

# Teknologisk Institut; Legionella-temadagen 2024

STATENS  
SERUM  
INSTITUT



## Legionella, legionærsygdom & analysemetoder

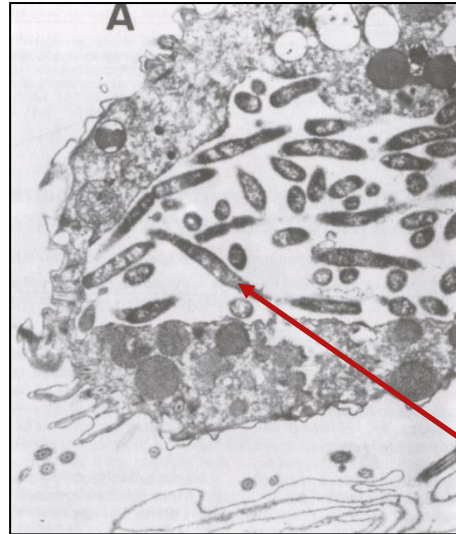
Søren Uldum, seniorforsker, SSI.

# Legionella - bakterierne

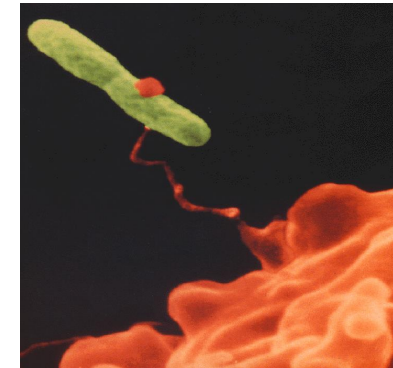
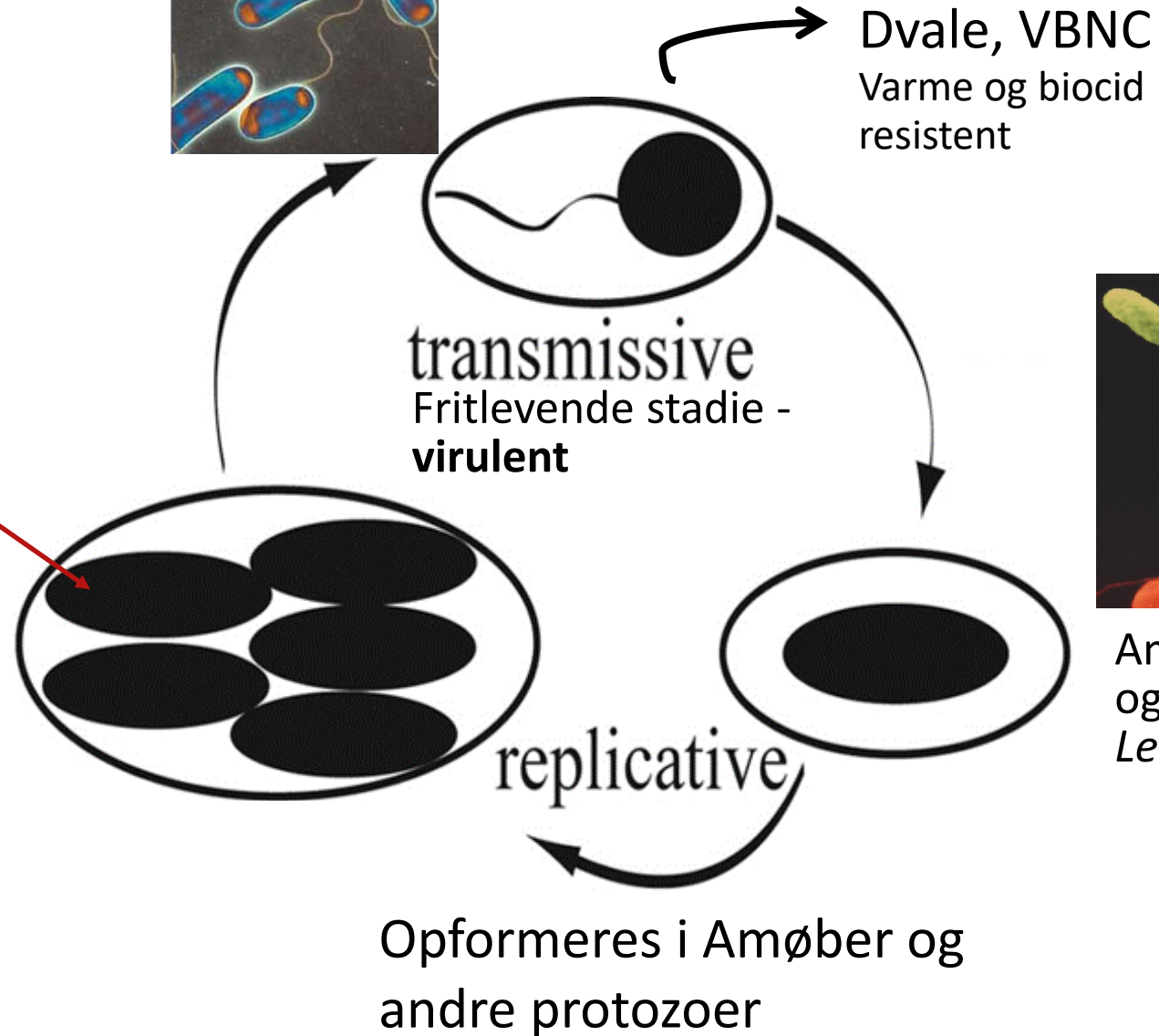
- Små stavformede Gram-negative bakterier (2µm), de fleste med en til to flageller
- Der er beskrevet mere end 60 arter, heraf har 25 har været associeret med sygdom hos mennesker
  - F.eks. – *L. pneumophila*, *L. bozemanæ*, *L. micdadei*, *L. longbeachæ* (kompost)
- *Legionella pneumophila* forårsager (måske) ca. 90% af alle tilfælde af legionærsygdom
- *L. pneumophila* kan opdeles i 16 serogrupper
- Serogruppe 1 forårsager 50-60% af alle dyrkningspåviste tilfælde i Danmark



# Legionella livscyklus



Amøbe med  
*Legionella*



Amøbe fanger  
og optager  
*Legionella*

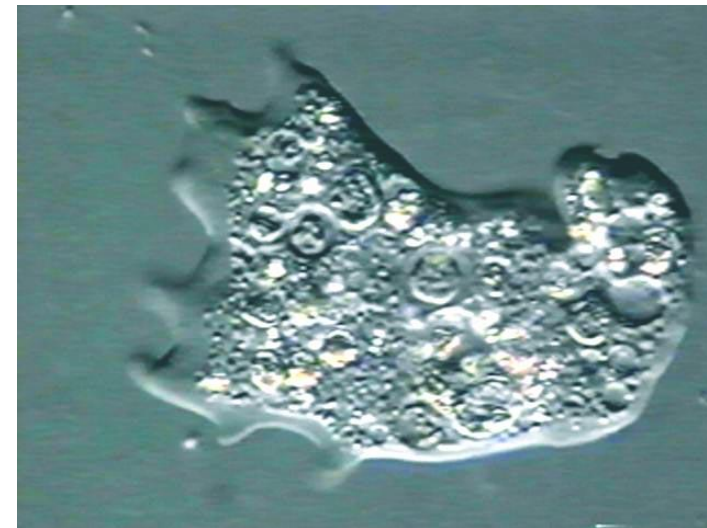
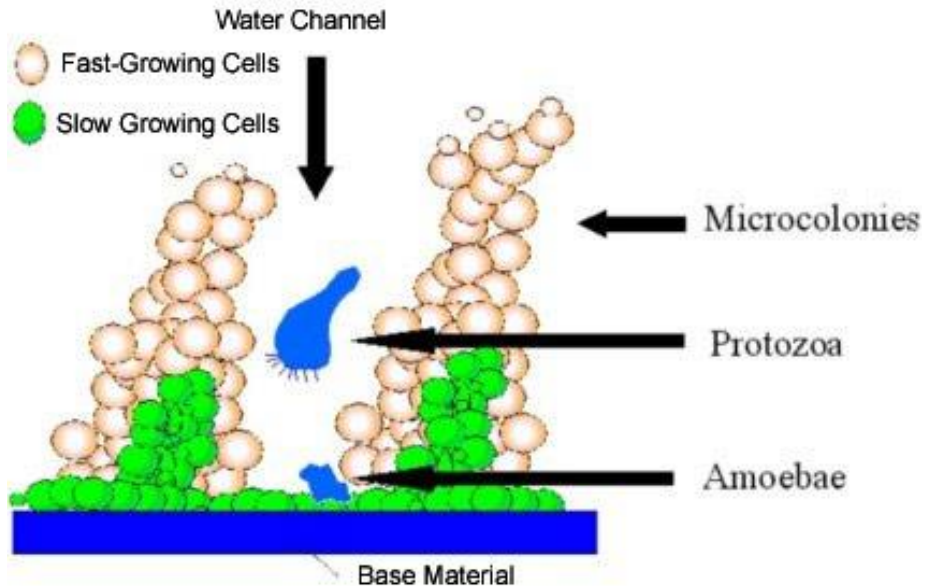


# Legionella og biofilm

## Biofilm - Protozoer



Ciliat

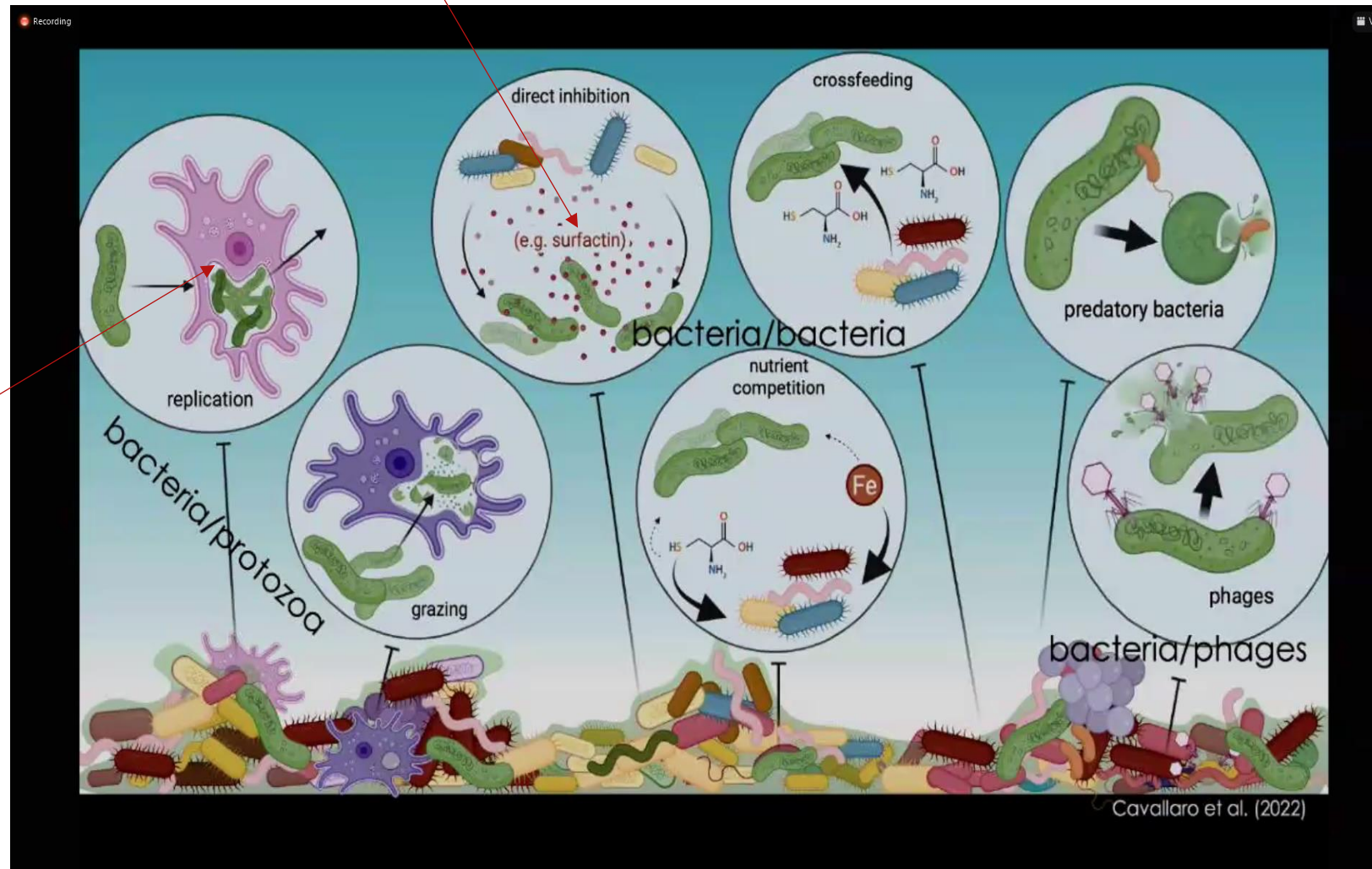


Amøbe

# Legionella og biofilm

Visse bakterier (fx *Pseudomonas*) har negativ betydning for tilstedeværelse af *Legionella*

De fleste protozoer (fx amøber og ciliater) har positiv betydning for tilstedeværelse af *Legionella*



