Activity report from 2011 to 2022

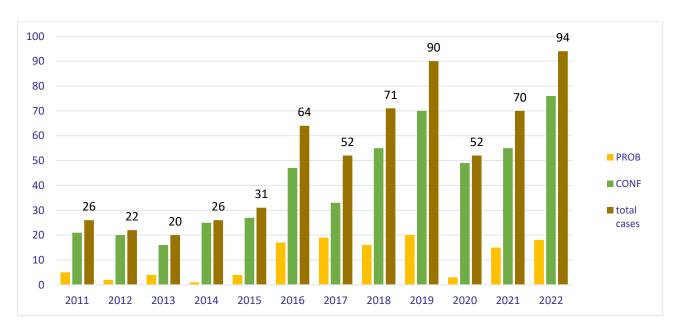
Reference centre for Legionella pneumophila

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The National Reference Centre (NRC) for *Legionella pneumophila* (*Lp*) has gathered data for 618 infections in Belgium between 2011 and 2022. The total cases of Legionnaire's disease (LD) confirmed by the NRC in Belgium have increased over the years (see graph.1), in accordance with the data from the European Centre for disease prevention and control (ECDC) (1). Indeed, a continuous increase over the years was observed except for 2020 and 2021 where a decrease was observed that could be related to the containment measures during the COVID-19 pandemic. In 2022, 94 new cases were reported; a number that is comparable to 90 cases in 2019 (Graph 1).



Graph 1. Cases of Legionnaire's disease confirmed by the NRC (2011-2022)

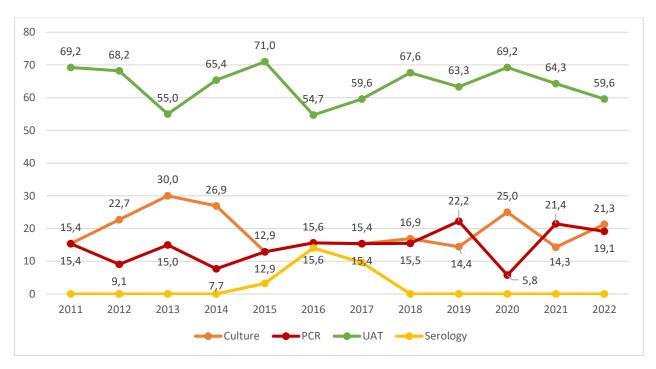
The NRC receives requests from external laboratories on voluntary basis for culture and PCR diagnosis. It receives also isolates for typing from patients and environmental investigations. The

data of infections by *Legionella* species collected within the last decade (2011-2022) were retrospectively gathered and analyzed.

Lp was responsible for 97.7 % (n=604) of the infections, among them 80.4% (n=484) were Lp serogroup 1 (SG-1). The other Legionella species represented 16 cases, with 10 cases for which the species could not be identified, as they were positive in PCR only. The remaining cases were culture positive and were identified as Legionella bozemanii (n=4), Legionella wadsworthii (n=1) and Legionella longbeachae (n=1).

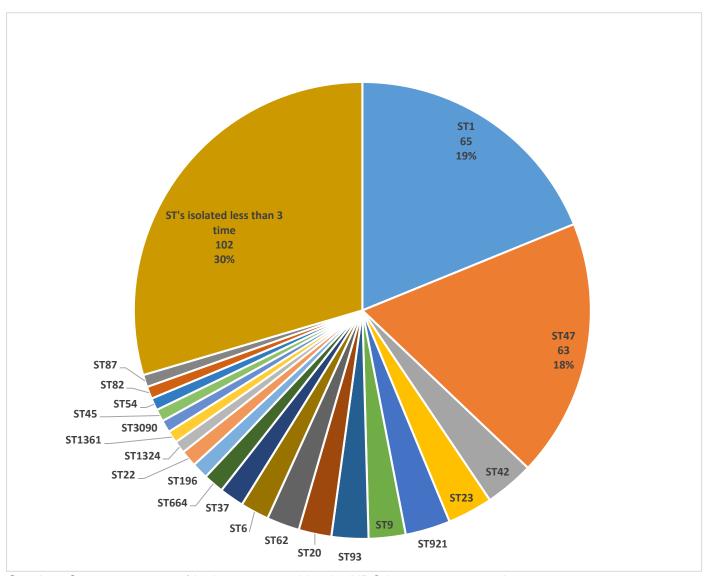
The majority of cases, 69.2% (n=392), were diagnosed by detection of the urinary antigen test (UAT) of *Lp.* SG-1 either before sending respiratory samples to the NRC or tested at the NRC (for a small proportion of the samples). For 12.5% (n=77) the UAT was negative and in 24% (149) of the cases no information was available whether the UAT was carried out or not. In 32.4% (n=200) of these cases both UAT and culture were positive. In 16% (n=99) of the cases only the PCR was positive and then set as "probable" given that the PCR alone is not yet recognized as a confirmation test by the ECDC.

