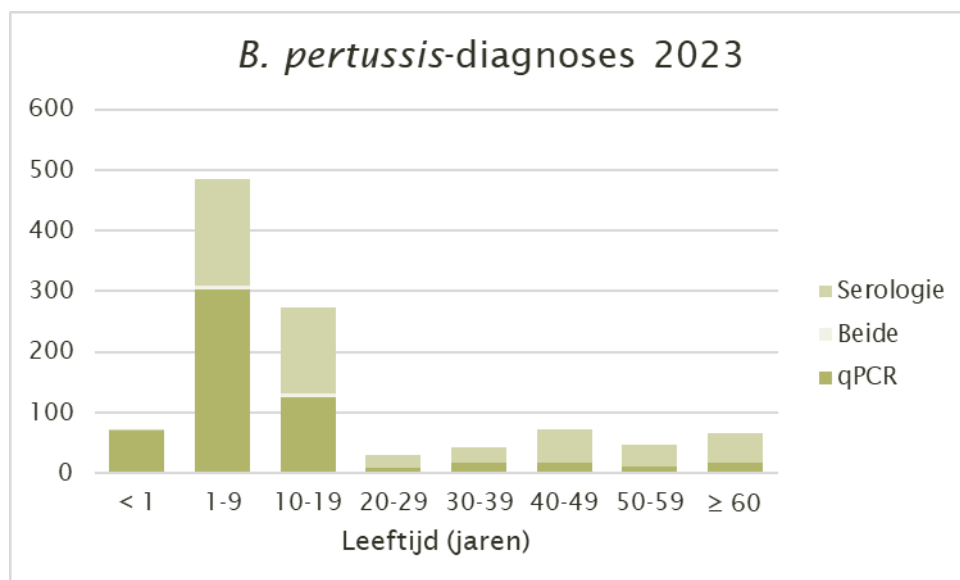


Figure 2: Age distribution of patients with pertussis confirmed by the NRC in 2023.

Antibiotic susceptibility testing

Macrolide resistance was determined by disk diffusion. All strains were found sensitive to both erythromycin and sulfamethoxazole/trimethoprim. Furthermore, the presence of the A2047G-mutation in the 23S rRNA gene, associated with erythromycin resistance, was determined using WGS. The mutation was not present in any of the strains.

Molecular typing

The following virulence genes were typed by whole genome sequencing (WGS) for 92 isolated strains from 2022 and 2023: pertussis toxin subunit 1 (*ptxS1*) and 3 (*ptxS3*), tracheal colonisation factor (*tcfA*), pertussis toxin promoter (*ptxP*), and pertactin (*prn*).

As in 2020, no variation was found for *ptxS1*, *ptxS3*, *tcfA* and *ptxP*, all strains retained the same types that were in the majority before.

Remarkably, almost all strains also show the same pertactin type. 87 strains carry *prn* type 2, 5 carry *prn* type 1. Where during the years before 2021 a rise in strains with *prn* deletion or mutations was evident, these no longer seem to be in circulation.