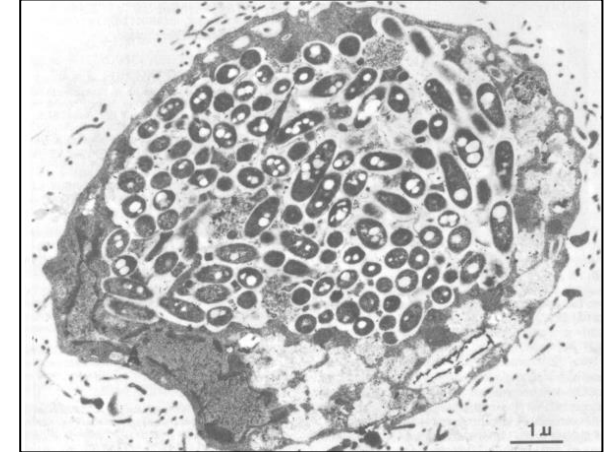
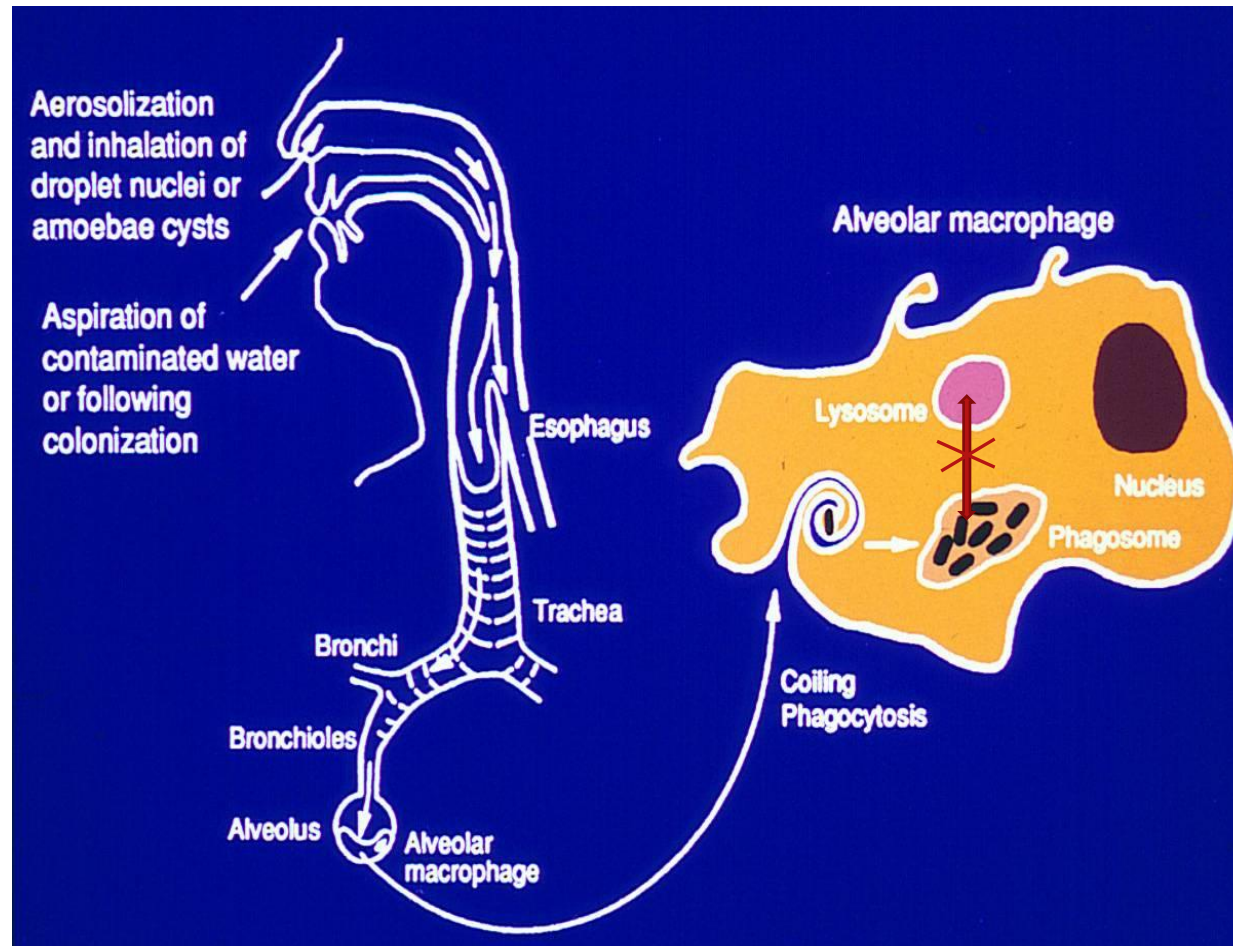
Hammer F. Legionella in complex biofilms: Collaborators and antagonists within the building plumbing microbiome. Oral presentation 10<sup>th</sup> International Legionella Conference, Japan, 2022



# Legionærsygdom



Overføres oftest til mennesker ved inhalation af kontaminerede vandpartikler (**aerosoler**)



Human makrofag  
(hvid blodcelle i lungerne)  
inficeret med *Legionella*

# Legionærsygdom

- Akut lungebetændelse
- Attack-rate < 5 % (ved udbrud)
- Dødelighed < 5 – > 35 % (10 – 15%)
- Inkubationstid 4 – 10 dage
- Ingen person – person smitte
- Kan behandles med antibiotika
  - (men ikke med penicillin!)
- Alder > 50 år
- Immunologisk svækkede
- Organtransplanteret
- Leukæmi
- Diabetes
- Tobak & alkohol
- Kronisk lunge & hjertesygdom
- Rejse/hotelophold
- Arbejde med aerosoliseret vand



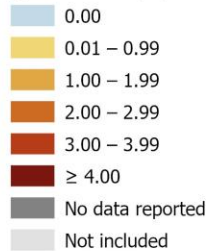
## Pontiac feber

- Influenza-lignende sygdom
  - Alle kan blive syge (raske og børn)
  - Varighed: 2 – 7 dage uden behandling
  - Attack rate ~ 100 %
  - Dødelighed 0 %
- Smitter kun ved indånding af aerosoler

# Age-standardised rates of Legionnaires' disease per 100 000 population, EU/EEA, 2023



Age-standardised rate  
(per 100 000 population)



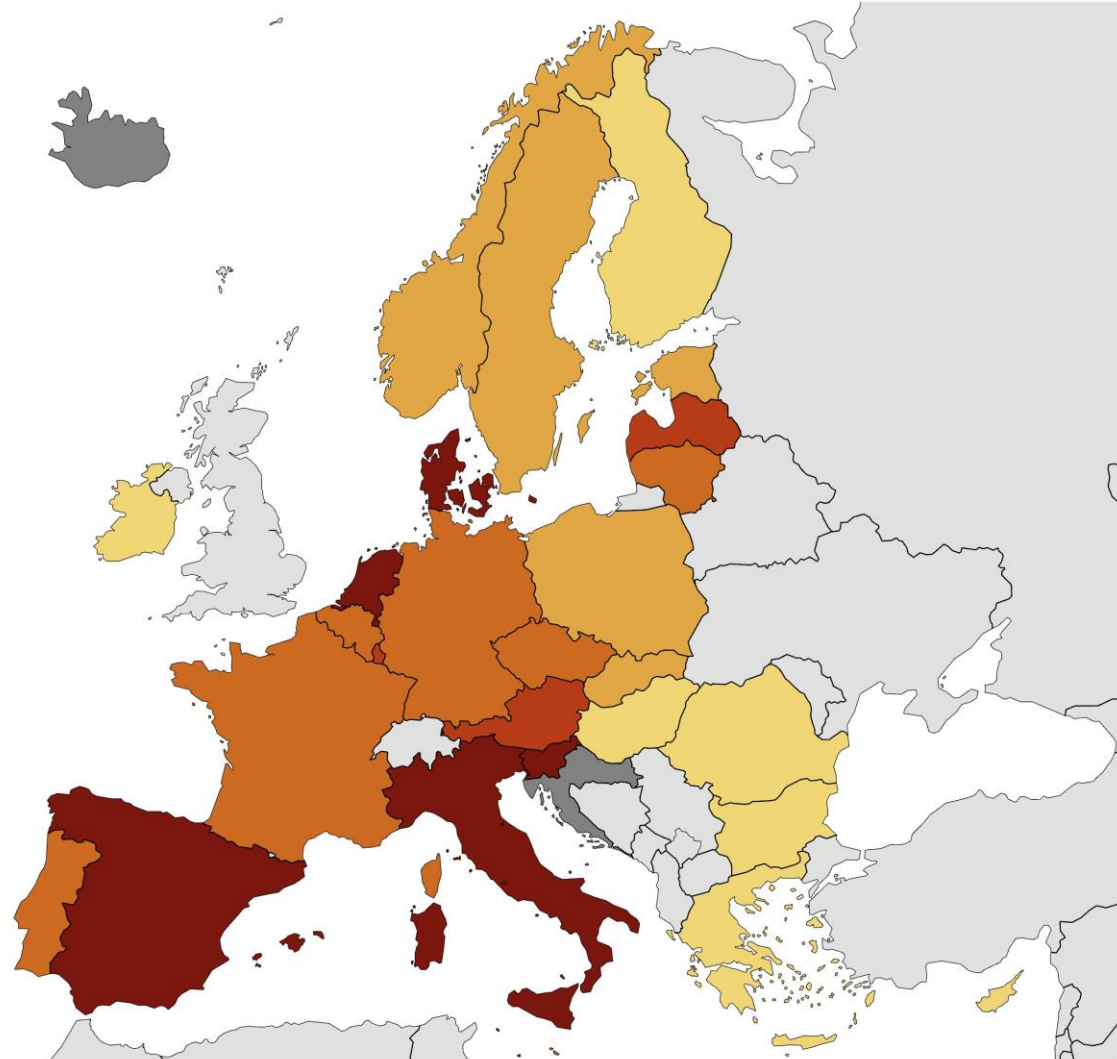
Countries not visible  
in the main map extent



Liechtenstein



Malta



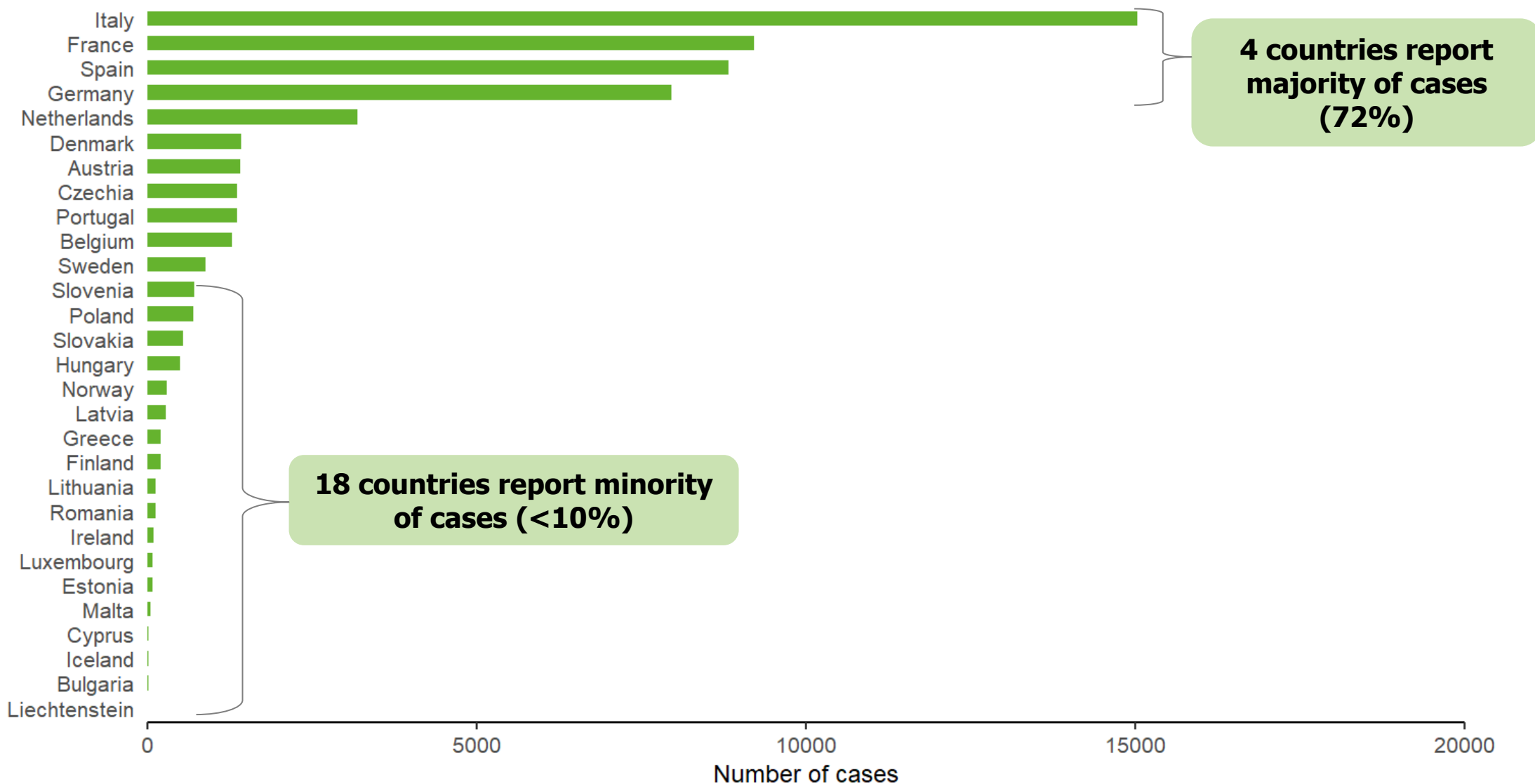
Administrative boundaries: © EuroGeographics. The boundaries and names shown on this map do not imply official endorsement or acceptance by the European Union. Map produced by ECDC on 2 September 2024

