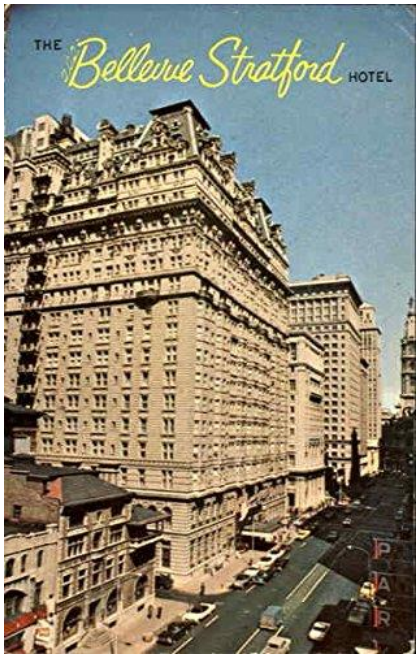


Legionella update

Olivier Denis

National Reference Centre for Legionella
Laboratoire de bactériologie
Service de Microbiologie
Hôpital Erasme

CORATA Belgique 23-24 septembre, Beaune

In collaboration with UZ-Brussels

M Santé

SANTÉ | Accès aux soins | Addictions | Financement de la santé | Fin de vie | Maternité | Nutrition

Huit cas de légionellose signalés à Montpellier dont un mortel

Le Monde.fr avec AFP | 11.09.2015 à 11h53

ÉTATS-UNIS

La légionellose cause la mort de sept personnes à New York

Accueil > Monde - mercredi 5 août 2015 06h00 - L'Avenir

LE FIGARO.fr

Actualité | Economie | Sport | Culture | Lifestyle | Madame | FigaroTV | Vox | Abonnez-vous

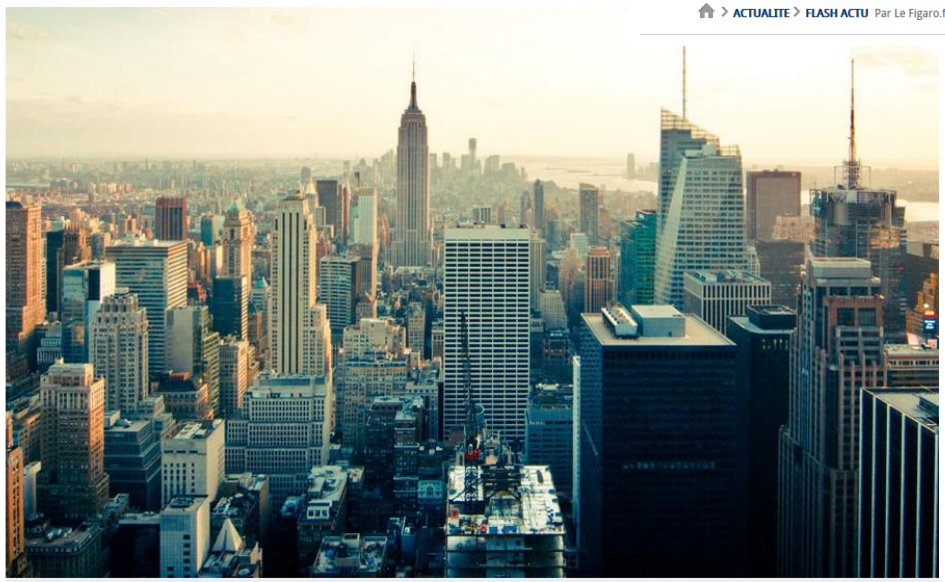
18h54 Scandale, Bourse, mea culpa : l'actu éco spécial Volkswagen

Légionellose à New York: le pic est passé

ACTUALITE > FLASH ACTU Par Le Figaro.fr avec AFP | Mis à jour le 07/08/2015 à 19:39 | Publié le 07/08/2015 à 19:33

Legionnaires' Disease Sickens 127

Largest outbreak ever in New York City kills 12 in South Bronx



CHECKING THE ROOFTOPS: Cooling towers are found on the rooftops of buildings throughout New York City. After initial testing in the South Bronx, city officials found five cooling towers contaminated with Legionella bacteria.

Actualité > Grand Sud > Aude > Espéraza

Légionellose : le stade Jean Biart frappé d'interdiction

LADEPECHE.fr

Recherche sur le site

mardi 22 septembre, 19:24, Saint Maurice

GRAND SUD | FRANCE - MONDE | FAITS DIVERS | SPORTS | SANTÉ | TV

Toulouse | Agen | Albi | Auch | Cahors | Carcassonne | Castres

Le stade jean Biart est interdit au moins pour un mois. /Photo DDM, O.L.

July 21st 1976 - Philadelphia

- 58th Convention of the American Legion at the Bellevue-Stratford Hotel
- > 4000 World War II Veterans with families & friends
- 600 persons staying at the hotel
- July 23nd: convention closed
- Explosive epidemic of febrile illness with pneumonia

⇒ Searching for the causative agent ? (David Fraser: CDC – Atlanta)

- Influenza virus?
- Heavy metal intoxication?
- Toxin?

- 2603 toxicology tests
- 5120 microscopy exams
- 990 serological tests

July 22nd – August 2nd

- **Clinical picture**
  - High fever
  - Coughing
  - Breathing difficulties
  - Chest pains

436 APPENDIX C

The New England
Journal of Medicine

Volume 297 DECEMBER 1, 1977 Number 22

LEGIONNAIRES' DISEASE

Description of an Epidemic of Pneumonia

DAVID W. FRASER, M.D., THEODORE R. TSAI, M.D., WALTER ORENSTEIN, M.D., WILLIAM E. PARKIN, D.V.M., DR. P.H., H. JAMES BEECHAM, M.D., ROBERT G. SHARRAR, M.D., JOHN HARRIS, M.D., GEORGE F. MALLISON, M.P.H., STANLEY M. MARTIN, M.S., JOSEPH E. MCDADE, PH.D., CHARLES C. SHEPARD, M.D., PHILIP S. BRACHMAN, M.D., AND THE FIELD INVESTIGATION TEAM*

Abstract An explosive, common-source outbreak of pneumonia caused by a previously unrecognized bacterium affected primarily persons attending an American Legion convention in Philadelphia in July, 1976. Twenty-nine of 182 cases were fatal. Spread of the bacterium appeared to be air borne. The source of the bacterium was not found, but epidemiologic analysis suggested that exposure may have occurred in the lobby of the headquarters hotel or in the area immediately surrounding the hotel. Person-to-person spread seemed not to have occurred. Many hotel employees appeared to be immune, suggesting that the agent may have been present in the vicinity, perhaps intermittently, for two or more years. (N Engl J Med 297:1189-1197, 1977)

NEW infectious diseases continue to be found with the aid of increasingly sophisticated laboratory methods for identifying microbial agents. Often, it is through investigation of an epidemic — as recently with Lassa fever[1] and Ebola-virus disease[2] — that new organisms and new diseases are identified. The occurrence of an epidemic signals the need for an investigation of a previously unrecognized problem and presents a cluster of cases in which, by means of appropriate comparisons with controls, a common epidemiologic, clinical, and microbiologic thread can be sought. On the centennial of Koch's discovery that bacteria caused anthrax, an explosive outbreak of pneumonia occurred in Pennsylvania, mostly in persons who had attended an American Legion convention. We describe the epidemic, the clinical illness and, in a companion paper,[3] the evidence that it is caused by a bacterium not previously recognized as a cause of human disease.

Address reprint requests to the Center for Disease Control (attention of Dr. Fraser) Bacterial Diseases Division, Bureau of Epidemiology, Atlanta, GA 30333.

*Dennis Bregman, M.S., Robert B. Craven, M.D., Michael J. DiMaio, M.D., Robert Gess, M.D., Marshall F. Goldberg, M.D., Mark J. Goldberger, M.D., Robert A. Gunn, M.D., Philip L. Graziani, D.D.S., William E. Halperin, M.D., Gregory F. Hayden, M.D., David L. Heymann, M.D., Richard A. Kaslow, M.D., Carlos E. Lopez, M.D., James S. Marks, M.D., William J. McKay, M.D., Vera Pabon, Dr. P.H., Philip J. Rettig, M.D., Cathryn L. Samples, M.D., John J. Seggerson, Michael D. Shelby, M.D., Jason D. Shelton, M.D., Edward W. P. Smith, M.D., and Stephen B. Thacker, M.D.

BACKGROUND

The 58th annual convention of the American Legion, Department of Pennsylvania, was held in Philadelphia July 21-24, 1976. The headquarters of the convention was in Hotel A. During the same period, the 56th annual convention of the American Legion Auxiliary, Department of Pennsylvania, was also held in Philadelphia, with headquarters in Hotel B. Persons who attended the conventions included American Legion delegates, delegates of the Ladies Auxiliary, members of the families of Legion and Auxiliary delegates, and other Legionnaires with no formal role at the conventions.

Official activities of the American Legion Convention incuded meetings for all delegates, a parade, a testimonial dinner, a dance, committee meetings, regional caucuses and a breakfast. Unofficial activity centered around the Hotel A lobby, a sidewalk in front of the hotel and several hospitality rooms. Each of the 13 candidates for major office reserved a room or a suite of rooms in Hotel A to serve as a hospitality room for entertaining delegates. Each district and many of the local posts had their own hospitality rooms, which were scattered throughout several hotels. Liquor — most commonly beer and whiskey with or without mixers and ice — was served along with simple snacks.