Year	<i>prn</i> 1	<i>prn</i> 2	<i>prn</i> 3	other type	<i>prn</i> -	<i>ptxS1</i> A	<i>ptxS1</i> B	<i>ptxS3</i> A	<i>ptxS3</i> B	<i>tcfA</i> 2	<i>tcfA</i> 3	other type	<i>tcfA</i> -	<i>ptxP</i> 1	<i>ptxP</i> 3	<i>ptxP</i> 15
1987	1	3	2	1	0	7	0	7	0	7	0	0	0	6	0	0
1988	1	0	0	0	0	1	0	1	0	1	0	0	0	1	0	0
1989	0	4	1	0	0	5	0	5	0	5	0	0	0	5	0	0
1990	3	3	1	0	0	7	0	7	0	5	0	2	0	7	0	0
1991	2	3	5	0	0	10	0	10	0	5	3	2	0	10	0	0
1992	0	5	1	1	0	6	1	7	0	6	1	0	0	7	0	0
1993	0	2	4	0	0	6	0	6	0	3	3	0	0	6	0	0
1994	0	4	2	0	0	6	0	6	0	4	2	0	0	6	0	0
1995	0	1	2	1	0	4	0	4	0	3	1	0	0	4	0	0
1996	1	2	1	0	0	4	0	4	0	3	1	0	0	4	0	0
1997	1	2	9	0	0	11	1	10	2	3	9	0	0	10	0	0
1998	5	4	9	0	0	17	1	14	4	7	9	1	1	9	3	0
1999	1	19	11	0	0	31	0	16	15	20	11	0	0	5	5	0
2000	4	38	12	0	0	54	0	22	32	41	12	0	1	5	6	0
2001	2	42	3	0	0	47	0	10	37	45	1	0	1	3	8	0
2002	2	41	1	1	0	45	0	4	41	43	0	1	1	3	8	0
2003	1	33	2	1	0	37	0	7	30	35	2	0	0	4	6	0
2004	3	45	1	1	0	50	0	4	46	49	1	0	0	3	6	0
2005	1	66	2	0	0	69	0	1	68	67	0	2	0	1	9	0
2006	0	71	0	0	0	71	0	0	71	70	0	0	0	0	10	0
2007	0	64	1	0	0	65	0	1	64	0	0	0	0	1	9	0
2008	0	51	2	0	0	53	0	3	50	53	0	0	0	2	8	0
2009	1	52	0	1	0	54	0	1	53	51	0	0	1	1	9	0
2010	0	30	0	2	0	32	0	0	32	32	0	0	0	0	9	0
2011	0	29	0	0	1	28	1	2	27	30	0	0	0	2	8	0
2012	0	61	0	1	0	62	0	0	62	62	0	0	0	0	60	2
2013	0	90	0	4	0	94	0	1	93	93	0	0	0	1	93	0
2014	1	125	0	0	1	127	0	1	126	126	0	0	0	1	126	0
2015	4	94	0	3	7	108	0	5	103	106	0	2	0	2	106	0
2016	1	100	1	2	14	118	0	2	116	116	0	2	0	1	117	0
2017	0	53	0	9	35	97	0	0	97	93	0	2	2	0	97	0
2018	0	45	0	12	43	100	0	0	100	99	0	0	1	0	100	0
2019	0	45	0	4	24	72	0	0	73	72	0	1	0	0	73	0
2020	0	7	0	2	5	14	0	0	14	14	0	0	0	0	14	0
2021	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
2022	0	3	0	0	0	3	0	0	3	3	0	0	0	0	3	0
2023	5	84	0	0	0	89	0	5	84	87	0	2	0	5	84	0

Table 2: Overview of typing results since 1987. The most frequently occurring types each year are highlighted, as are the pertactin-negative (large deletions or insertions in the *prn* gene).

Phylogenetic relationship

A phylogenetic analysis was performed based on core genome SNP analysis. This suggests a very limited genetic diversity. A number of different clusters of very similar strains are observable, however, within these clusters the strains have different geographic origins.