**EU/EEA ASR rate:**  
2.7 per 100 000 population

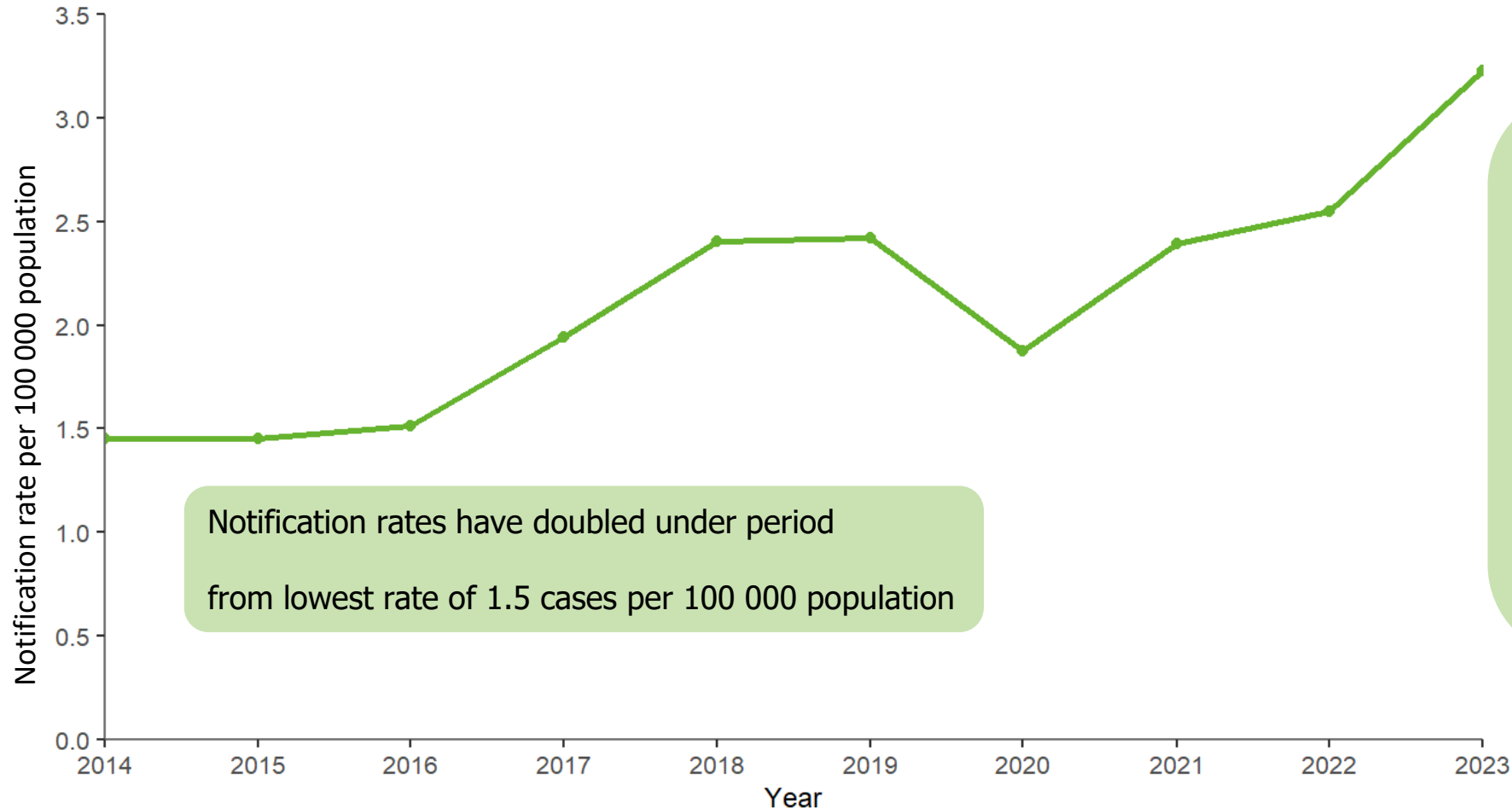
| 2023<br>ASR ranking | ASR rate<br>per 100 000<br>population |
|---------------------|---------------------------------------|
| 1. Slovenia (SI)    | 6.9                                   |
| 2. Italy (IT)       | 5.0                                   |
| 3. Denmark (DK)     | 4.6                                   |
| 4. Netherlands (NL) | 4.4                                   |
| 5. Spain (ES)       | 4.0                                   |



# Number of Legionnaires' disease cases, by reporting country, EU/EEA, 2019-2023 (n=56 001)



# Annual notification rates of Legionnaires' disease, EU/EEA, 2014-2023



Notification rates have doubled under period  
from lowest rate of 1.5 cases per 100 000 population

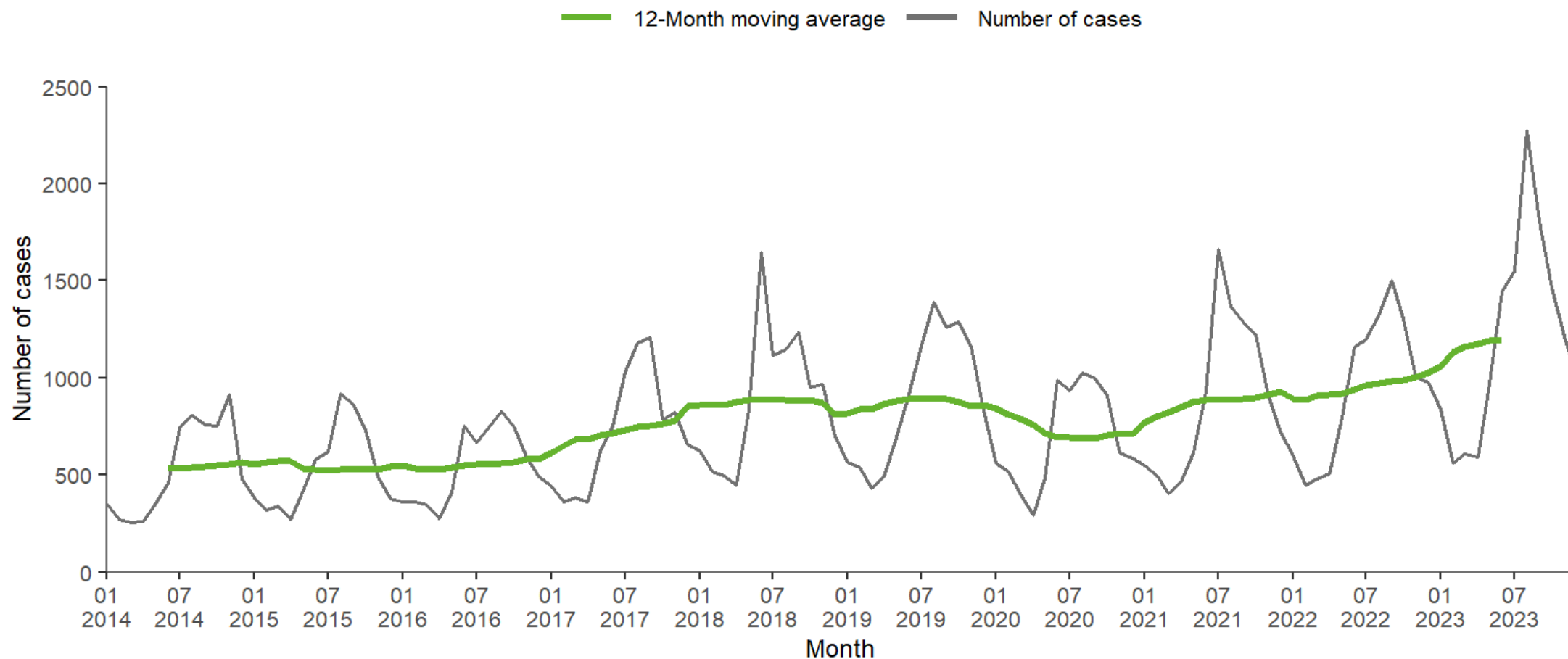
**2023, EU/EEA**

- **highest rate to date**  
= 3.2 per 100, 000 population
- **highest observed numbers**  
= 14 537 reported cases  
= **27%** increase on 2022

Source: Country reports from Austria, Belgium, Bulgaria, Croatia, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Liechtenstein, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden.

Data source: TESSy 2023 annual LEGI dataset, as of 6 August 2024. For comparative purpose, UK data reported in 2019 are excluded. Time period based on variable 'DateUsedForStatistics'.

# Trend of Legionnaires' disease, EU/EEA, 2014-2023

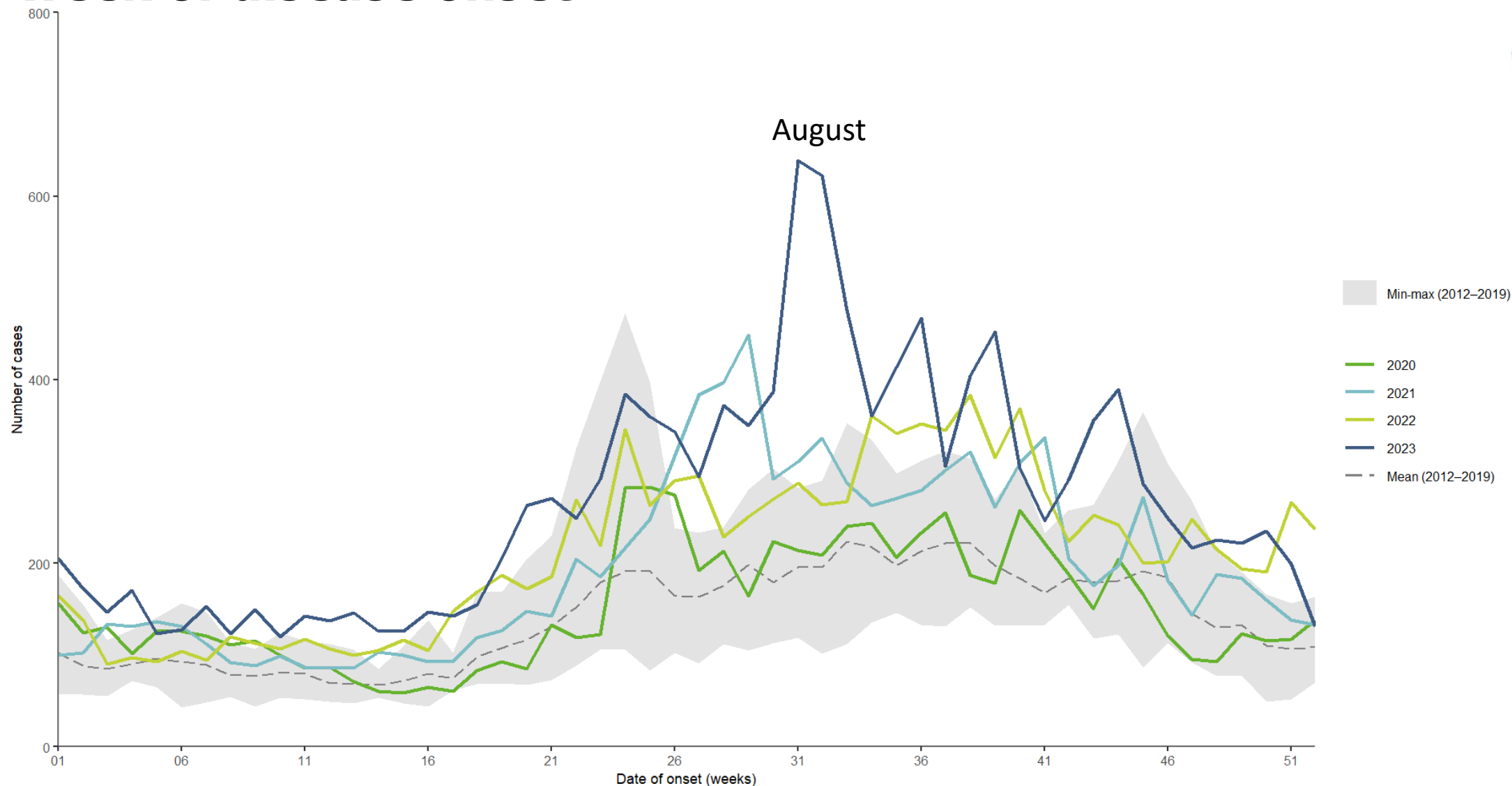


**2023 :**  
highest summer peak observed since 2014

Source: Austria, Belgium, Czechia, Denmark, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden