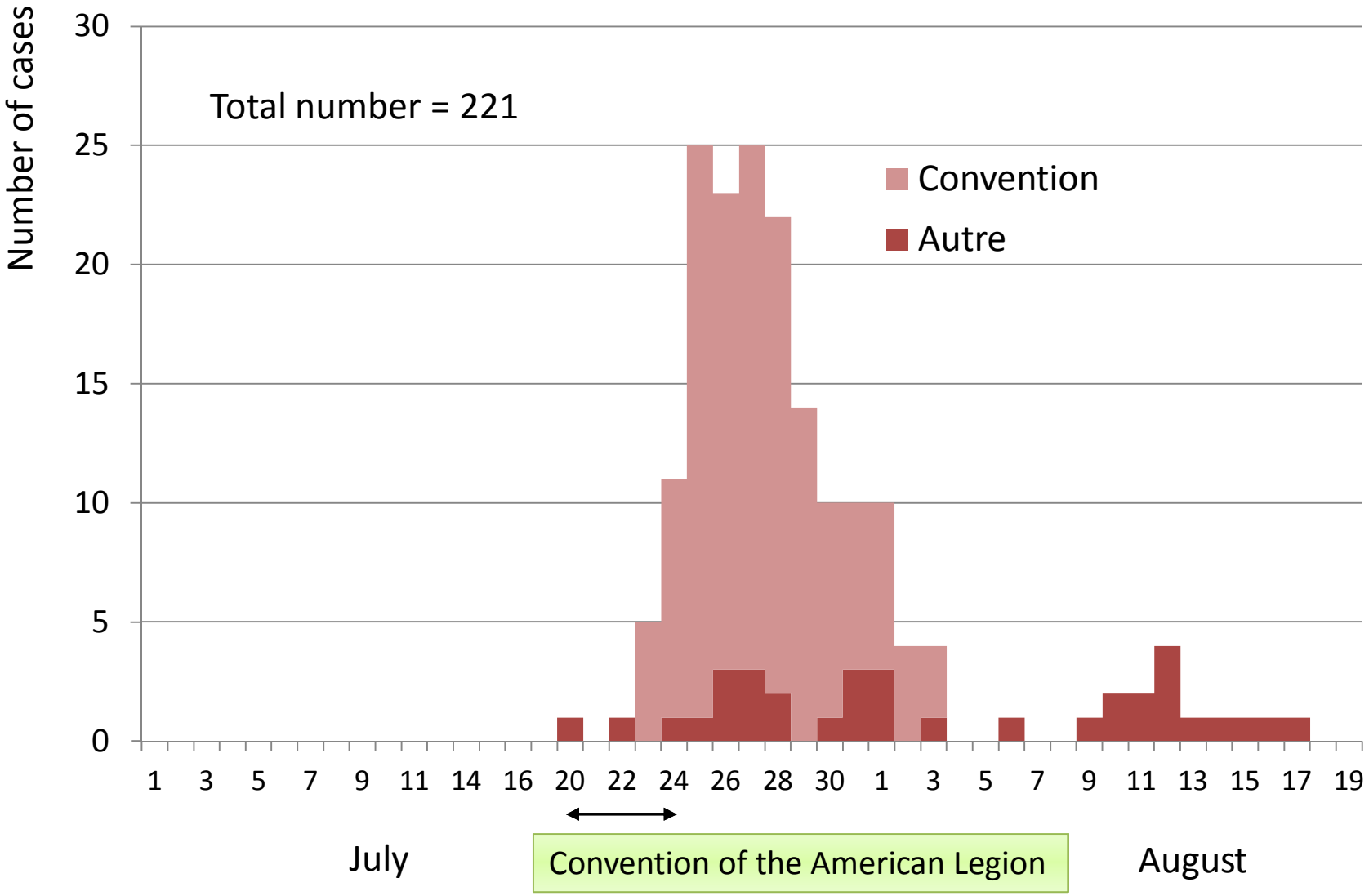
Hotel A was constructed in 1904 and has been extensively modified and renovated since. Hotel guests were housed in approximately 700 rooms on the sec-

ENVIRONMENTAL MEDICINE

Exposed Population = people staying in the lobby or outside the Bellevue Stratford Hotel ≪Broad Street Pneumonia≫

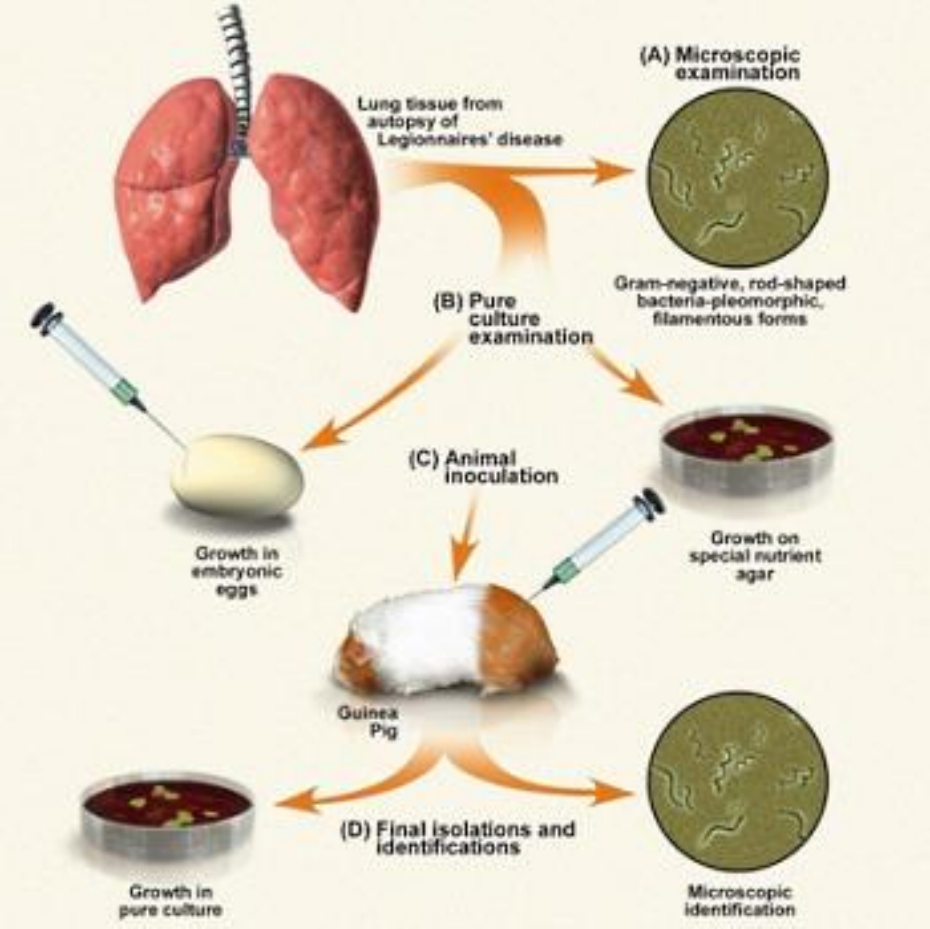
- 221 persons were infected (182+39 ≪Broad Street Pneumonia≫)
- 34 patients died (29+5)

Outbreak, Philadelphia, 1976



December 1976 – January 1977

Å Joseph McDade – discovery of new bacteria genus



⇒ ***Legionella pneumophila***

Development of diagnostic tests including Serological assays and culture

95% of infected patients had positive titer for *L. pneumophila*

Looking back

Previously unresolved outbreaks

- 1968 in Pontiac, Michigan: 95% of the persons working in the same building, 144 cases
- 1965 in Washington: outbreak with 81 cases including 14 death in a hospital
- 1957 in Austin, Minnesota: 78 cases
- 1942 Fort Bragg, North Carolina: 40 soldiers

1st Outbreaks in Europe

Spain 1973 and 1980

In 2015, ≅ 58 distinct species

Legionella dumoffii

Legionella jordanis

Legionella wadsv

Legionella hackel

Legionella feelei

Legionella mac

Legionella birm

Legionella cincinnatiensis

Legionella gormanii

Legionella sainthelensi

Legionella tucsonensis

Legionella anisa

Legionella lansingensis

Legionella erythra

Legionella parisiensis

Legionella oakridgensis

Legionella spiritensis

Legionella jamestowniensis

Legionella santicrucis

Legionella cherrii

Legionella steigerwaltii

Legionella rubrilucens

Legionella brunensis

Legionella adelaidensis

Legionella fairfielddensis

Legionella shakespearei

Legionella waltersii

Legionella genomospecies

Legionella quateirensis

Legionella worsleiensis

Legionella geestiana

Legionella nautarum

Legionella londiniensis

Legionella taurinensis

Legionella lytica

Legionella drozanskii

Legionella rowbothamii

Legionella fallonii

la gresilensis

la beliardensis

Legionella busaniensis

rancourtii

onii

Legionella yabuuchiae

Legionella impletisoli

Legionella dresdeniensis

Legionella nagasakiensis

Legionella steelei

Legionella tunisiensis

Legionella massiliensis

Legionella cardiaca

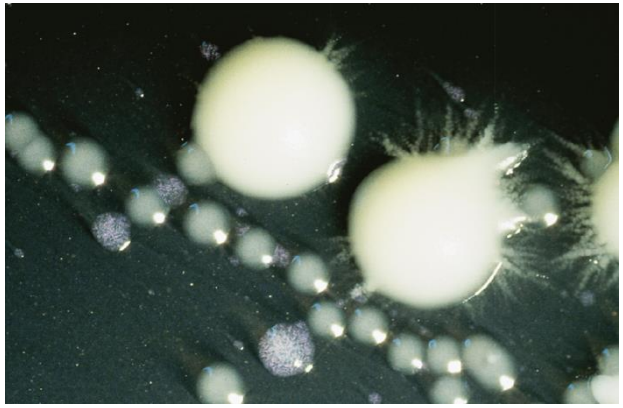
95% of infections caused by *L. pneumophila* serogroup 1

except *L. longbeachae* frequent in Australia and New-Zeeland

Microbiological characteristics

- Aerobic Gram negative coccobacilli
  - Mobile, non-sporulating, noncapsulated, facultative intracellular parasite
  - LPS O antigens ⇒ serogroups more than 60s
- Catalase +, Oxydase variable
- Inert
  - No fermentation of carbohydrates, no nitrate reduction, no urease
- Nutritionally fastidious
  - in vitro nutritional requirement for growth
  - Auxophotrophism
  - Aminoacids as C-source: L-cystein and other aa
  - Iron salt