These data confirm that it remains important to perform the three diagnostic methods for each suspicious case, as they all have their advantages and pitfalls. Only 2.4% (n=15) of the cases were reported based on serology that was only performed in an epidemic context as recommended by the ECDC (Graph 2). In total, 73.8% (n=456) of the tested samples are PCR positive and 50.5% (n=312) are culture positive. Since 2016, both methods have in average the same impact on diagnostics at the NRC as shown on graph 2, except for 2020 as aforementioned NRC activities were slowed down during the pandemic. It is nevertheless important to take into account that the requesters often send isolates/samples to the NRC without notifying if they have performed an UAT or PCR test prior to sending; and whether the result was positive for these tests.



Graph 2. Method allowing primary diagnosis of Legionnaires' disease (2011-22)

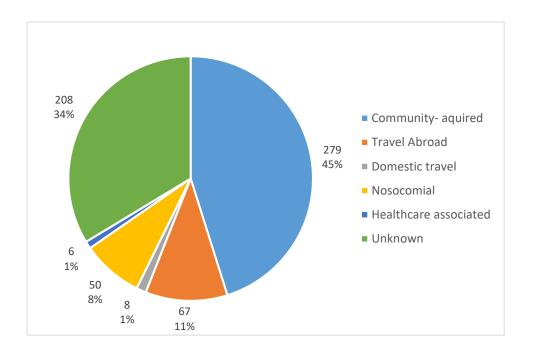
Altogether, 345 isolates were typed by sequence-based typing (SBT), 104 different sequence-types were found. The most common ST's in Belgium were ST1 and ST47, representing respectively 19 and 18% of all identified ST's. This is in accordance with what was previously described regarding the Belgian data from the previous decade (2). ST 42 (12%), ST23 (11%) and ST921(11%) are the other most represented STs, ST42 and ST23 are known to be related to clinical cases worldwide, while ST921 is a rare ST related to an epidemic in our country. Interestingly, ST20 was identified in 8 cases during the previous decade but was also reported in France in 2019 as one of the major ST's (3) (Graph 3). ST1 represented 24% (n=12) of the nosocomial cases while ST47 was associated with only one nosocomial case. For these ST's, no epidemiological link could be confirmed, because they are ubiquitous in the environment. SBT is not able to discriminate isolates with sufficient accuracy to confirm the source. Of note, SBT can be performed on strains but also on samples if the culture is negative. The Nested-SBT, as it is called, where DNA amplification is performed directly from the respiratory sample, not from isolates, is less sensitive than the one performed on culture but can help resolve uncertain cases. It was introduced in 2017; it was performed 121 times and gave complete profile in 39 cases and partial profile (2 to 6 genes out of 7) in 42 cases.



Graph 3. Sequence types of isolates reported by the NRC between 2011 and 2022.

The infection was acquired in the community in 45% (n=279), by travelling abroad in 11% (n=67) and nosocomial in 8 % (n=50) of the cases respectively. The source remains undocumented in 34% (n=208) of the cases because the information is missing on the NRC forms (Graph 4).

Forty environmental investigations resulted in isolates being sent to the NRC; among them 15 were matching the clinical isolate, 12 were also probably matching but this could not be confirmed, either because they belonged to the most prevalent STs, ST1 and ST47 (2) or because of partial SBT results. Seven investigations were non-matching and the remaining were inconclusive.