# LD cases, EU/EEA, 2020-2023 and 2012-2019 comparison, by week of disease onset

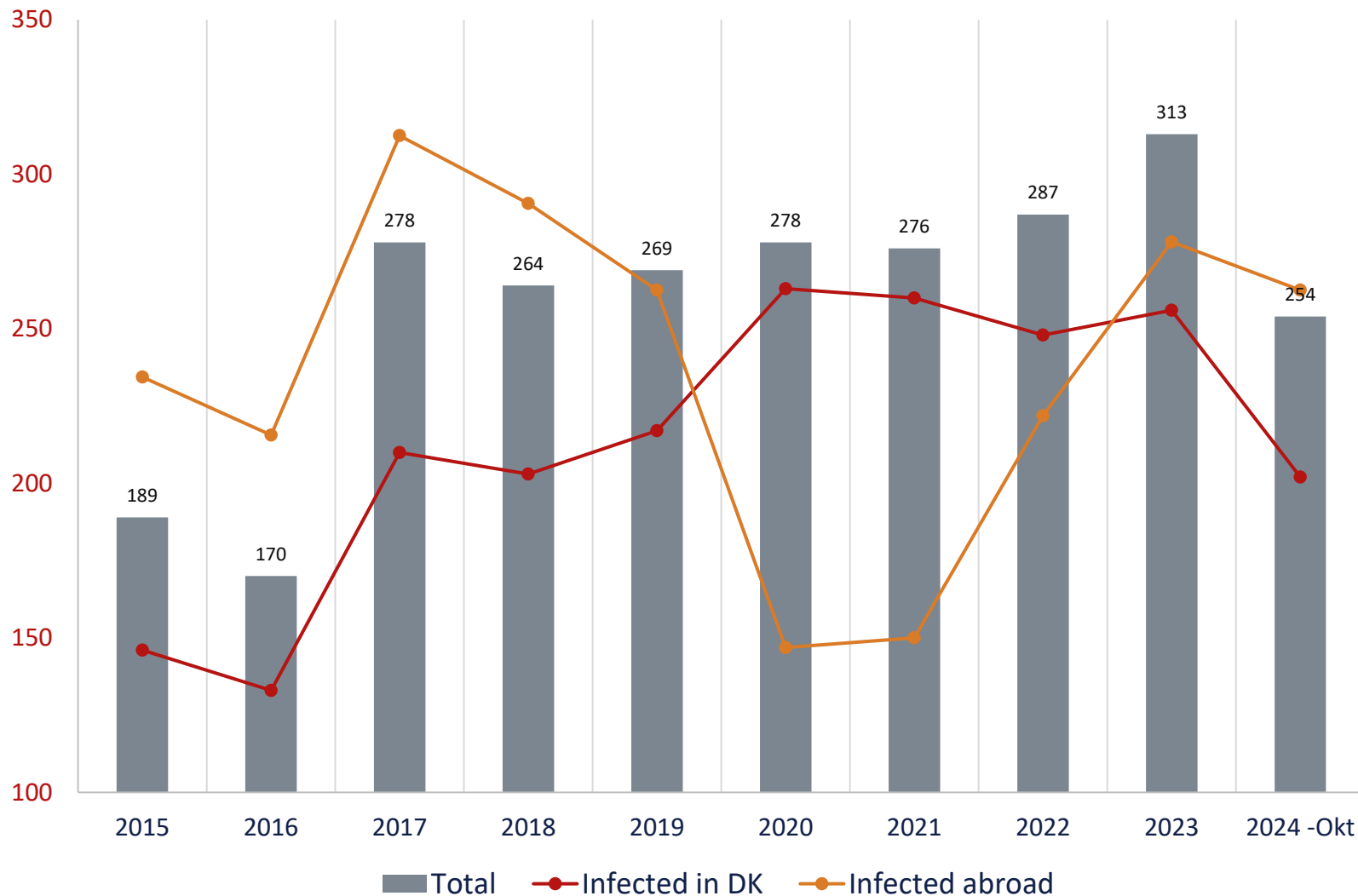


Data source: EU/EEA 29 countries of Austria, Belgium, Bulgaria, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Liechtenstein, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, and Sweden. TESSy 2023 annual LEGI dataset, as of 6 August 2024. For comparative purpose, UK data are excluded. Time period based on variable 'DateOfOnset'.

# Legionærsygdom Danmark 2015 – 2024 (okt.)

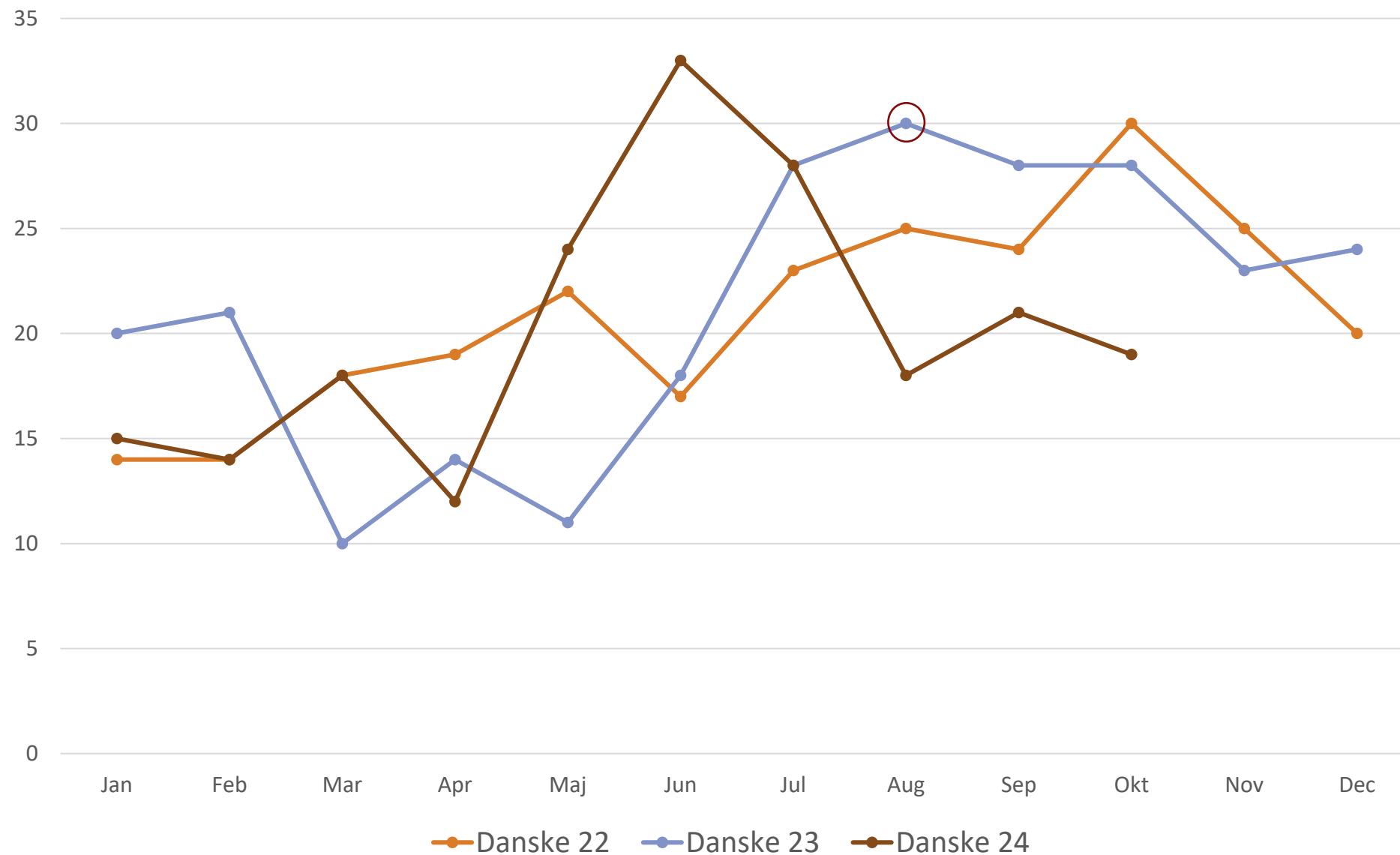


Tilfælde smittet  
i alt og i  
Danmark.



Tilfælde smittet  
i udlandet.

# Dansksmittede tilfælde pr. måned 2022, 2023 og 2024 (okt.)



# Tilfælde af legionella-pneumoni, fordelt på formodet smittekategori og vitalstatus 2023

| Smittekategori  | Samlet antal | Døde | Døde % | Median alder |
|-----------------|--------------|------|--------|--------------|
| Samfund DK      | 202          | 20   | 9,9    | 73           |
| Rejse DK        | 11           | 1    | 9,1    | 65           |
| Hospital        | 12           | 3    | 25     | 80,5         |
| Inst./plejehjem | 22           | 9    | 40,9   | 84           |
| Ukendt          | 9            | 2    | 22,2   | 59           |
| Rejse udland    | 57           | 0    | 0      | 63           |
| I alt           | 313          | 35   | 11,2   | 71           |

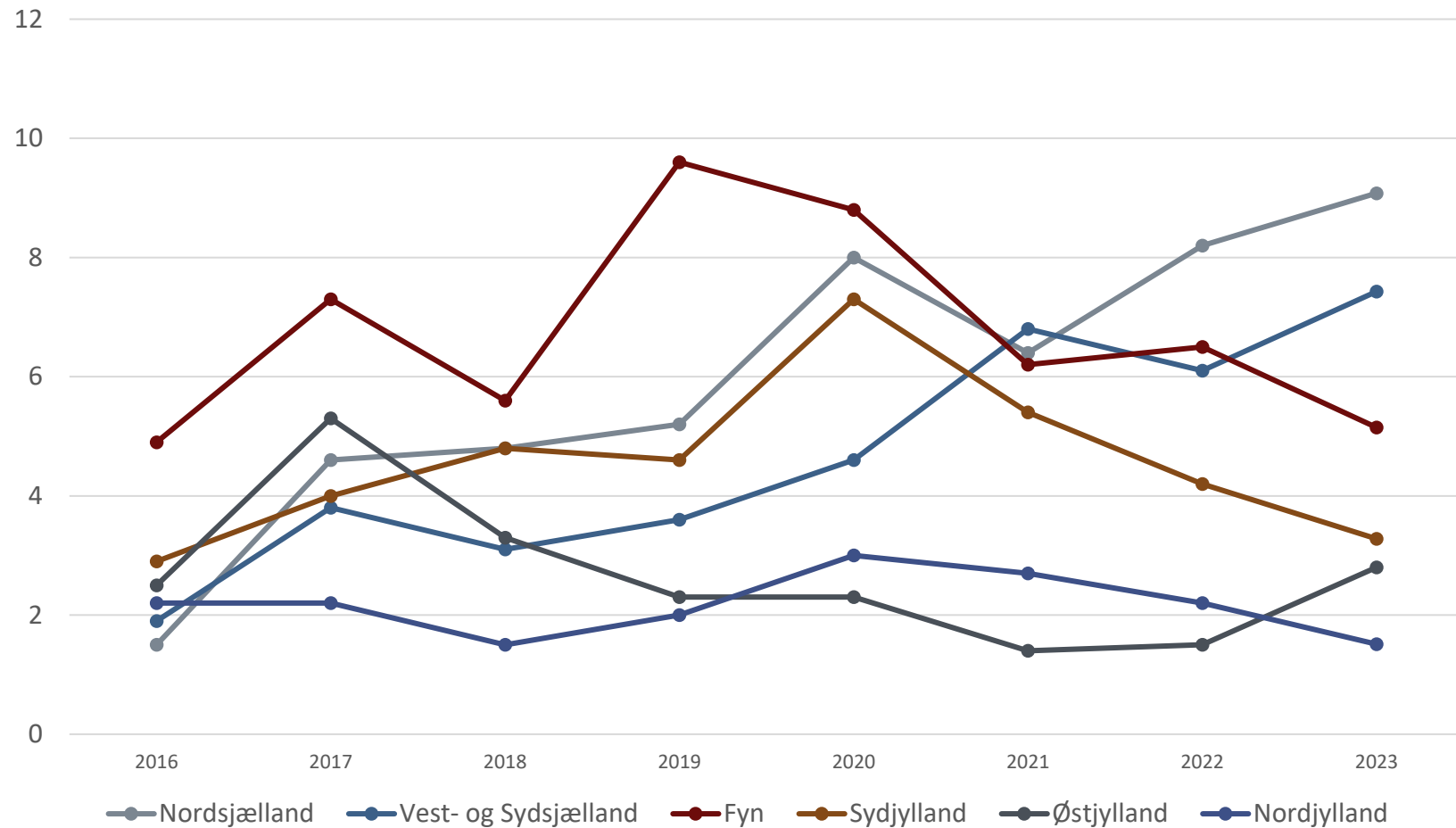


## Antal tilfælde og incidens pr. 100.000 erhvervet i Danmark 2023 fordelt på region og landsdel

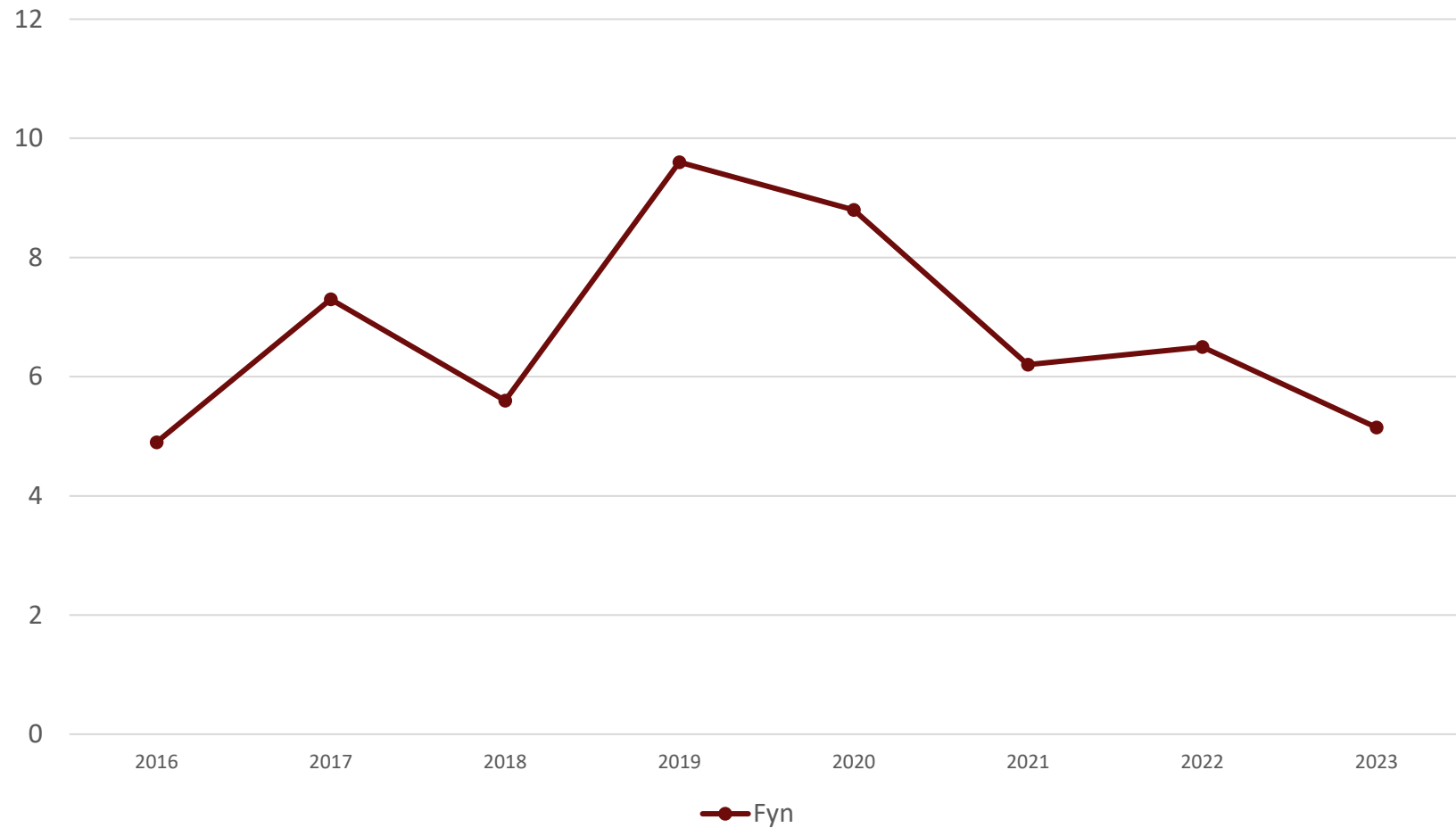


| Region      | Landsdel             | Antal 2023 | Incidens |
|-------------|----------------------|------------|----------|
| Hovedstaden | København by         | 27         | 3,31     |
|             | København omegn      | 33         | 5,87     |
|             | Nordsjælland         | 43         | 9,08     |
|             | Bornholm             | 1          | 2,52     |
| Sjælland    | Østsjælland          | 9          | 3,49     |
|             | Vest- og Sydsjælland | 44         | 7,43     |
| Syddanmark  | Fyn                  | 26         | 5,15     |
|             | Syddanmark           | 24         | 3,28     |
| Midtjylland | Østjylland           | 26         | 2,80     |
|             | Vestjylland          | 14         | 3,25     |
| Nordjylland | Nordjylland          | 9          | 1,51     |
| Danmark     | I alt                | 256        | 4,32     |