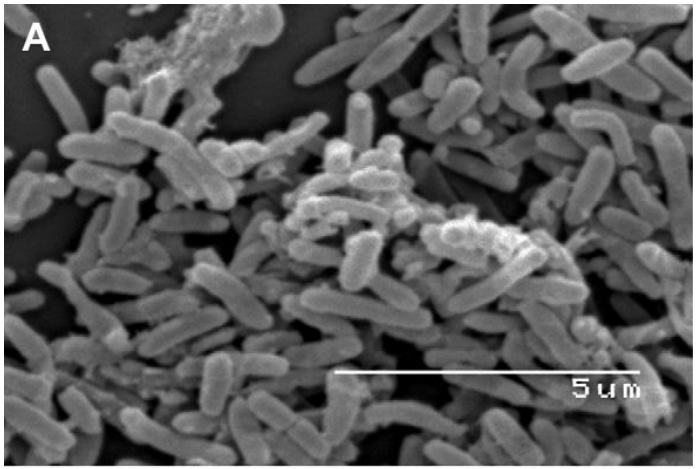
Specific media for culture

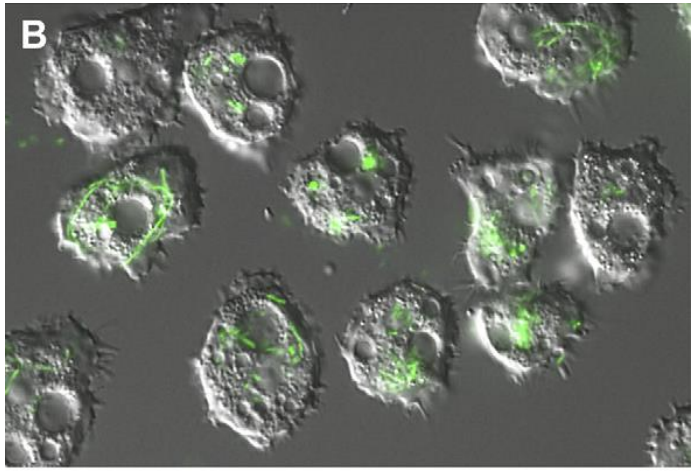


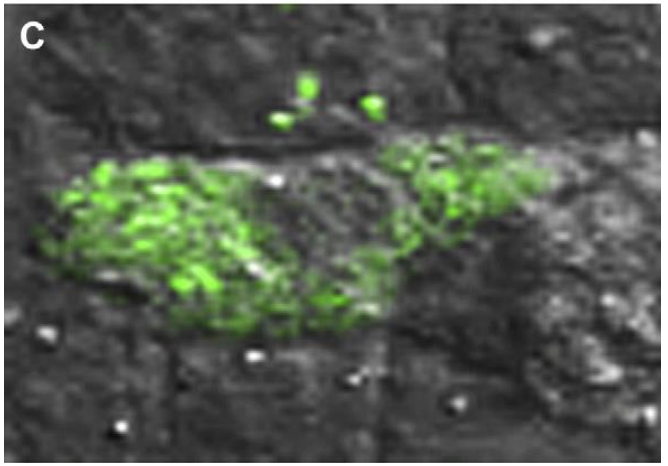
Environnements

Associated with complex biofilm communities

- Adapted for survival and replication within protozoa
  - Including amoeba and ciliates
- Nutrient supply
  - Including amino acids
- Protection against
  - Temperature alteration, flow effects and chemicals







Epidemiology

- Both sporadic and epidemic form

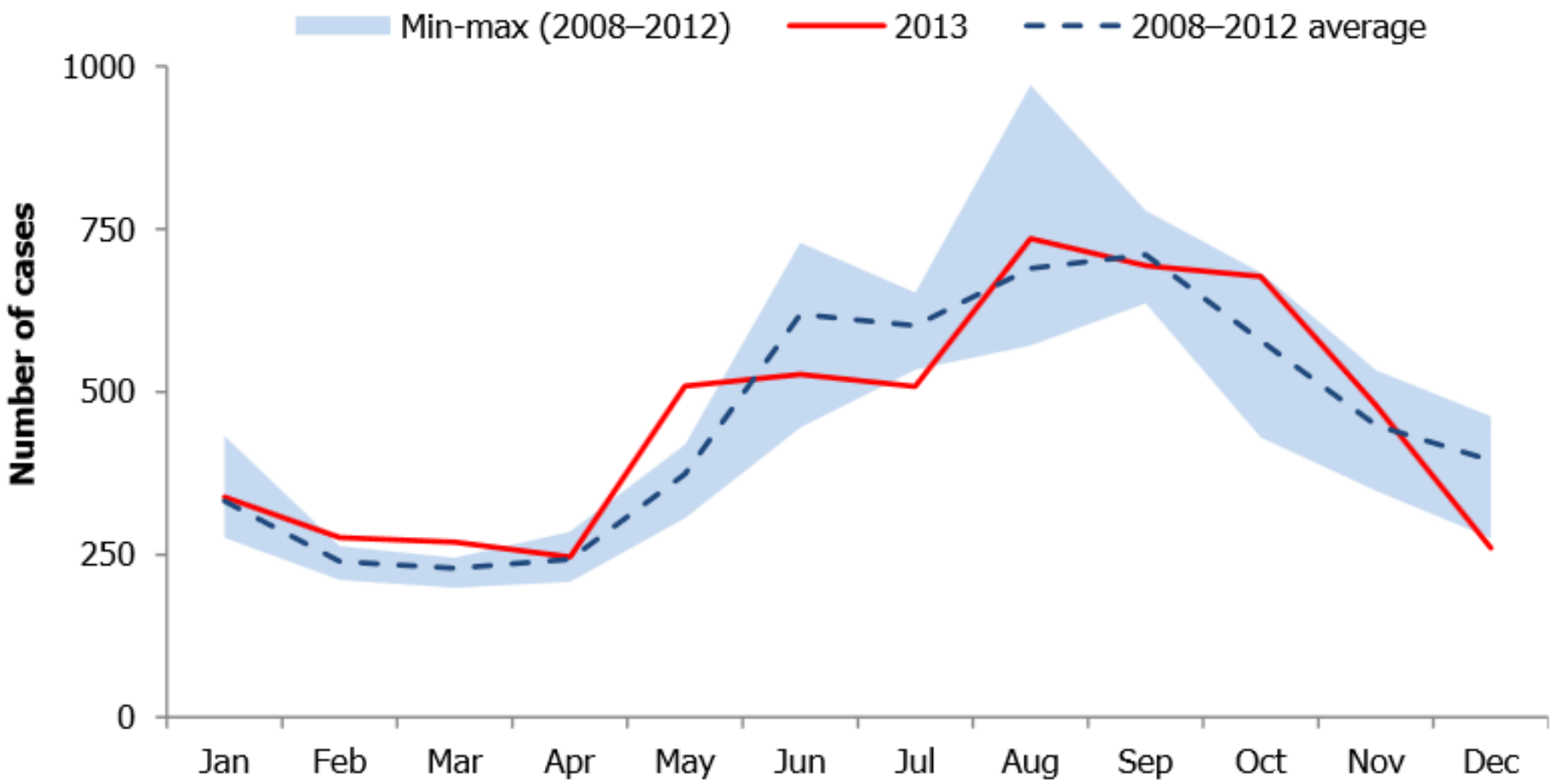
Table 6. Ten largest reported clusters of Legionnaires' disease, 2008–2013

Rank	Reporting country	Year of reporting	Number of cases	Probable setting of infection	Probable source
1	Spain	2010	51	Community-acquired	Cooling tower
2	Spain	2012	39	Community-acquired	Decorative fountain
3	Portugal	2012	36	Community-acquired	Unknown
4	Spain	2009	25	Community-acquired	Unknown
5	United Kingdom	2012	23	Community-acquired	Spa pool
6	Spain	2010	22	Community-acquired	Water system
7	Poland	2010	19	Community-acquired	Water system
8	Spain	2012	18	Travel-associated	Pool
9	United Kingdom	2010	15	Community-acquired	Multiple unknown sources
10	Spain	2008	14	Community-acquired	Unknown