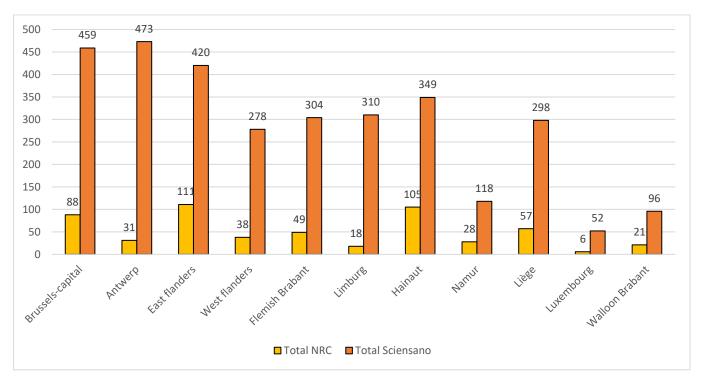
Graph 4. Infection sources of Legionnaire's disease reported by the NRC in Belgium 2011-2022

The gender ratio of the patients was 2.66/1 (male/female). Median age of the patients was 61 (11-94). The mortality rate cannot be evaluated, as the information is often not known when the samples reach the laboratory or is not completed on NRC forms.

Clinical symptoms are often not mentioned on NRC forms, they were lacking in 31.6% (n=195) of cases. Pneumonia or respiratory symptoms were reported for 66.2% (n=409), two hemoptysis were reported. Extra pulmonary cases were scarce (that we know of), of note two hepatitis and one neurological presentation, 8 clinical presentation of Pontiac fever and a cellulitis (but associated to a pneumonia).

East Flanders, Brussels Capital Region and Hainaut gathered the majority of the cases reported to the NRC. Limburg, Namur, West Flanders, Walloon Brabant and Antwerp seem to have fewer cases. LD is known to have hot spots of occurrence like in South of France, Italy or Slovenia in Europe. However, considering the small size of Belgium and its general weather, the difference of incidence between the provinces is not expected to be very high. In addition, the non-proportionality of demographic rates in relation to the total cases in some provinces would suggest that the observed difference between the provinces is rather due to differences in the habits of the laboratories; some do not send samples systematically to the NRC. Indeed, when compared to the regional incidence in Belgium, as reported by Sciensano (based on the total notifications of LD to Sciensano), the majority of the cases were from East Flanders, Brussels Capital and Antwerp (Graph 5). The NRC

receives samples for only 17% of this total, with only 7% and 6% of the total in Antwerp area and Limburg respectively.



<u>Graph 5</u>. Belgian provinces where cases were reported between 2011-2022 by both Sciensano and the NRC.

Five community-acquired epidemics were investigated during the period observed, a cluster travel associated was also observed.

- 2016: an outbreak occurred in Dendermonde (East Flanders), for which 1 respiratory sample and 16 serum samples were sent to the NRC. Ten out the 17 cases were confirmed LD cases, 3 were probable and 4 possible cases. Lp SG-1 was cultured in the single available respiratory sample and was identified as ST48. The most likely source of this outbreak was a cooling tower but the human isolate could not be matched with environmental strains due to due to delay in sampling of environmental sources (4).
- 2017: in a potatoes factory of Nieukwerke (West Flanders) where 127 cases of Pontiac fever were associated (76 in France and 51 in Belgium) thanks to serology and PCR tests. No ST was associated as no Lp isolate was available for typing.
- From April to June 2019 an outbreak of *L. pneumophila* SG-1 occurred around an industrial zone in Ghent (East Flanders). In total 33 LD cases were observed of whom two cases died. Respiratory samples from 19 cases were available for typing at the NRC. Nine cases were confirmed to be ST921, two cases could only be partially typed but seemed to correspond to ST921 as well. A different ST was observed for two probable cases, excluding them from the outbreak cluster and the

typing failed in six cases. An identical ST921 strain could be detected in the samples from a cooling tower at a recycled paper factory, linking this source to the outbreak cluster (5).

- In 2021,
 - o Five patients from Herenthals (Turnhout area) got infected (4 confirmed and one probable), a swimming pool was the probable source. The isolates found were ST54 and the environmental investigation resulted in matching isolates.
 - o Two other cases were reported from a travel to Sardini, the patients were unrelated but travelled to the same area at the same time.
- In 2022, 6 confirmed cases were reported between August and September in the city of Mouscron. The clone responsible was Lp SG-1 but the typing was available only for two patients isolates for which ST703 and ST2697 were found. Two other patients had a partial SBT result that could correspond to ST703 suggesting that this ST was probably the responsible for the epidemic. Unfortunately, no isolate was received from the environmental investigation and consequently no infection source could be highlighted.

In conclusion, the continuous increase of LD cases might become a threat in the coming decade. Therefore, the collection of isolates and data sharing should be done in a more optimal way by clinical laboratories in order to enable an adequate follow-up of the epidemiological data and environmental investigations.

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