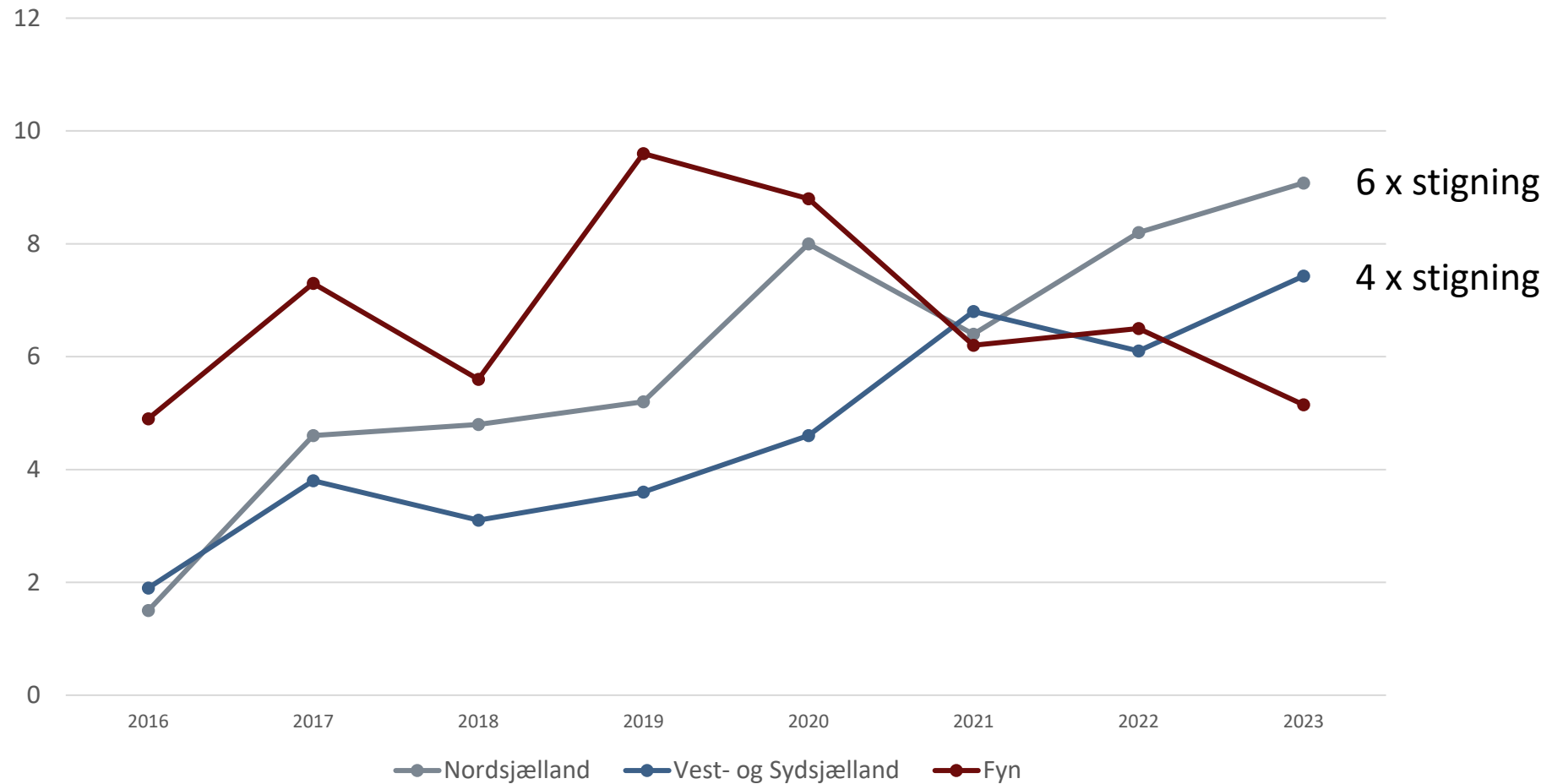
# Incidens pr. 100.000 erhvervet i Danmark fordelt på udvalgte landsdele 2016 til 2023



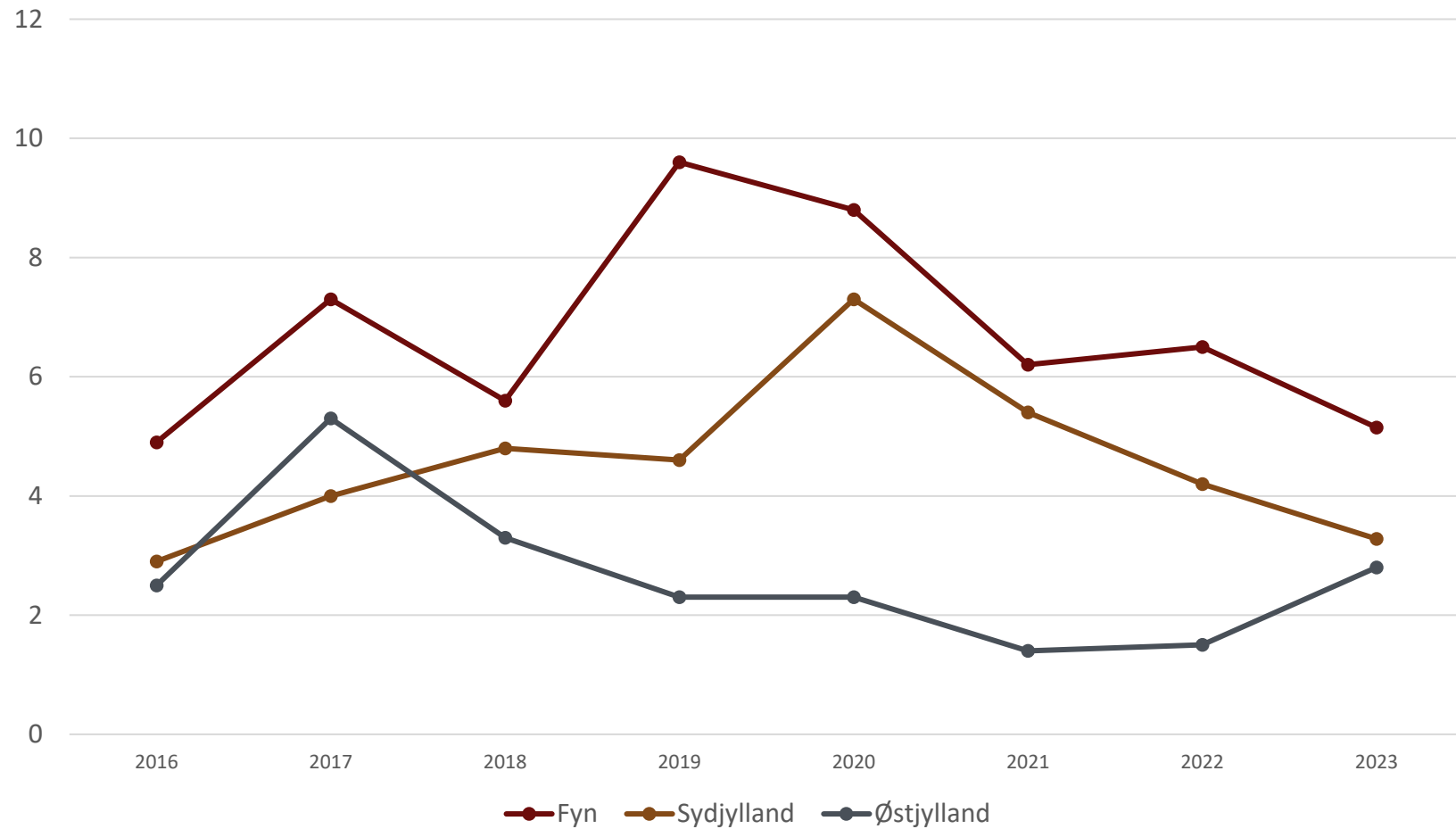
# Incidens pr. 100.000 erhvervet i Danmark fordelt på udvalgte landsdele 2016 til 2023