- Pneumonia (Legionnaire's disease): **mandatory reporting**
- Europe in 2013
  - 6012 cases reported by 29 countries with 11,4 notifications per millions inhabitants
  - 0,5 to 5% community-acquired pneumonia
  - 1st cause travel-associated pneumonia : 787 reported cases
  - <1% nosocomial pneumoniae (outbreak)

http://ecdc.europa.eu/en/publications/Publications/legionnaires-disease-2015.pdf

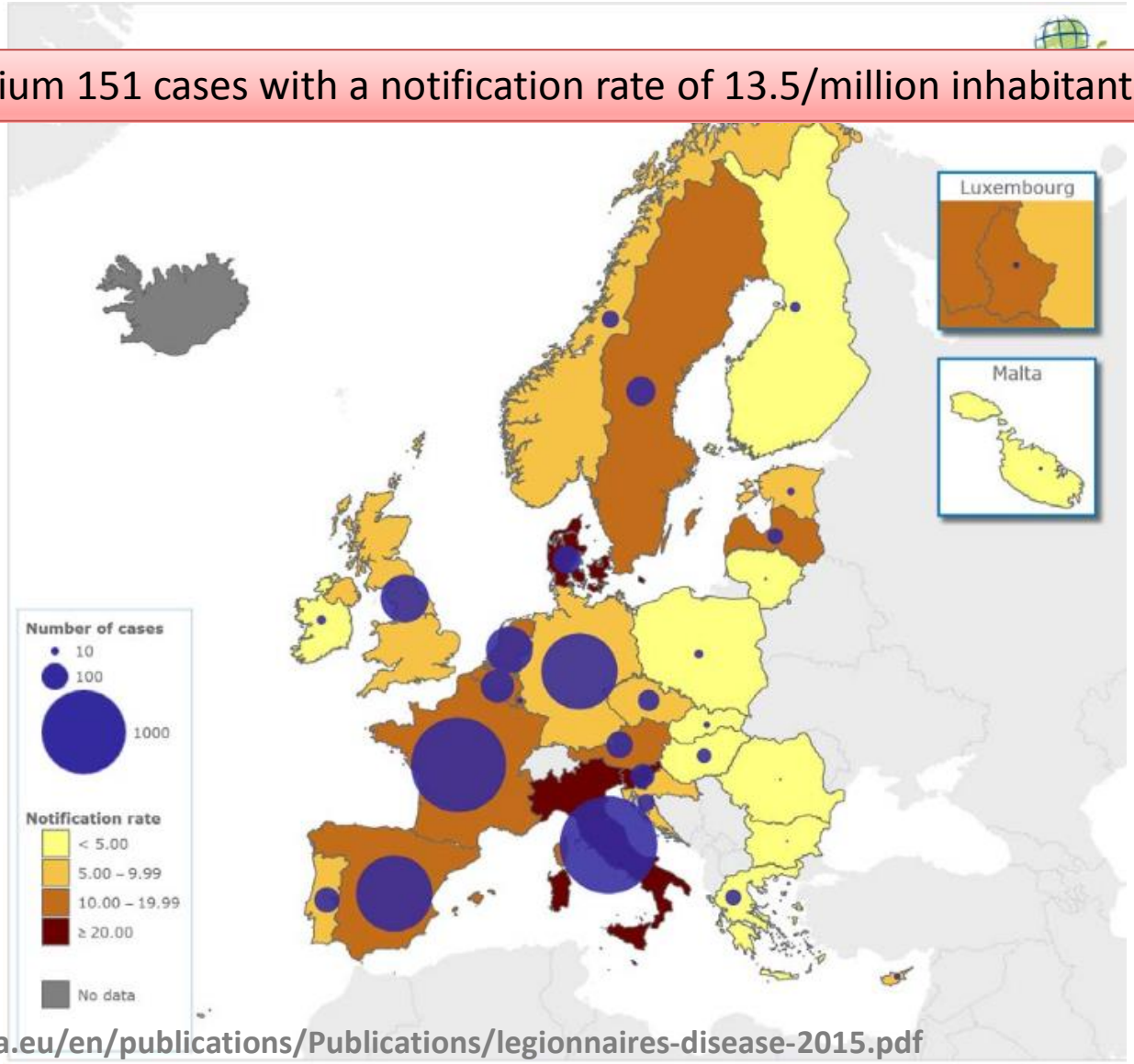
Reported cases of Legionnaires' disease by month, UE, 2008-13



http://ecdc.europa.eu/en/publications/Publications/legionnaires-disease-2015.pdf

Reported cases of Legionnaire's Disease per million, by reporting country, EU/EEA, 2013

Belgium 151 cases with a notification rate of 13.5/million inhabitants



http://ecdc.europa.eu/en/publications/Publications/legionnaires-disease-2015.pdf

RESEARCH Open Acces

Legionnaires' disease: overview of the situation concerning notification in Wallonia (Belgium) in 2012, a retrospective descriptive study based on a capture-recapture method

Stéphanie Jacquinet[1*], Olivier Denis[2,3], Filomena Valente Soares[3] and Carole Schirvel[1]

Abstract

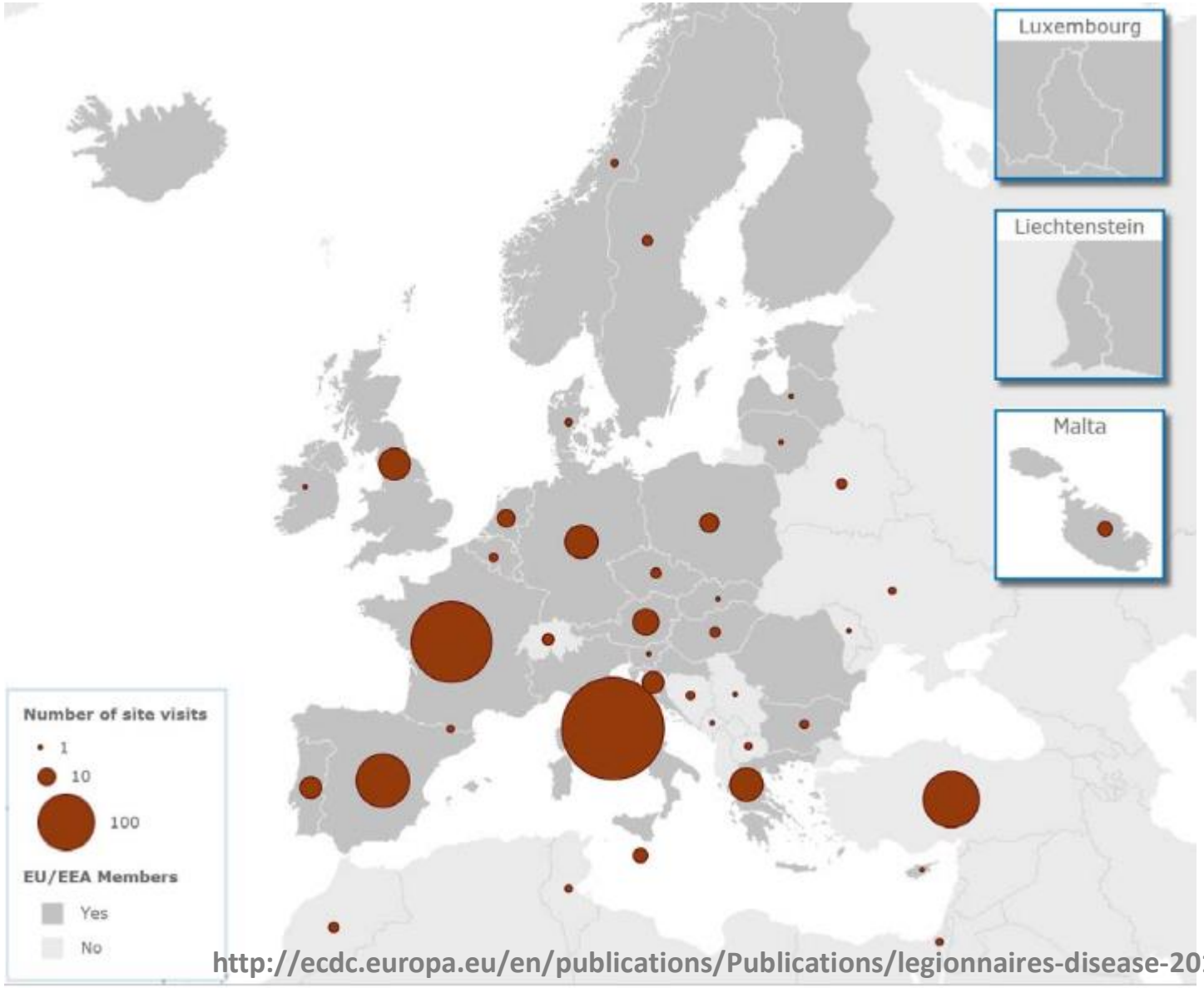
Background: Legionnaires' disease is a severe form of pneumonia, and although public health medical inspectors must be notified, it is often under-reported. The objectives of this study were to determine the completeness rate of notification of Legionnaires' disease and to estimate the incidence of this disease in Wallonia, the southern part of Belgium, in 2012.

Method: This retrospective, transversal descriptive study was based on a capture-recapture method using two sources. An estimation of the total number of Legionnaires' disease cases was calculated using Chapman and Seber's estimators for small numbers, thereby allowing us to estimate the real incidence of this disease in Wallonia as well as the completeness rate of notification.

Conclusions: The notification rate of *Legionella* must be improved in Wallonia. Doctors should be made aware of the importance of diagnosing and reporting Legionnaires' disease.