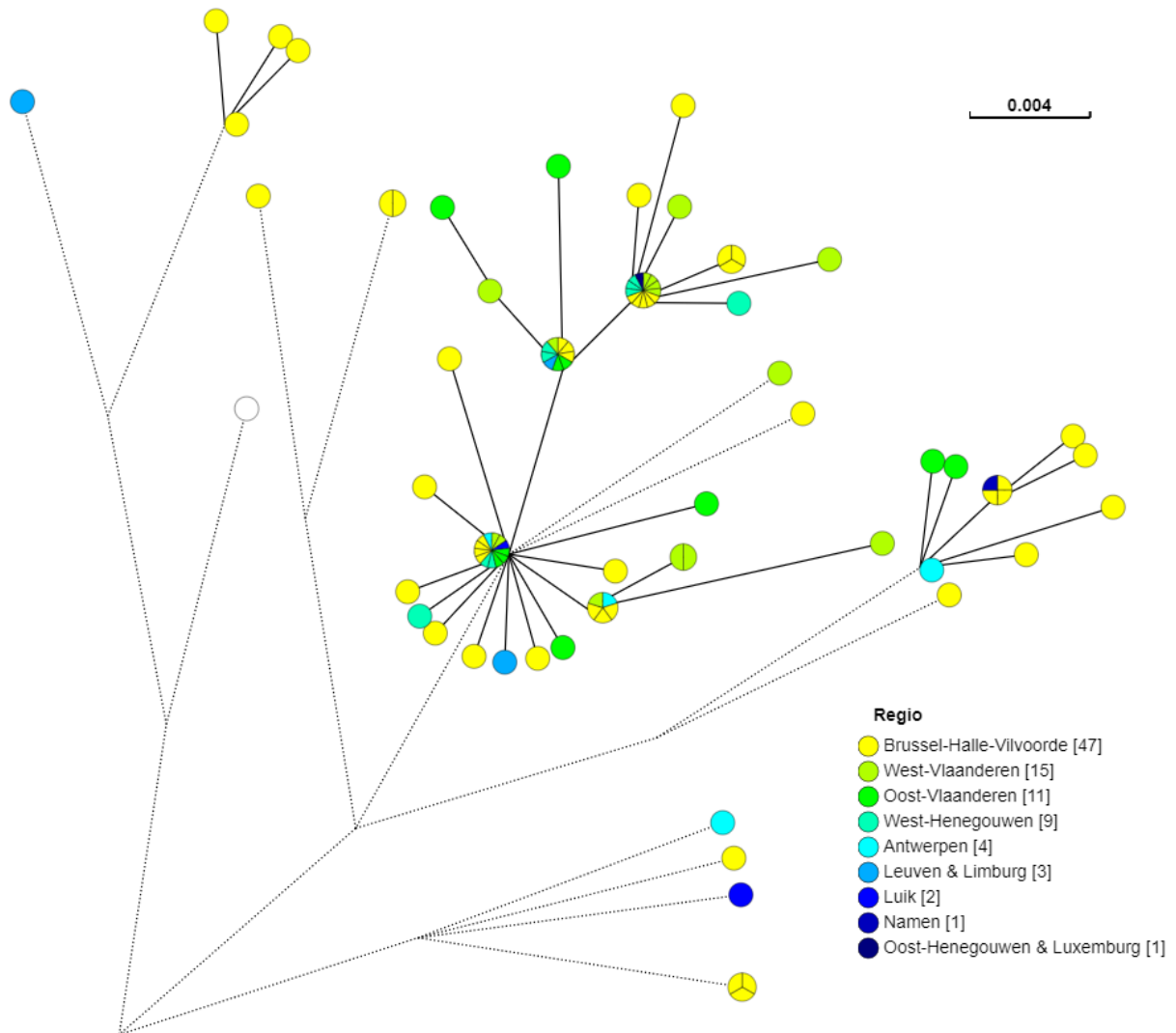


Figure 3: Approximate maximum likelihood tree generated with Fasttree 2.1.11 and visualised by Grapetree, Contigs were aligned with Snippy 4.6.0 using the Tohama-stam as reference (GenBank accession NC002929). Gubbins 2.4.1 was used to filter recombinant regions. Branch length represents the average number of nucleotide substitutions per site, however, branches with length > 0,008 were shortened for visualisation purposes (dotted lines).

Conclusion

After the extremely low *B. pertussis* incidence during the period 2020-2022, caused by the COVID-19 pandemic and related measures, 2023 shows a very abrupt rise in number of cases, to numbers much higher than the years immediately preceding the COVID period. This is the case for Belgium but also the rest of Europe (ECDC, 2023).

In Belgium, 1048 cases of pertussis were confirmed by the NRC in 2023, about 50% more than in 2019 before the COVID epidemic. Moreover, no peak was seen during the summer months, but the upswing seems to plateau or even continue after the end of the year.

The return of whooping cough after cessation of COVID-related measures was expected. The very steep upsurge on a short term, and the rise to numbers beyond those during the period pre-COVID, can be due to factors such as reduced population immunity due to long-term low circulation of the pathogen, as well as possibly a suboptimal vaccination coverage.

WGS analysis of isolated strains seems to show a pause in the rise of pertactin negative strains. Likewise reassuring is the continued absence of macrolide resistant strains.

Phylogenetic analysis shows some clusters of strongly related strains, but geographic origins are spread.

Referenties:

Clark TA. Changing Pertussis Epidemiology: Everything Old is New Again. *The Journal of Infectious Diseases*, 2014.

Copan. Veiligheidskennisgeving eSwab® FSN-2021-001 Ref. FSCA-2021-001. 19 oktober 2021.

ECDC. Weekly Communicable Disease Threats Report, Week 51, 17-23 December 2023.