# Incidens pr. 100.000 erhvervet i Danmark fordelt på udvalgte landsdele 2016 til 2023

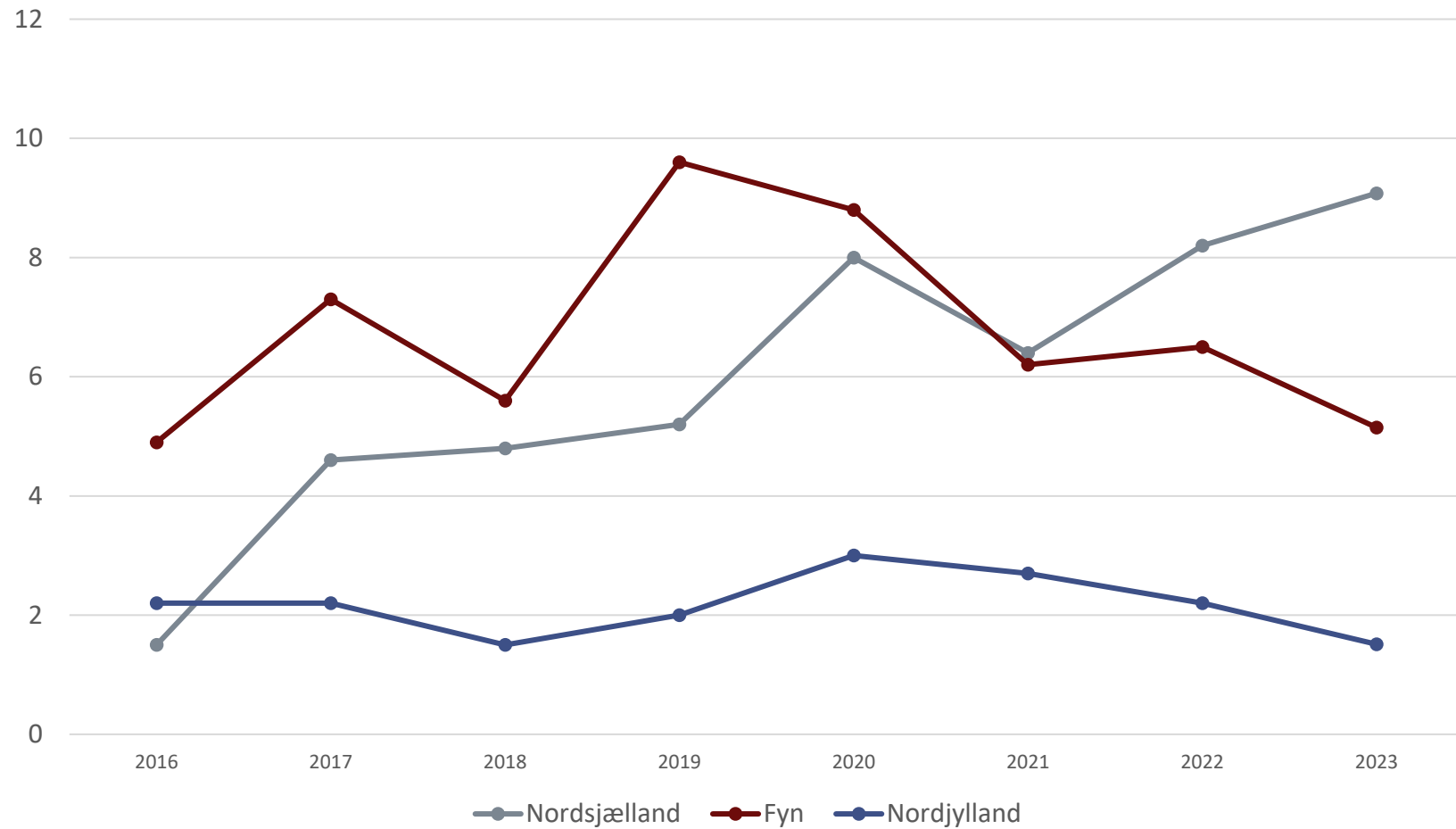


# Incidens pr. 100.000 erhvervet i Danmark fordelt på udvalgte landsdele 2016 til 2023

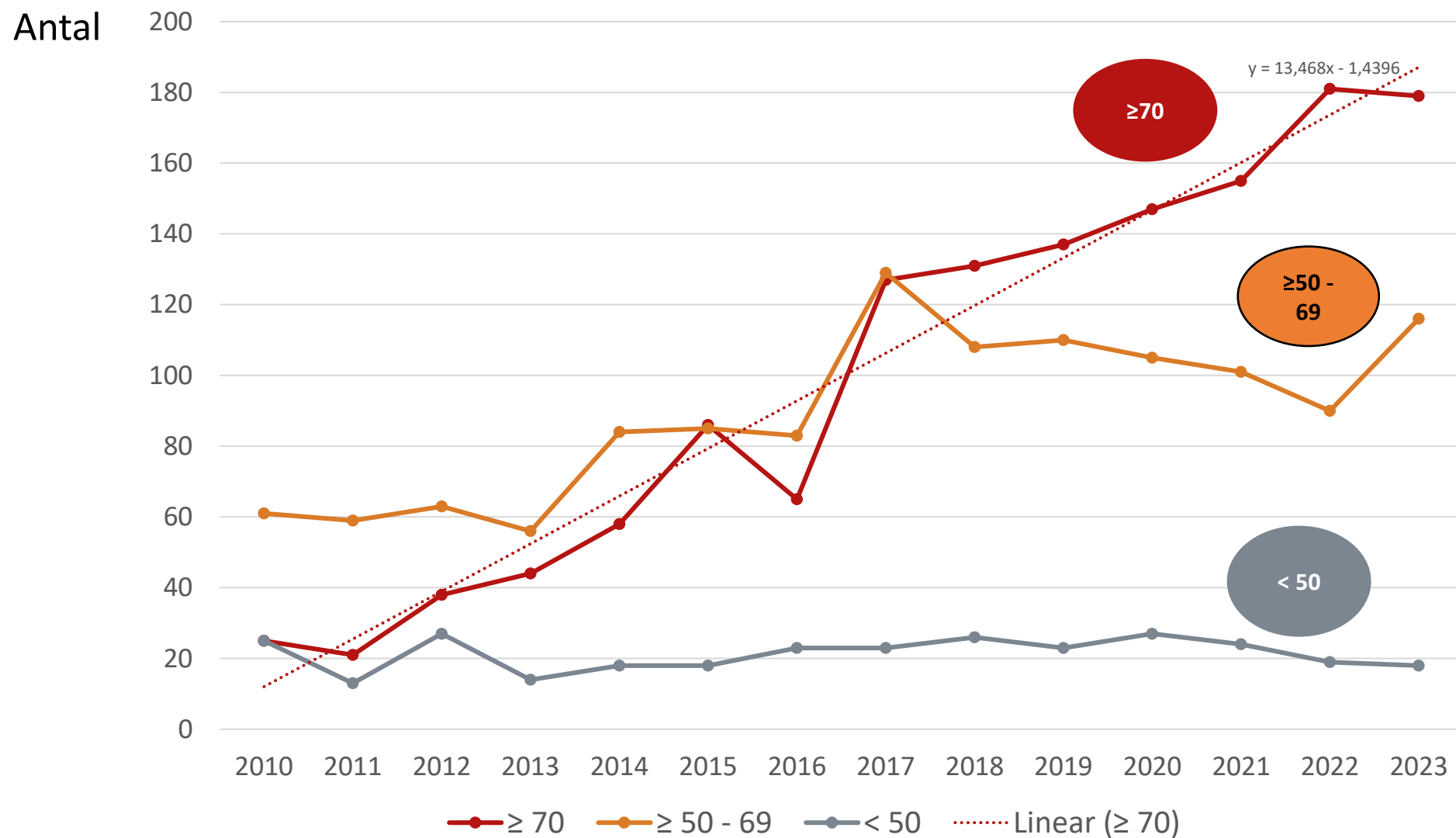




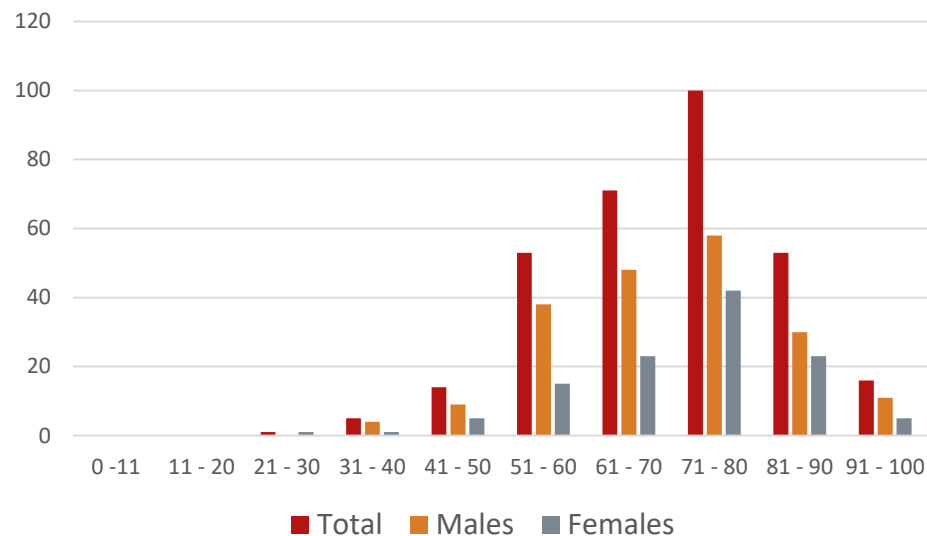
# Incidens pr. 100.000 erhvervet i Danmark fordelt på udvalgte landsdele 2016 til 2023



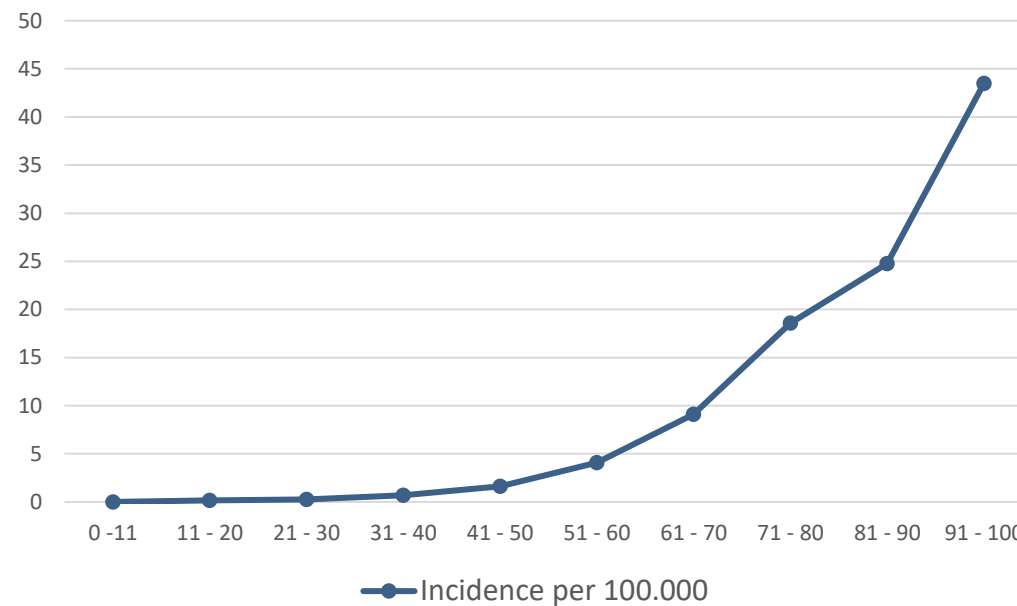
# Aldersgruppefordeling 2010 til 2023.



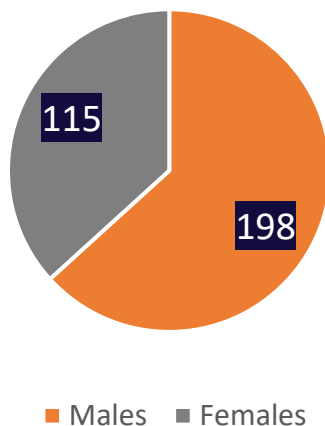
# Alders- og kønsfordeling samt alderskorrigeret incidens- 2023



Incidence per age groups per 100.000, LD 2023

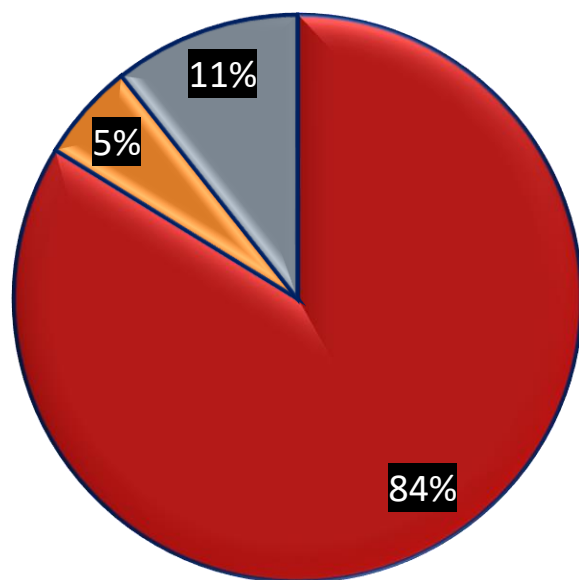


Mand-kvinde ratio: 1,7:1

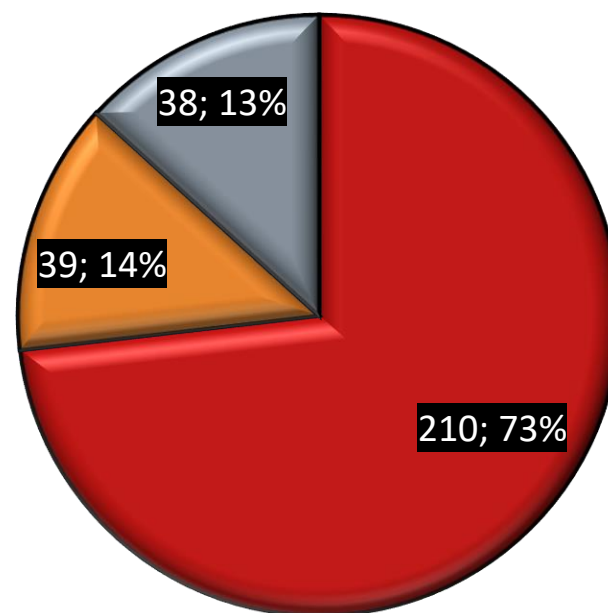


# Smittekategori og mortalitet

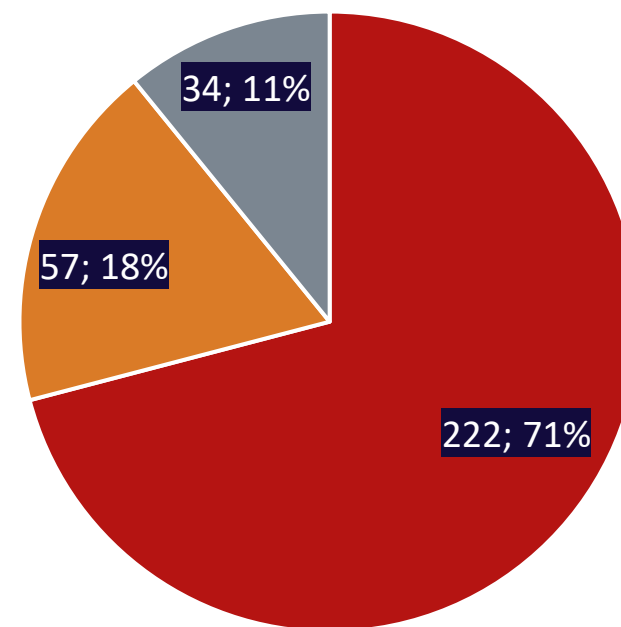
2020 - 2021;  
554 tilfælde  
Døde 14,5 %



2022;  
287 tilfælde  
Døde 19,5 %



2023;  
313 tilfælde  
Døde 11,2 %

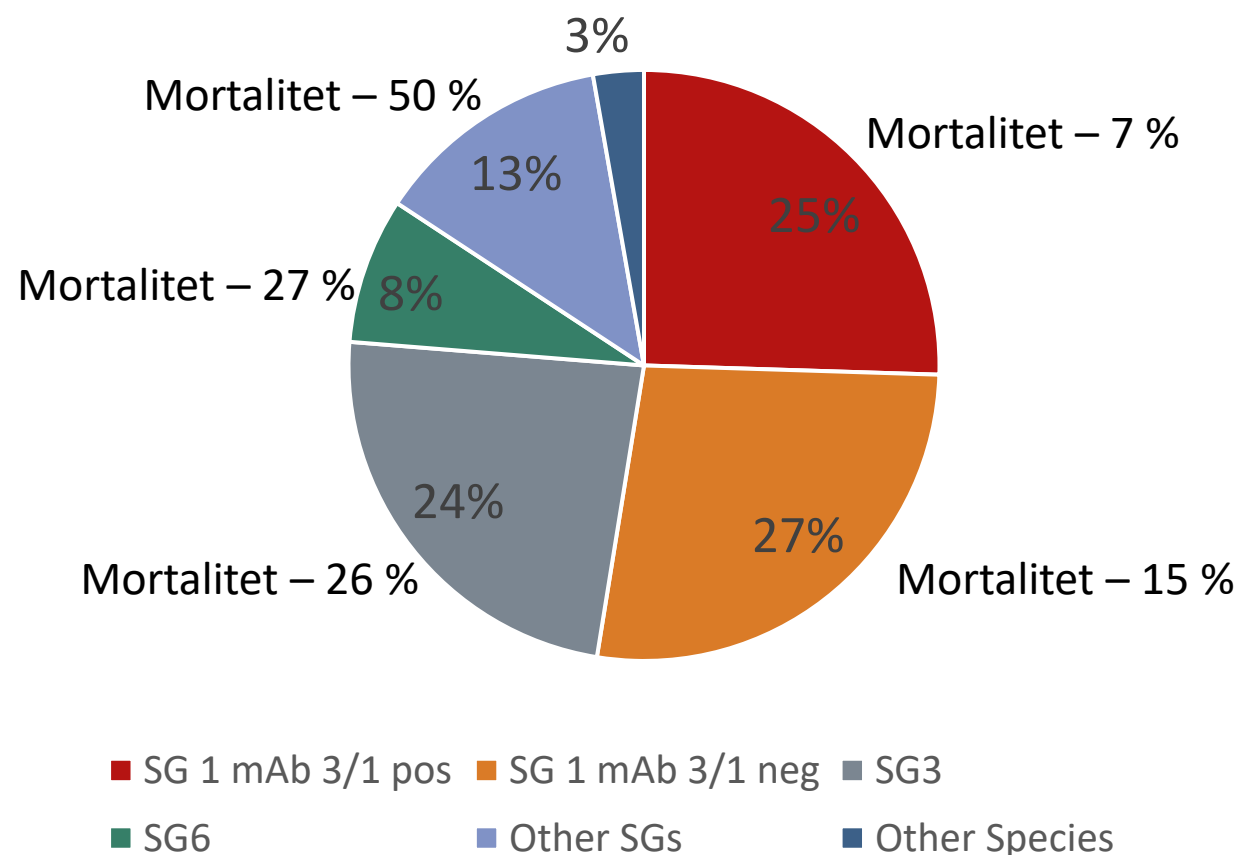


Rød – Samfund Danmark (ink. rejse Danmark)  
Orange – Rejse udland  
Grå - Hospital og institution