Keywords: Legionnaires' disease, Surveillance, Belgium

Travel-associated cases of Legionnaires' disease per destination country, EU/EEA, 2013, n=835



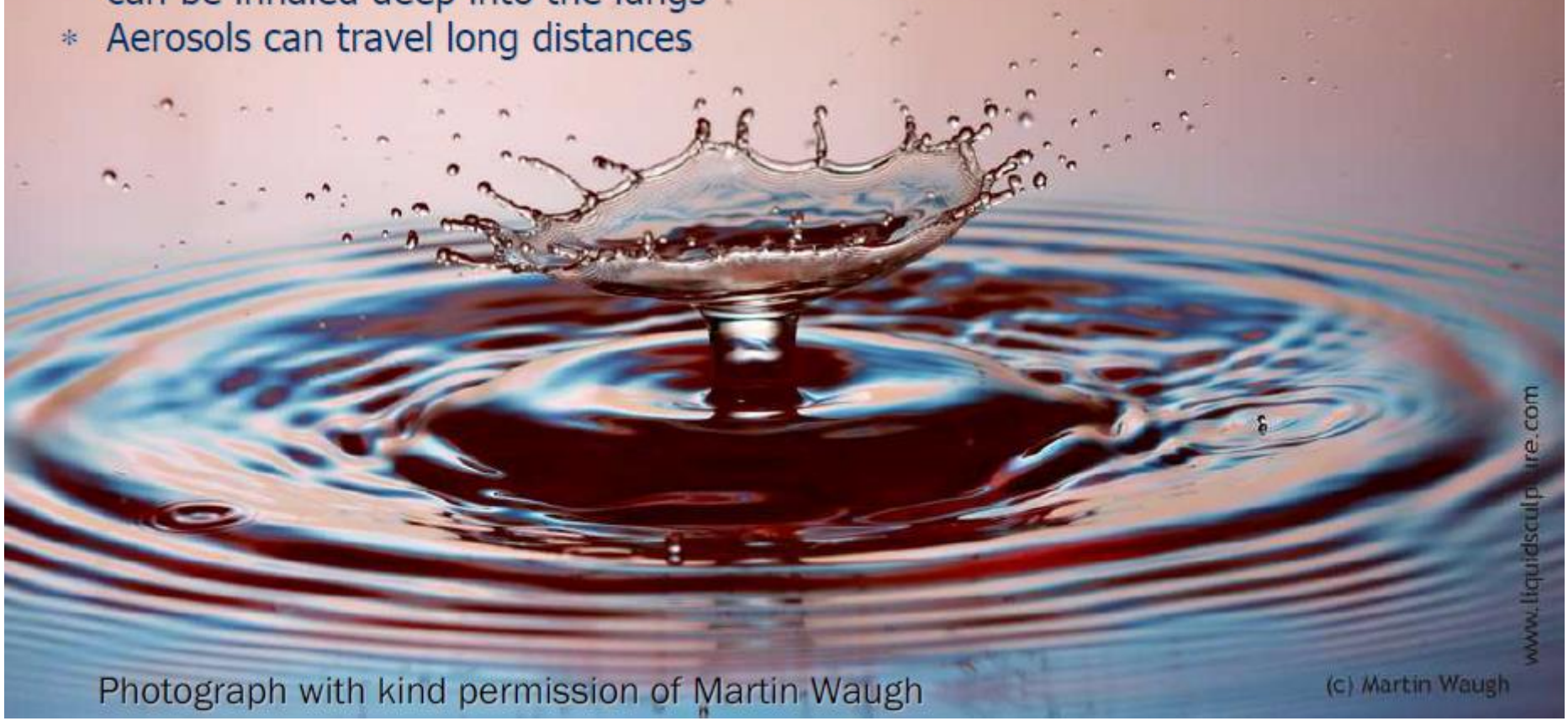
http://ecdc.europa.eu/en/publications/Publications/legionnaires-disease-2015.pdf

Mode of transmission

- The source is always the environment
- Inhalation of Legionella-containing aerosols generated by
  - Man-made structures such as fountains, building water systems, cooling towers, …
  - Infection is caused if *Legionellae* can reach the lungs, or, rarely, other organs
  - *Legionellae* infect most often previously unhealthy, but also healthy persons.
- Person-to-person transmission never been reported unlike other pathogens including *Mycoplasma* and *Chlamydophila*

Aerosol

* An aerosol is not a spray although it can be formed from a spray by small droplets drying to leave suspended droplet nuclei
* Aerosols are formed by bubbles released at a water surface (concentration effect)
* An aerosol is not visible
* Small particles <5μm can remain in suspension in air for prolonged periods and can be inhaled deep into the lungs
* Aerosols can travel long distances

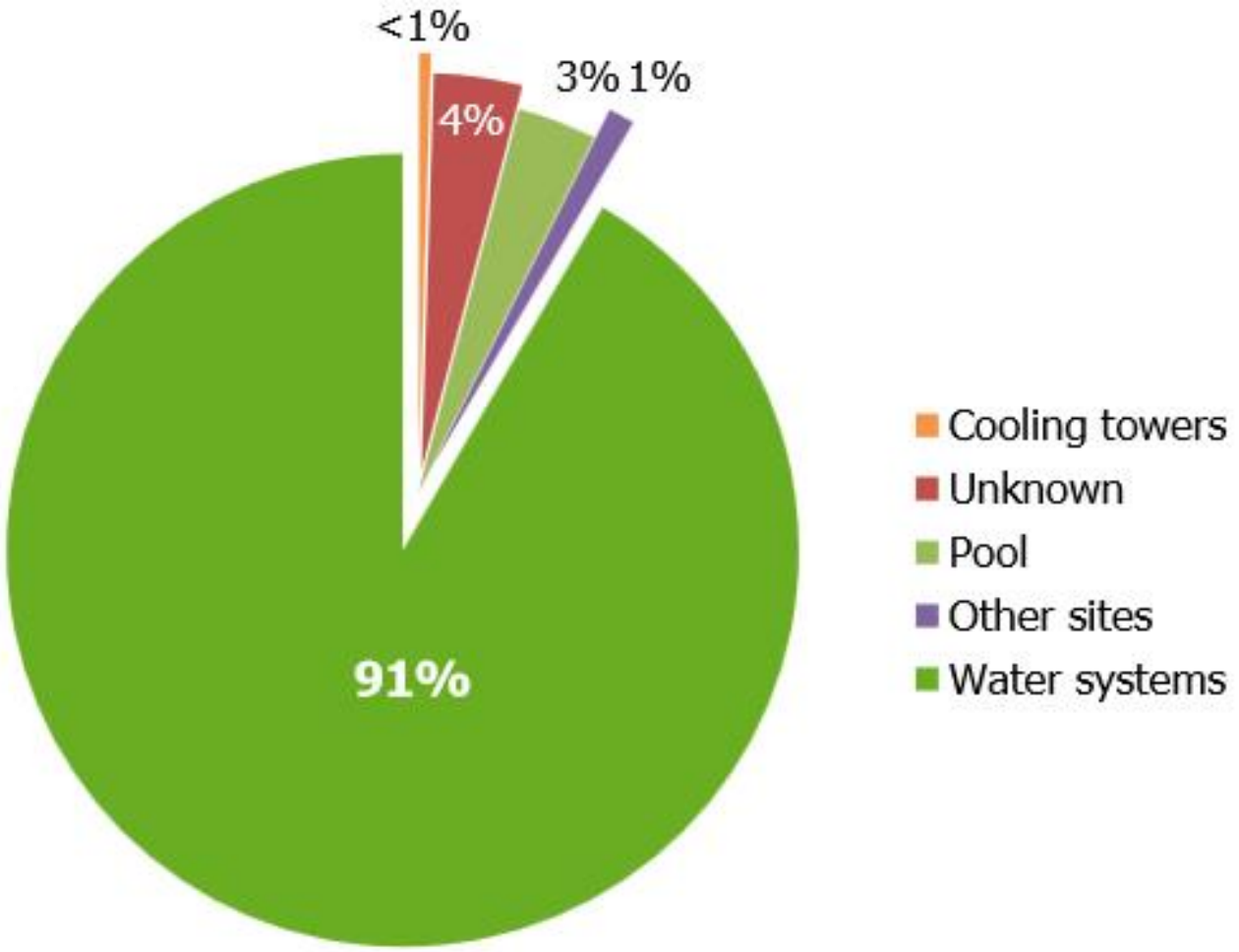


Photograph with kind permission of Martin Waugh

Aerosol formation

- Water drops falling onto a hard surface
- Bubbles rising to the water surface and bursting
- Rain
- Running a tap
- Running shower
- Flushing a toilet
- Spraying plants
- Humidifiers
- Water running over pack of cooling towers
- Wave formation

Distribution of sampling sites testing positive for Legionella, EU, 2013



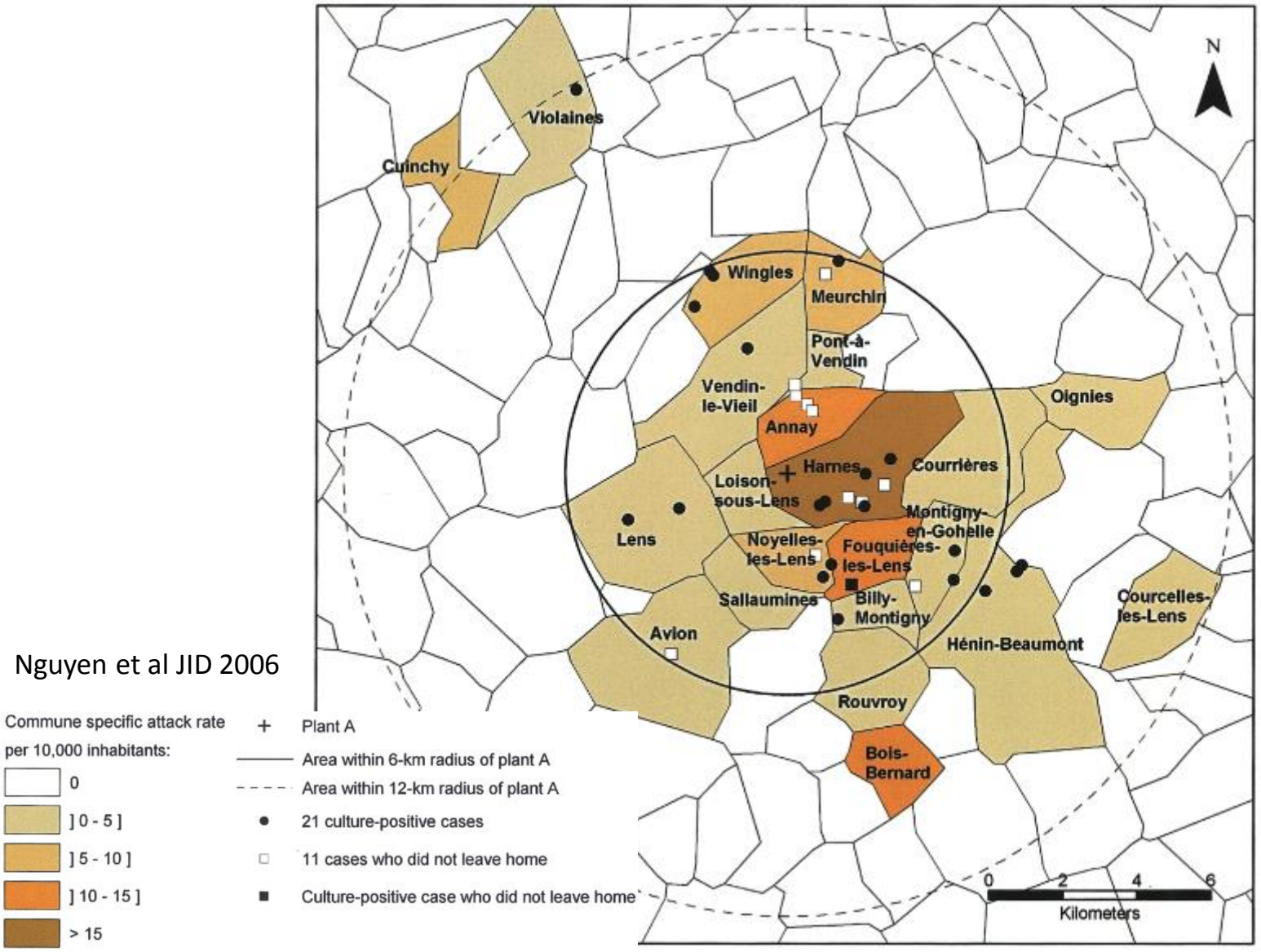
http://ecdc.europa.eu/en/publications/Publications/legionnaires-disease-2015.pdf

Transmission from cooling towers

Distance	Outbreak	Reference
150 m	85 cases medical centre Vermont	Am J Epidemiol 1984
30 m	15 cases at hospital Rhode Island	JAMA 1985
400 m	Delaware community outbreak caused by hospital cooling tower	Inter J Epidem 1999
1,7 km	33 cases in Glasgow	Lancet 1986
3,2 km	29 cases in Wisconsin	Am J Epidemiol 1989
≥ 6 km	86 cases (18 death) Lens, France	J Inf Dis 2006

Long distance

Commune-specific attack rates and geographical distribution of selected cases of legionnaires disease, Pas-de-Calais, France, November 2003–January 2004



Nguyen et al JID 2006

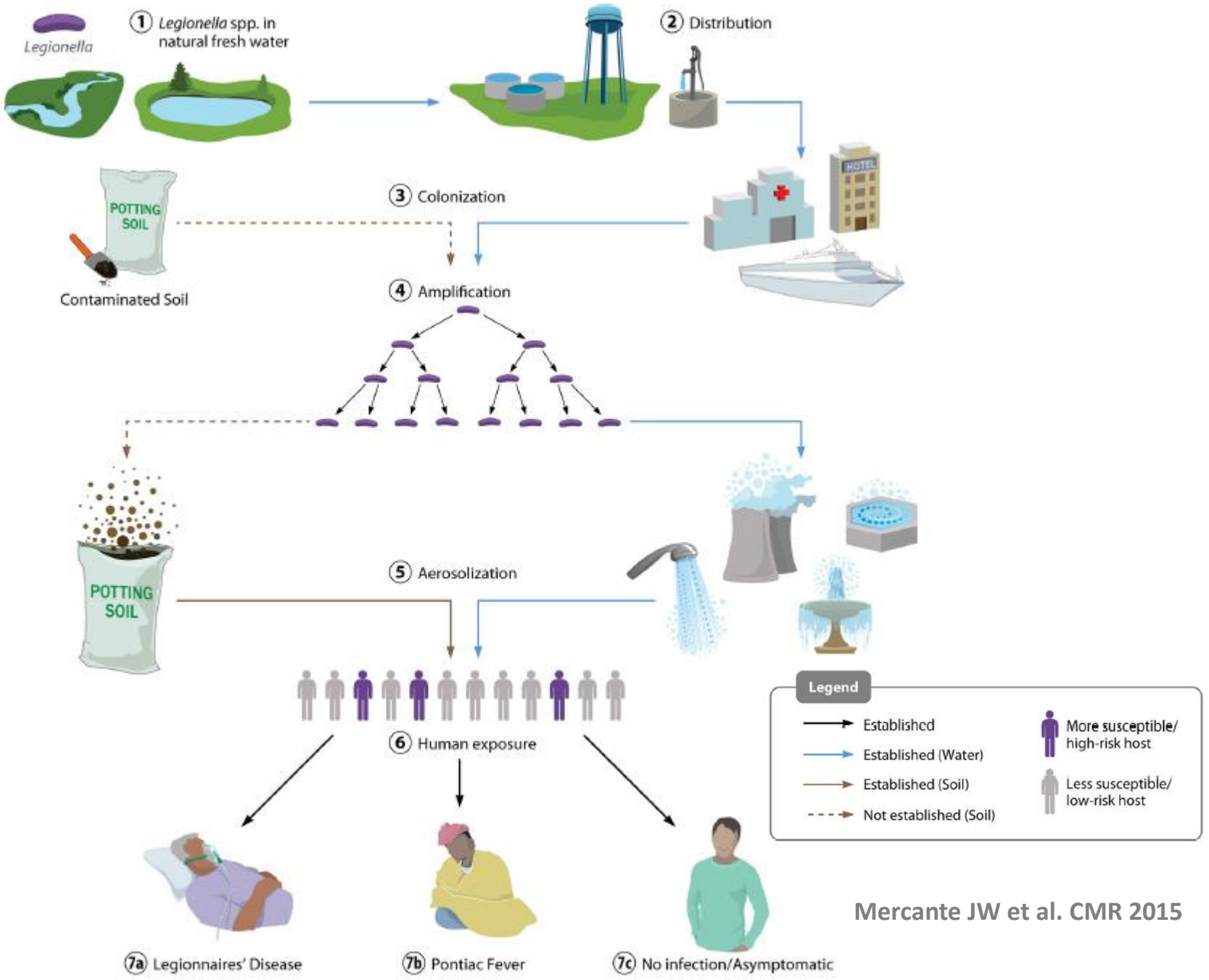
Clinical manifestations

Pneumonia or Legionnaires' Disease

- **Incubation**
  - 2 to 10 days
- **Clinical and radiographic presentations**
  - Indistinguishable from other cause of pneumonia
  - mild to severe illness
  - With initially unilateral and patchy infiltrates evolving to bilateral consolidation
  - Small pleural effusion (rare)
  - Other: T°; abdominal pain, nausea, vomiting and diarrhea; neurological symptoms; hyponatremia; hepatic dysfunction
- **High case-fatality rate**
  - Up to 30%, higher than other cause of CAP
  - Evolution dependent of host factors and therapy

Other clinical manifestations

- **Pontiac fever**
  - Incubation: 1 to 2 days
  - Self-limited, short-duration febrile illness,
  - Fever, chills, headache, myalgia's, malaise, for less than one week
  - Usually diagnosed during an outbreak
- **Extra-pulmonary infections**
  - Rare always in immunocompromised patients
  - Often occur as metastatic complications of pneumonia
  - Miscellaneous findings: abscesses and other infections of the brain, spleen or lymph nodes, and skeletal and myocardial muscles, … prosthetic valve endocarditis
- **Asymptomatic**
  - Frequent
  - Diagnosed in outbreak setting



Mercante JW et al. CMR 2015

Risks factors

Individual

- **Immunocompromised patients*** (organ transplantation, chemotherapy, radiotherapy, corticosteroid; uncommon in HIV infection)
- **Elderly***
- Male> female
- Dialysis patients
- Host with chronic disease (heart, lung, kidney, diabetes)
- Alcoholics and **smokers** (COPD!)
- Patients undergoing major surgery
- Neonates with VAP

* = higher risk to develop fatal infection

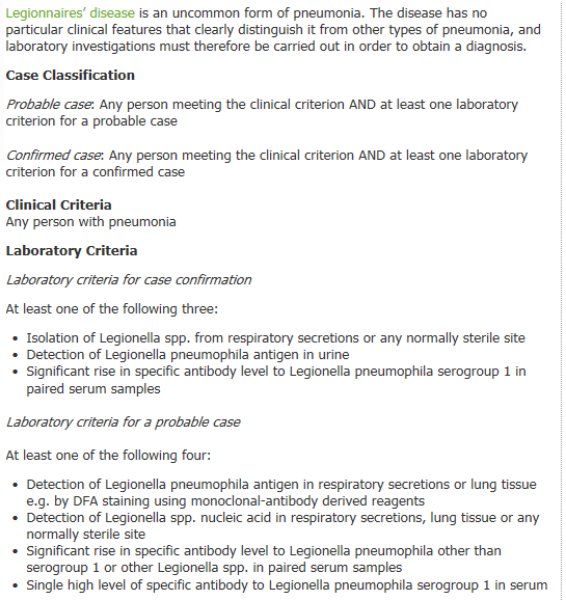
Environmental

- Travel (inside country or abroad)
- Stay at proximity of aerosol producing systems (whirlpools, cooling towers, industrial complexes)

EU case definition

Case definition

- Probable case
  - Clinical criterion **+** at least one laboratory criterion for a probable case
- Confirmed case
  - Clinical criterion + at least one laboratory criterion for a confirmed case



Legionnaires' disease is an uncommon form of pneumonia. The disease has no particular clinical features that clearly distinguish it from other types of pneumonia, and laboratory investigations must therefore be carried out in order to obtain a diagnosis.