# Stor forskel mellem hvad der findes i miljøet og hvad man smittes med – og hvad man dør af

- Mere end 80 % af varmtvandssystemer i Danmark er kontamineret med *Legionella pneumophila*
- < 1 % med *L. pneumophila* **SG 1 mAb 3/1 positive** stammer (nogle steder mere)
- < 10 % med *L. pneumophila* **SG1 mAb 3/1 negative** stammer (nogle steder mere)
- Omkring 50 % med *L. pneumophila* **SG 3**
- Omkring 20 % med *L. pneumophila* **SG 6**
- Omkring 40 % med **andre *L. pneumophila* serogrupper**
- Op mod 100 % med andre *Legionella* arter
- Stor variation fra område til område

Smittet i Danmark 2010-2022, 902 kliniske isolater



# ***Legionella longbeachae* cluster with probable link to gardening products in Denmark, 2024**

Caroline Eves (1), Jonas Steenholdt Sørensen (2), Charlotte Kjelsø (1), Hanne Klink Epstein (3), Søren A. Uldum (2)



(1) Department of Infectious Disease Epidemiology and Prevention, Statens Serum Institut, Copenhagen, Denmark

(2) Department for Bacteria, Parasites and Fungi, Statens Serum Institut, Copenhagen, Denmark

(3) Supervision and Guidance - East, Danish Patient Safety Authority, Copenhagen, Denmark



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## **Background**

- Legionnaires' disease is clinically and microbiologically notifiable in Denmark
- During the summer of 2024, Statens Serum Institut (SSI) and the Danish Patient Safety Authority (STPS) observed an increase in cases caused by *L. longbeachae*
- Interviews conducted by STPS revealed handling of gardening products prior to disease onset, an association also observed abroad (1, 2)

## **Methods**

- Cases were asked to send samples of gardening products used to SSI for *Legionella* culture
- Whole Genome Sequencing and Single Nucleotide Polymorphism (SNP) analysis were performed on three clinical isolates (from Cases 3, 4, and 6) and 15 environmental isolates from Brands X, Y, and Z

## **Case Definition**

1. Positive for *Legionella* species non-*pneumophila* by PCR
2. Test date between June 10 - August 10, 2024 (weeks 24 - 32)



## Findings

- 11 cases were confirmed positive for *Legionella* species non-pneumophila (Figure 1)
  - Four cases were culture positive for *L. longbeachae*
  - Two cases were positive for *L. longbeachae* by molecular methods only
  - An additional five cases were confirmed positive for *Legionella* species non-pneumophila in the study period and negative for *L. longbeachae* (all were culture negative):
    - By sequencing one case had *L. feeleii* (best match), one had *L. lytica* (best match), one had *L. micdadei*, one had *L. rubrilucens* and one had unidentified *Legionella* species
- Among the six confirmed cases of *L. longbeachae* (Table 1):
  - Median age was 68.5, the majority were male and resided in the Capital Region
  - All six cases were hospitalized
  - All six cases reported using gardening products from different brands during the incubation period, at least three of which contained peat imported from Sweden

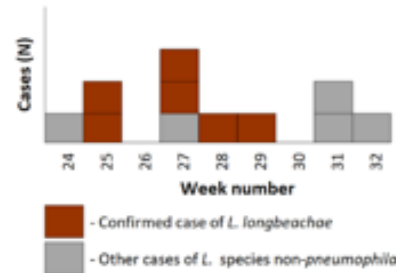


Figure 1. Epidemiological curve

- Gardening product samples:
  - Product samples were collected from 5 out of 6 confirmed cases of *L. longbeachae*
  - The samples were from at least three different brands
  - All 5 samples were culture positive for *L. longbeachae*

Table 1. Confirmed clinical cases of *L. longbeachae* and product sample results

| Case | Week nr. | Age group | Molecular methods     | Culture               | Reported contact with gardening products | Product brand | Species detected in product sample(s)                                      | CFU/gram <i>Legionella</i> |
|------|----------|-----------|-----------------------|-----------------------|------------------------------------------|---------------|----------------------------------------------------------------------------|----------------------------|
| 1    | 25       | 55-59     | <i>L. longbeachae</i> | Negative              | Yes                                      | Brand X       | No environmental sample collected                                          | -                          |
| 2    | 25       | 75-79     | <i>L. longbeachae</i> | <i>L. longbeachae</i> | Yes                                      | Brand unknown | <i>L. longbeachae</i> , <i>L. bozemaniae</i> , <i>L. saintelensis</i>      | 240,000                    |
| 3    | 27       | 65-69     | <i>L. longbeachae</i> | <i>L. longbeachae</i> | Yes                                      | Brand X       | <i>L. longbeachae</i> , <i>L. bozemaniae</i>                               | 60,000                     |
| 4    | 27       | 65-69     | <i>L. longbeachae</i> | <i>L. longbeachae</i> | Yes                                      | Brand Y       | <i>L. longbeachae</i>                                                      | 1,800,000                  |
| 5    | 28       | 55-59     | <i>L. longbeachae</i> | Negative              | Yes                                      | Brand Y       | <i>L. longbeachae</i>                                                      | 600                        |
| 6    | 29       | 80-84     | <i>L. longbeachae</i> | <i>L. longbeachae</i> | Yes                                      | Brand Z       | <i>L. longbeachae</i> , <i>L. bozemaniae</i> , <i>L. pneumophila</i> SG 16 | 1,800,000                  |

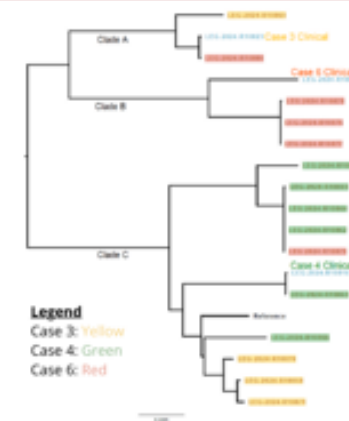


Figure 2. Phylogenetic tree based on SNP analysis with recombinant regions removed (Gubbins)

- The 18 isolates were grouped into three main clades: A, B, and C (Figure 2)
- Each clinical isolate belonged to a different clade, indicating that the cases did not originate from a common source
- Clade A included isolates from Brands X and Z, as well as the clinical isolate from Case 3, who used Brand X
- Clade B contained the isolate from Brand Z and the clinical isolate from Case 6, who used Brand Z
- Clade C contained isolates from Brands X, Y, and Z, as well as the clinical isolate from Case 4, who used Brand Y
- The clinical isolate from Case 4 closely matched one isolate from Brand Y, with only a single SNP difference
- The clinical isolate from Case 3, however, had a close match (just two SNP differences) with an isolate from Brand Z, which was used by Case 6

## Conclusion

- This is the first recorded cluster of *L. longbeachae* infection associated with the use of gardening products in Denmark
- Whole genome sequencing was used on isolates from cases and environmental samples to investigate potential links
- SNP differences in themselves for *L. longbeachae* most likely cannot be used to confirm nor reject the source of infection
- Future investigations will focus on assessing the risk of infection among various gardening products and the use of preventive measures

**Acknowledgments:** We sincerely thank the microbiological laboratories for sending samples to SSI. Special thanks to the Legionella Reference Laboratory, the SSI PCR and DNA Laboratory, and the Department of Bioinformatics for their essential contributions and dedication.

1. Loh, C. H., & Soni, R. (2020). Exposure to potting soils and compost material as potential sources of *Legionella pneumophila* in Australia. *Respiratory medicine case reports*, 31, 101156. <https://doi.org/10.1016/j.rmcr.2020.101156>  
 2. The Public Health Agency of Sweden. (2024). *Legionellainfektion* (Sverige maj 2024-). <https://www.folkhalsomyndigheten.se/smittskydd-beredskap/utbrott/aktuella-utbrott/legionellainfektion-sverige-maj-2024/>



# Nyt EU-drikkevandsdirektiv - Directive (EU) 2020/2184

- Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 **on the quality of water intended for human consumption** was adopted by the European Parliament and the Council of the European Union on 16 December 2020 and **entered into force on 12 January 2021**.
  - Legionella er ny parameter (fordelingsnettet – ink. varmt brugsvand)
  - Vand fra prioriterede ejendomme/bygninger skal regelmæssigt undersøges
  - Prøver skal undersøges iht. DS/ISO 11731 - kvantitativ dyrkning (ved spredningspladekultur), men kan suppleres (ikke erstattes) med andre metoder
  - Parameterværdien er 1.000 CFU/L (alle *Legionella* arter)
    - I DK primært målt i prøver fra varmt brugsvand tappet ved konstant temperatur
- SSI anbefaler at ovenstående parameterværdi generelt er gældende i Danmark, det er dog pt. kun en anbefaling.

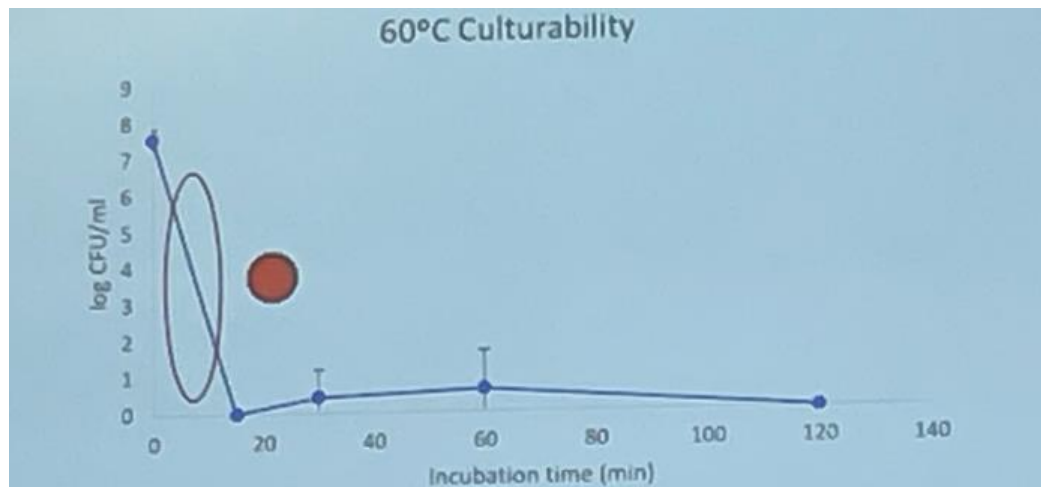
# Bekendtgørelse om indberetning af Legionellaprøver og -foranstaltninger

- Implementeret i Danmark af Social- og Boligstyrelse: BEK nr 721 af 11/06/2024
- <https://www.sbst.dk/byggeri/legionella/faq-om-indberetning>
- Prioriterede ejendomme i Danmark er sygehuse og plejehjem
- Mindst hvert 3. år skal der tages vandprøver hvor resultater indberettes til kommunen
- Udover de prioriterede ejendomme kan kommunen udpege andre ejendomme/bygninger, hvor der er risiko for smitte med *Legionella*. Disse skal overholde samme krav som prioriterede ejendomme – eller eventuelt skærpede.
- Analysemetoden er i henhold til DS/ISO 11731 (alternative er ikke nævnt)
- SSI har udarbejdet en vejledning i prøvetagning fra prioriterede ejendomme
- <https://hygiejne.ssi.dk/retningslinjer/legionella-proevetagning>
- SSI er ved at udarbejde en generel *Legionella* vejledning vedr. forebyggelse og kontrol (NIR) - gældende primært for prioriterede ejendomme.