Case Classification

Probable case: Any person meeting the clinical criterion AND at least one laboratory criterion for a probable case

Confirmed case: Any person meeting the clinical criterion AND at least one laboratory criterion for a confirmed case

Clinical Criteria
Any person with pneumonia

Laboratory Criteria

Laboratory criteria for case confirmation

At least one of the following three:

- Isolation of Legionella spp. from respiratory secretions or any normally sterile site
- Detection of Legionella pneumophila antigen in urine
- Significant rise in specific antibody level to Legionella pneumophila serogroup 1 in paired serum samples

Laboratory criteria for a probable case

At least one of the following four:

- Detection of Legionella pneumophila antigen in respiratory secretions or lung tissue e.g. by DFA staining using monoclonal-antibody derived reagents
- Detection of Legionella spp. nucleic acid in respiratory secretions, lung tissue or any normally sterile site
- Significant rise in specific antibody level to Legionella pneumophila other than serogroup 1 or other Legionella spp. in paired serum samples
- Single high level of specific antibody to Legionella pneumophila serogroup 1 in serum

Clinical Criteria

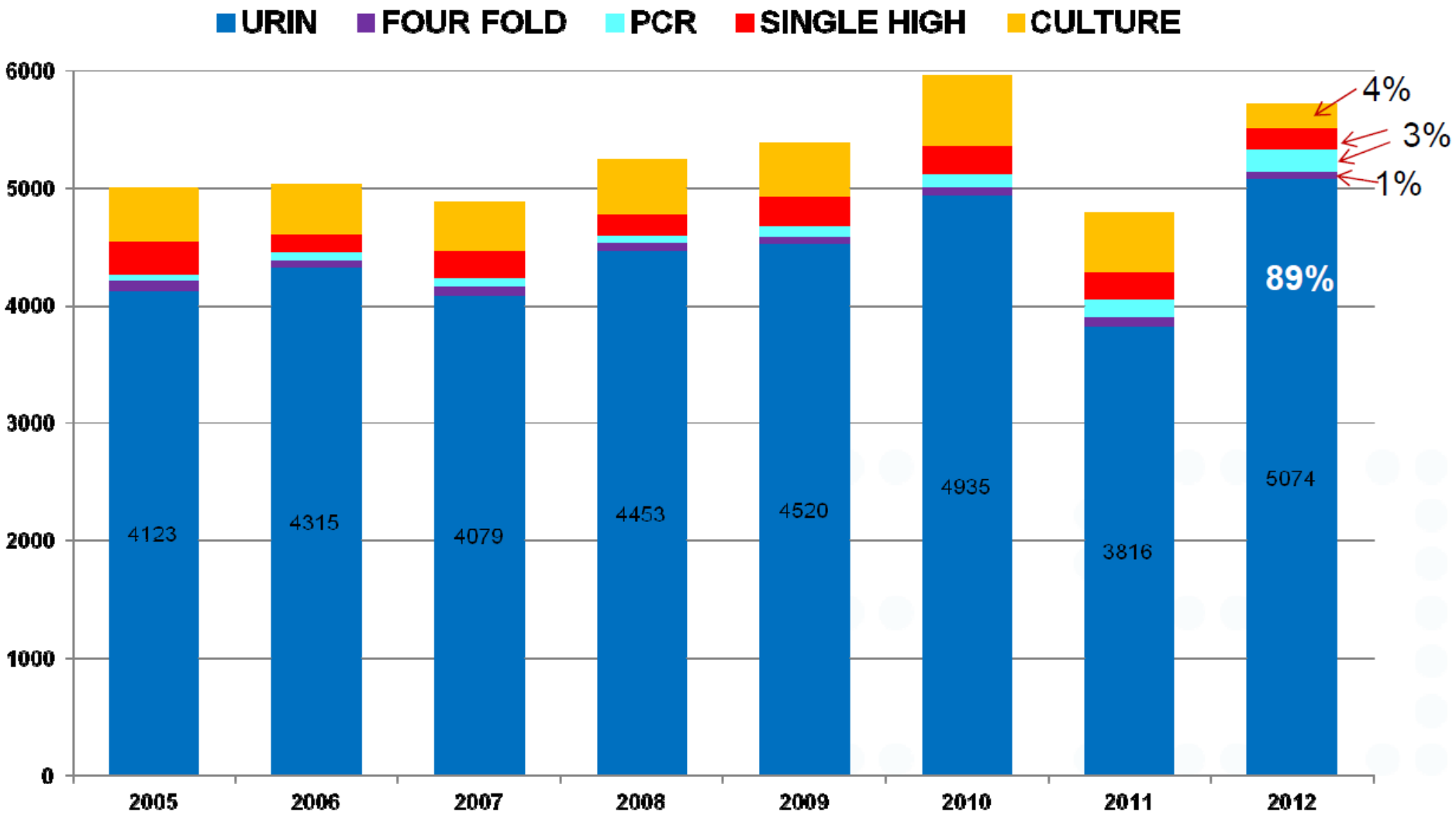
- Any person with pneumonia

Laboratory criteria

- For case confirmation
  - Positive culture of *Legionella spp*
  - Positive urinary Ag for *L. pneumophila*
  - Significant rise in specific antibody level to *L. pneumophila* type 1 in paired serum
- For probable case
  - Positive PCR for Legionella spp
  - DFA staining with monoclonal antibody
  - Significant rise in specific antibody level to other Legionella spp
  - Single high level of specific antibody to L. pneumophila

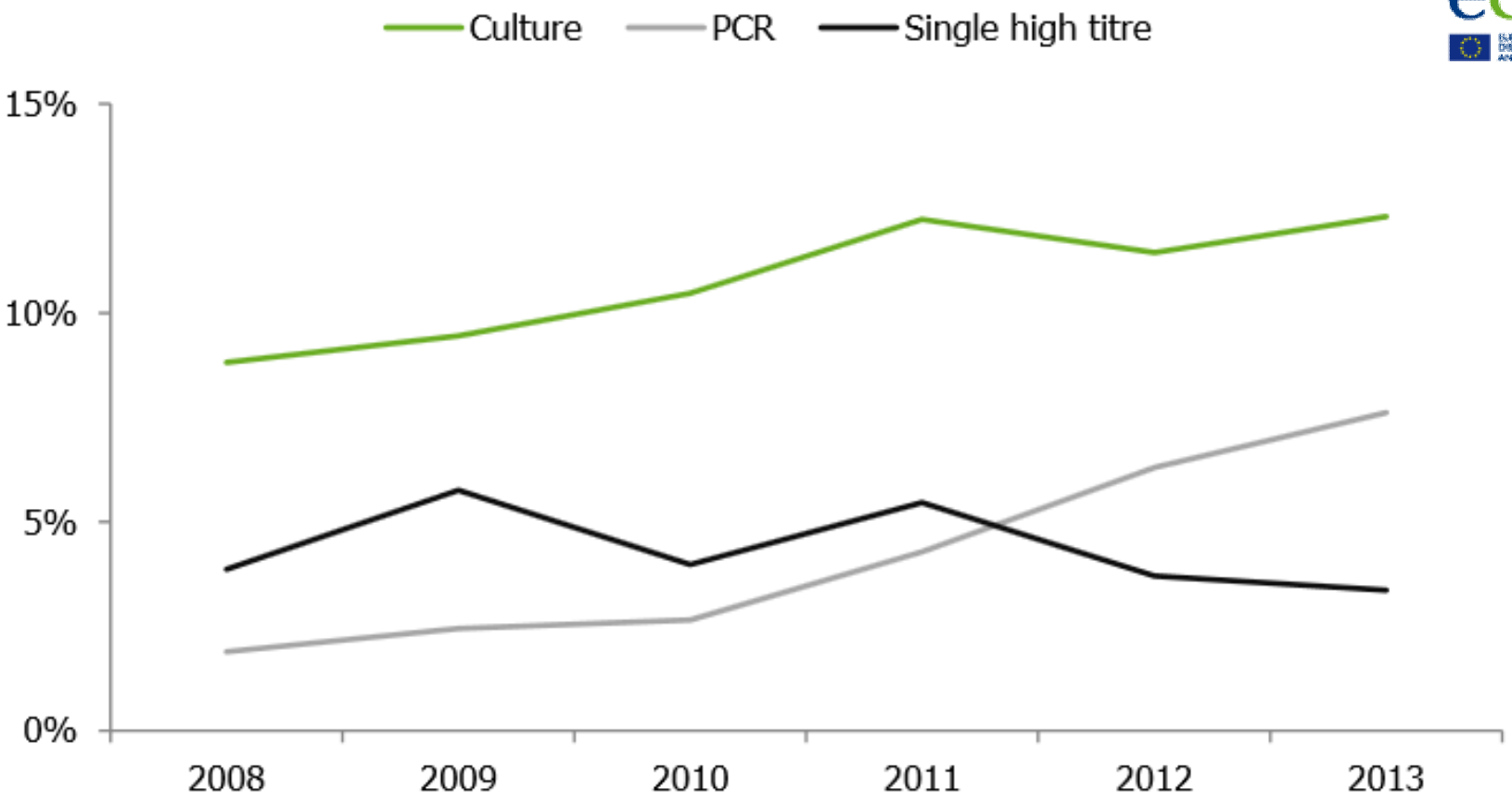
http://ecdc.europa.eu/en/activities/surveillance/ELDSNet/Pages/EU%20case%20definition.aspx

Diagnostic laboratory methods, 2005-2012, EU



http://ecdc.europa.eu/en/publications/Publications/legionnaires-disease-2015.pdf

Proportion of cases reported with culture, PCR and single high titre, 2008-13, EU



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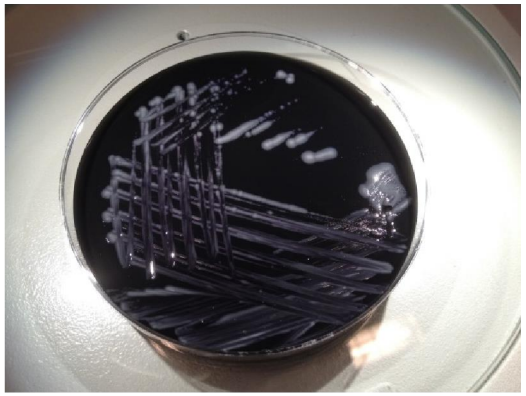
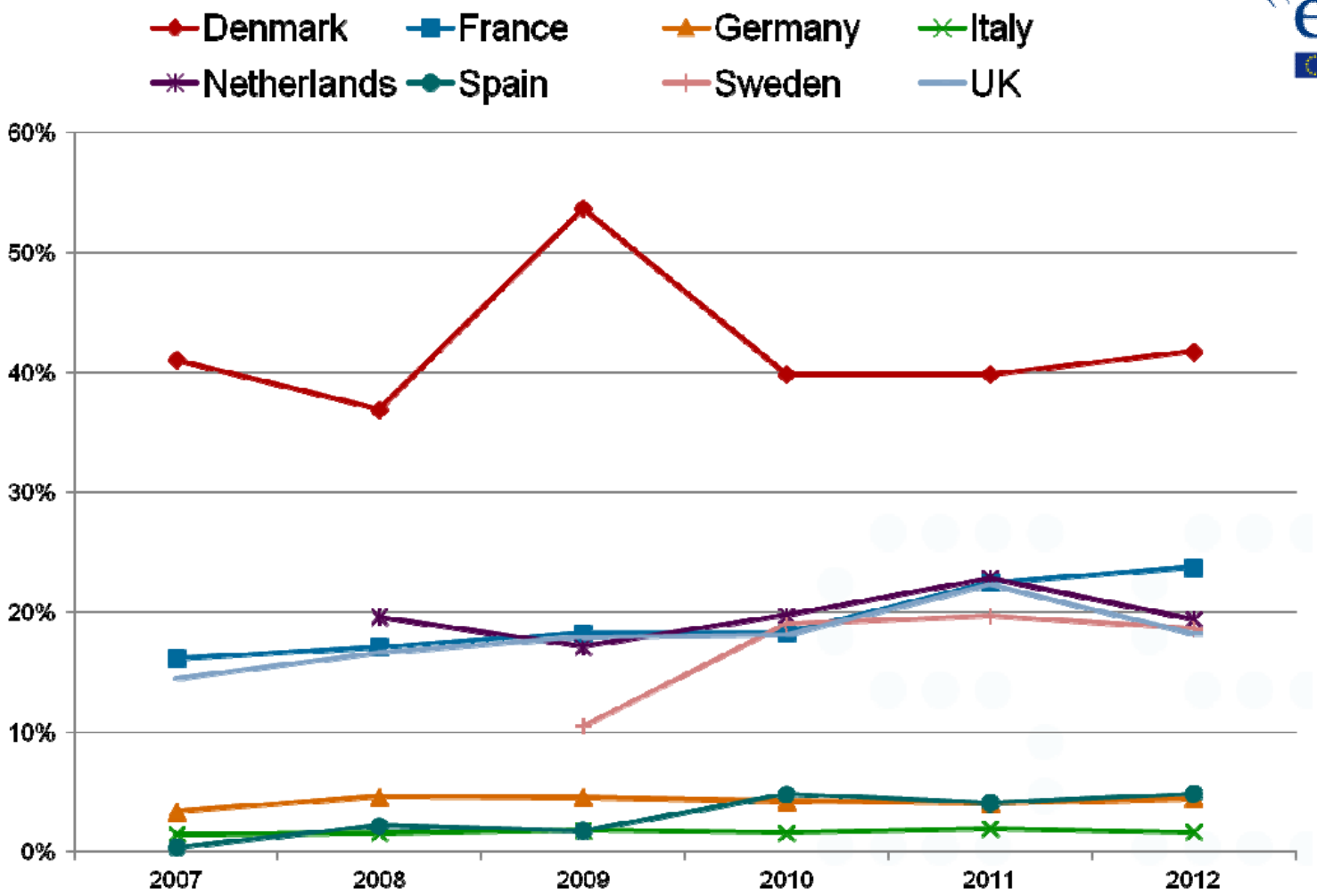
Culture methods



- **Method**
  - Samples from lower respiratory tract
  - Special media is needed (BCYE with and without antibiotics)
- **Performance**
  - Sensitivity High >60% and 100% specific
  - All *L. pneumophila* serogroups can be detected
  - The method is confirmatory
  - Isolation of the causative agent for genotyping in case of outbreak
- **Limitations**
  - Culture depending on the quality of the material obtained from the patient, quality of media, procedure and lab experience
  - Other *Legionella spp* appear as uncharacteristic colonies after several days
  - Growth is generally slow (3 to 7 days)

Proportion of cases diagnosed by culture: selected countries, EU



http://ecdc.europa.eu/en/publications/Publications/legionnaires-disease-2015.pdf

Identification of *Legionella spp*

- **Standard phenotypic methods**
  - Morphology of the colonies
  - Grows on Blood agar (or without cystein)
  - Autofluorescence ?
  - Latex agglutination test
    - *L. pneumophila* (Lp1/Lp 2-15)
    - Further tests are required for other *Legionella spp*
- **Maldi-Tof**
  - Highly discriminant
  - Good performance for species identification
- **Molecular methods**
  - Sequence analysis of *mip* gene
  - Free database hosted by Public Health England (access still available via www.ewgli.org)

Urinary antigen test

Å Methods

- ï Urine by immunochromatographic assay or ELISA
- ï 15 minutes assay

Å Performance

- ï Good sensitivity (60-95%) and excellent specificity (>99%)
- ï Concentration of urine increases sensitivity
- ï Positive detection one day after onset of symptoms
- ï The method is confirmatory

Å Limitation

- ï Only detection of *L. pneumophila* serotype 1
- ï Not possible to (sub)type the causative agent for outbreak investigation
- ï Samples can be false positive (especially if not heat treated)
- ï Possible prolonged Ag excretion for several months especially in immunocompromised patients

Serology

Å Methods

- ï Serum, commercial assay can be automated

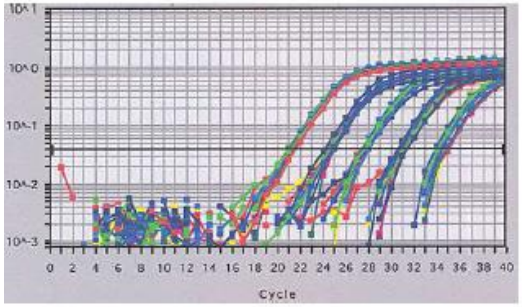
Å Performance

- ï High sensitivity (> 75%) if the right antigens are used and samples are collected timely
- ï All *L. pneumophila* serogroups (and species) can be detected
- ï Confirmatory if significant rise in antibody for *L. pneumophila* sg 1 demonstrated
- ï Outbreak investigation

Å Limitation

- ï Dependent of the patient´s antibody response, the time can vary considerably from patient to patient (few days to weeks) before positive levels can be detected
- ï Dependent on well timed samples: one acute phase and one convalescent phase sample
- ï high antibody level can persist for a long period (weeks, month and years)
- ï High proportion of “false” positive samples are seen especially for *L. pneumophila* non-serogroup 1 serogroups and species
- ï Asymptomatic and mild infection can also elicit a response

Molecular methods



Methods

- PCR on respiratory samples including sputum, BAL, …
- Method fast in few hours (faster than culture)

Performance

- High sensitive (75-95%) and good specificity (>95%)
- All *L. pneumophila* serogroups (and species) can be detected
- The infection can be diagnosed from the first day of admission to hospital
- DNA typing (SBT) can be performed directly on the patient sample
- *Legionella* can be cultured directly from the PCR positive sample

Limitations

- Not confirmatory
- The method requires expensive laboratory equipment
- False positive reactions due to contamination
- Not all patients produce sputum in the acute phase

Conclusion

- Environmental origin
- *Legionella pneumoniae* cause severe disease particularly in immunocompromised patients
- First cause of travel-associated pneumonia
- Difficult diagnosis
  - Requiring combination of methods
  - Including Ag detection, PCR and culture
- Mandatory reporting
  - Underreported and probably underdiagnosed

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