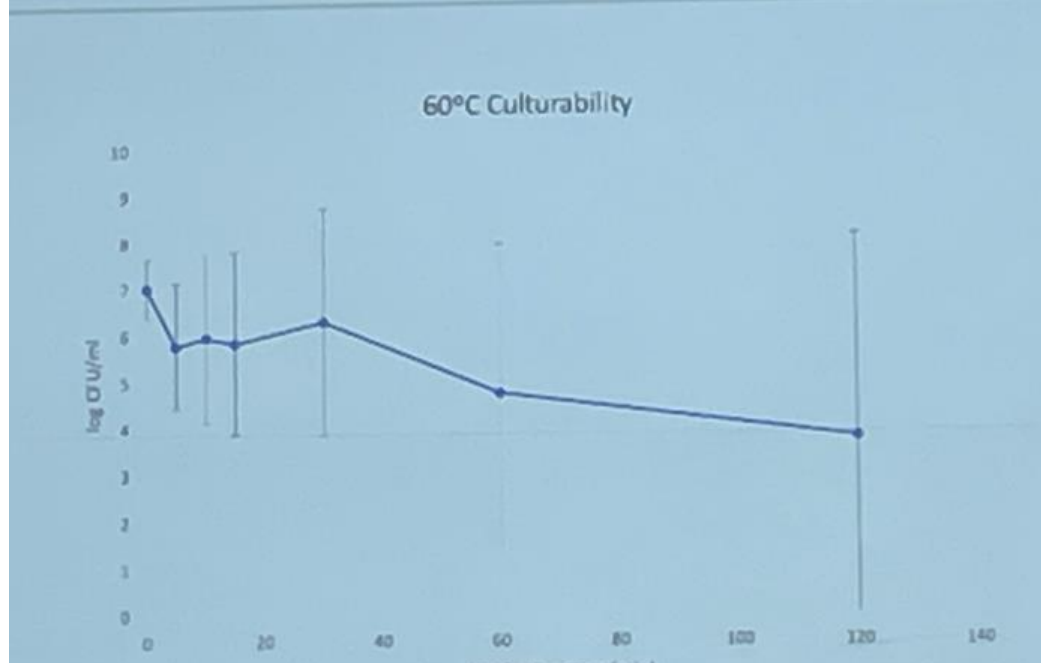
# *Legionella* dyrkbarhed – nedkøling 0 °C/stuetemperatur



0 °C



Stuetemp.



# Legiolert til påvisning af *L. pneumophila*



## Legiolert\*

For detection of *Legionella pneumophila*



- *Legionella* test for all serotypes of *Legionella pneumophila* with equal or greater accuracy than traditional spread plate culture methods.<sup>1-12</sup>
- Significantly improves *Legionella* testing workflow, leading to higher productivity.
- Liquid culture test; isolates can be stored and/or serotyped.
- Reports in MPN, equivalent to CFU per ISO 6107:2021.
- Published by ASTM International as Standard D8429-21.
- Received NF Validation by AFNOR Certification, reference number IDX 33/06-06/19.



Legiolert er en test, der kan bruges til at detektere *Legionella pneumophila* i vandprøver. Testen er baseret på en bakteriel enzymdetektionsteknologi, der signalerer tilstedeværelsen af *L. pneumophila* gennem anvendelse af et substrat, der er til stede i Legiolert-reagenset.

Testen kan detektere *L. pneumophila* ved 1 organisme i 100 mL inden for 7 dage.

### Fordele:

- Generel bedre sensitivitet end traditionel dyrkning
- Ingen koncentreringstrin (intet tab).
- Kendskab til *Legionella* morfologi ikke nødvendigt.
- Kun 100 ml vandprøver i stedet for 1 liters vandprøver.

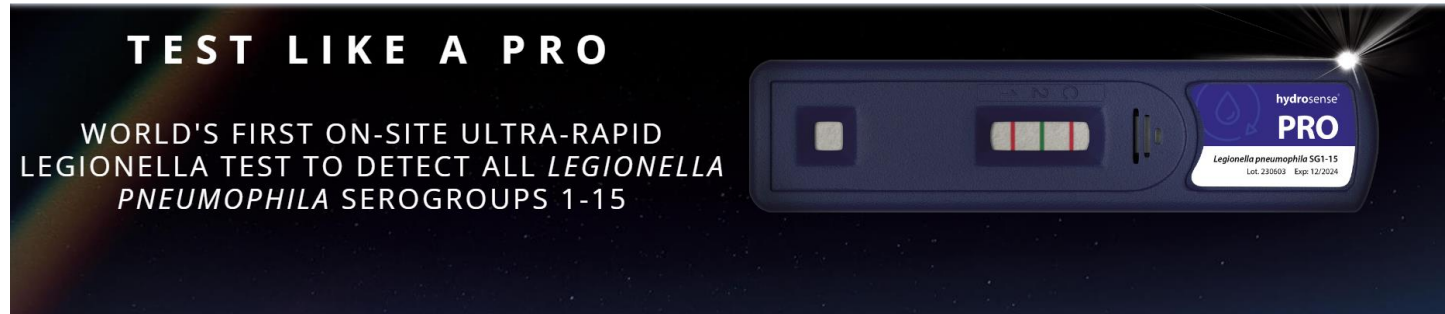
### Ulemper:

- Kan ikke påvise andre arter, hvilket kan være nødvendigt og er et krav i hht. drikkevandsdirektivet.
- Identificering af serogrupper og DNA typer kræver sekundær udsåning for at få enkeltkolonier.



# Andre metoder til påvisning af *Legionella pneumophila*

## Immunokromatografiske metoder



Ny metode der kan påvise alle *L. pneumophila* serogrupper

## Metoder baseret på klik-kemi\*

### MICA *Legionella*

MICA *Legionella* enumerates *Legionella pneumophila* in water (Domestic Water, Cooling Tower Water, Sanitary Water...) in CFU/liter and after only 48 hours of incubation.



Testen er baseret på en metabolisk inkorporation af et syntetisk sukkermolekyle (DIAMIDEX pLeg-N3) ved vækst af bakterierne. Ved brug af "klik-kemi" kan cellerne (mikrokolonier) ses og tælles ved brug af fluorescens.

\*Danske Nobelpristager: Morten P. Meldal, professor ved Kemisk Institut, Københavns Universitet.

# Legionærsygdom forårsaget af andre arter end *Legionella pneumophila*

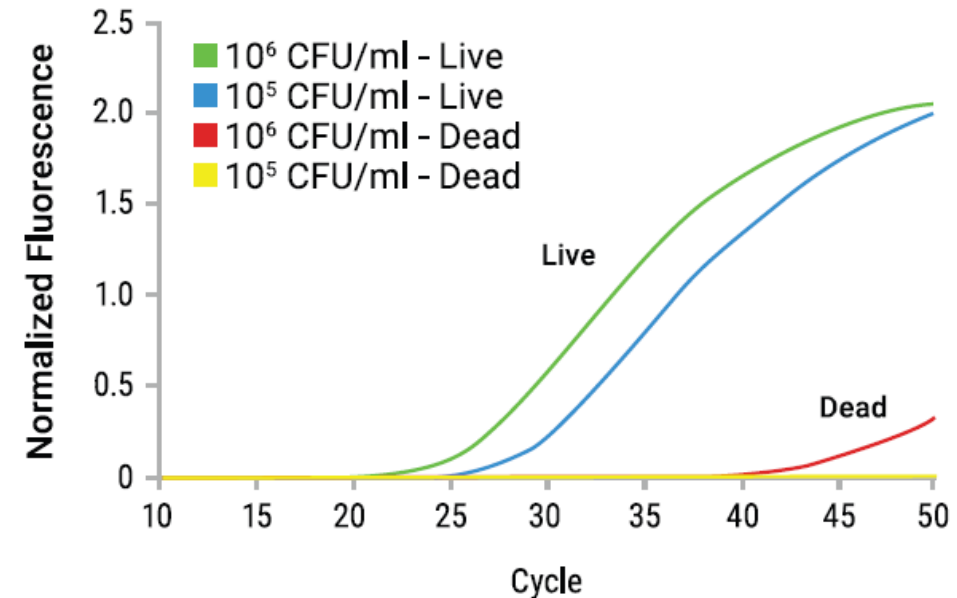
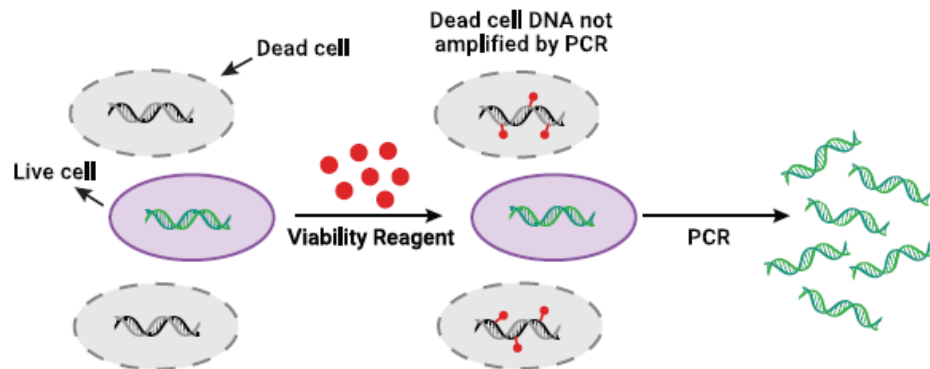
- En stigende andel af patienter er smittet med *Legionella non-pneumophila*, I 2023 var ca. 16% (n=46) af alle tilfælde smittet med anden art. Hertil skal det bemærkes, at det ikke er noget der undersøges for på alle KMAer.
- Disse arter er vanskelige at dyrke – både fra klinisk materiale og miljøprøver, vi har derfor ikke et godt overblik over hvilke andre arter der er årsag til infektion.
- Det er vi nu ved at råde bod på ved at have indført en dyrkningsfri DNA-sekventeringsmetode til arts ID.
- Foreløbige resultater i 2024:
  - *L. longbeachae* – 10; smitter antageligt udelukkende fra jord/kompost
  - *L. bozemanæ* – 3; findes både i jord/kompost og vand
  - *L. anisa* – 2; næst hyppigste art påvist i vandsystemer (koldt/blandet vand)
  - *L. feeleeii* – 1
  - *L. micdadei* – 1
  - *L. shakespearei* – 1
  - *L. rubrilucens* – 1
  - *L. lytica* – 1
    - (dødeligheden for infektion med andre arter er høj – ca. 20 %)



# Legionella viability qPCR - Promega

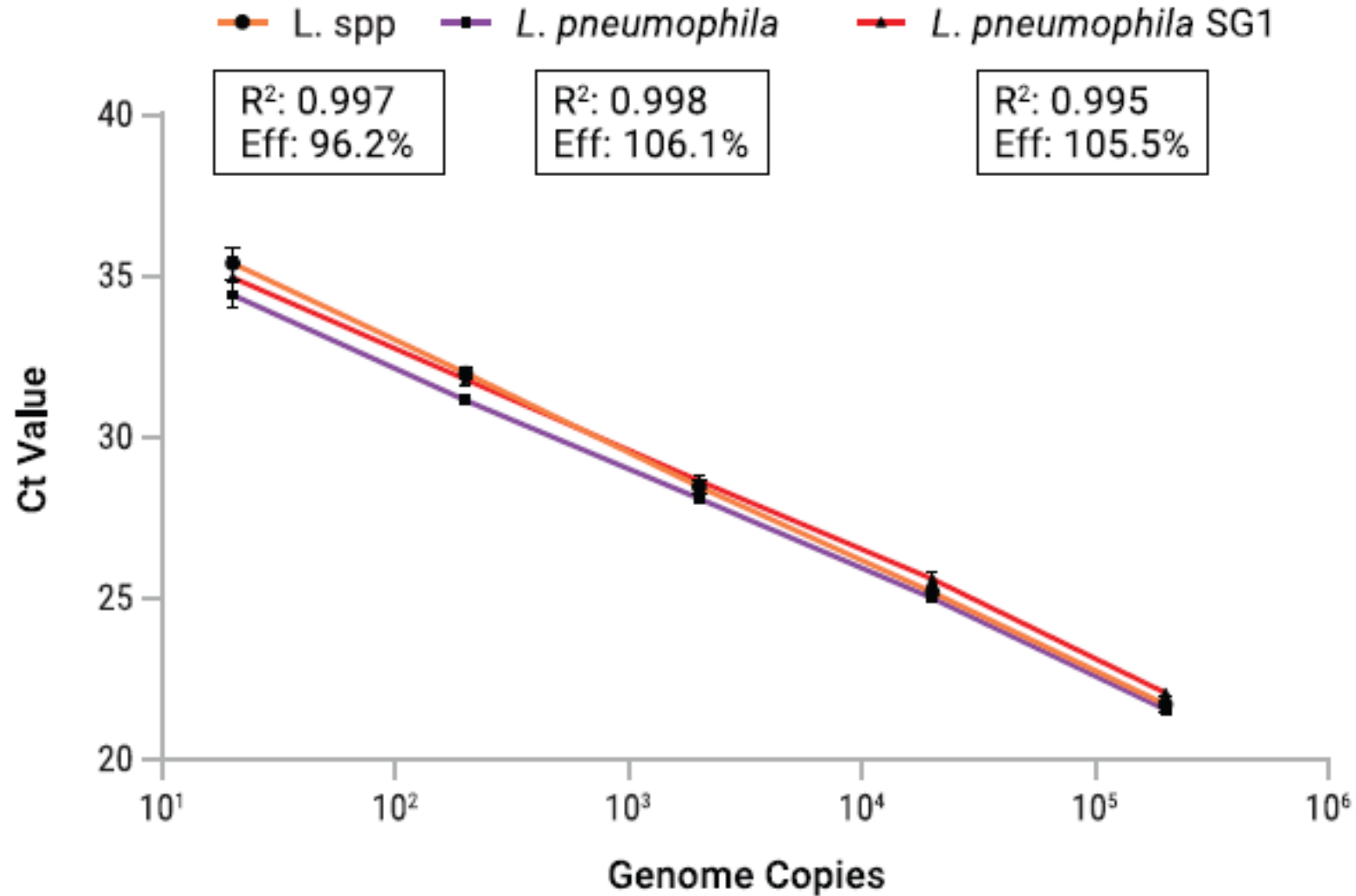
## Detection of Viable *Legionella* in Hours, Not Days

The *Legionella* Viability qPCR Kit differentiates between live/dead cells with an intercalating crosslinker that exclusively binds to DNA from membrane-compromised, dead, dying, or lysed cells. Once bound, the molecule irreversibly crosslinks DNA, making it not amplifiable. This ensures that signal from the qPCR detection reaction is exclusively from viable cells and not from dead cells, therefore eliminating false-positive results.



Example data from **untreated** and **heat treated** *Legionella pneumophila* culture.

# Legionella viability qPCR - Promega





Tak for jeres tid

