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Under the auspices of the Belgian Association of Public Health
# PROGRAMME

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ABSTRACTS OF PRESENTATIONS
DENIS PIÉRARD

BIOGRAPHY
Prof. Denis Piérard graduated as medical doctor at ULB in 1981 and trained in the Clinical Biology at the Universitair Ziekenhuis Brussel (formerly AZ-VUB). In 1986, he started his career in the department Microbiology and Infection Control under leading of prof. Sabine Lauwers. He is interested in many aspects of clinical microbiology, and defended in 1998 a thesis entitled “Epidemiology, clinical impact and virulence factors of verocytotoxin-producing Escherichia coli in Belgium” at the Vrije University Brussel (VUB). From 2016, he is head of the Department Clinical Biology of the Universitair Ziekenhuis Brussel, coordinator of the Laboratory Microbiology & Hospital Hygiene of the Universitair Ziekenhuis Brussel and coordinator for the AIDS reference laboratories of the Vrije Universiteit Brussel. Since 2001, he is associate professor at the Faculty of Medicine and Pharmacy of VUB and teaches among others Basis concept of disease: Microbiology and Infection and Pharmaceutical Microbiology. In the frame of the program of National reference Centers for Microbiology, organized for RIZIV/INAMI by Sciensano, he is coordinator of NRC Bordetella, NRC Burkholderia, NRC Diphtheria, NRC Legionella and NRC STEC/VTEC.

TRANSPORTATION OF SAMPLES TO NRCS

Biological substances are often transported between clinical laboratories without the elementary precautions of safety. The NRCS are particularly concerned, in particular those who deal with the most infectious pathogens, a fortiori when cultures are sent. In practice, most complains are registered by the NRC mycobacteria and the NRC Brucella, as the risk of aerogenic spread of Mycobacterium tuberculosis complex and Brucella spp. is particularly high. The packaging of infectious substances for transport must therefore be designed to minimize the potential for damage during transport. In addition, the packaging must ensure the integrity of the materials and so, in turn, timely and accurate processing of specimens.

- Several international organisations as WHO are giving guidance on regulations for the transport of infectious substances. In Belgium, information can be found on the Belgian Biosafety server. In addition, Sciensano has issued some recommendations for sending clinical samples in a circular letter. See addresses of websites in the references.
- Infectious substances are classified in two categories: A, labelled with hazard label for infectious substance concerns pathogens which are transported in a form that, when exposure to it occurs, are capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals. Besides biological substances containing pathogens like Ebola, most other pathogens enter in this category only after culture. Transport must be by private carriers, as sending by
post is not allowed. Cat. B (labelled UN3373) comprises other infectious substances.

- For both categories, basic triple packaging system is required: a primary watertight, leak-proof receptacle containing the specimen, packaged with enough absorbent material to absorb all fluid in case of breakage or leakage, a secondary durable, watertight, leak-proof packaging to enclose and protect the primary receptacle(s). Outer packagings with suitable cushioning material protect their contents from outside influences, such as physical damage, while in transit.

- All transport of biological samples containing potentially infectious substances stay under the entire responsibility of the sender. All personne

REFERENCES

2. Belgian Biosafety Server: https://www.biosafety.be/ Go to «Specific biosafety topics», then to «Safety measures ...»
WESLEY MATTHEUS

BIOGRAPHY

In 2010 Wesley Mattheus obtained his PhD at the Faculty of Bioengineering Sciences of the KULeuven for the genetic characterization and modification of biosynthetic clusters of new antibiotics. In 2010 he started as responsible of the National Reference Laboratory Antibiotic Resistance in foodborne pathogens. Since 2011, he heads the Human National Reference Centers for Salmonella, Shigella, Listeria and Neisseria associated with the unit ‘Gastrointestinal diseases and Bacterial meningitides’. He is responsible for the quality assurance of the diagnostic and surveillance activities carried out here under ISO15189 and ISO17025 accreditation.

USE OF WGS IN A NATIONAL REFERENCE CENTRE

The Bacterial Diseases scientific service at Sciensano hosts four National Reference Centres (Neisseria meningitidis, Listeria monocytogenes, Salmonella/Shigella and Mycobacteria). The role of a National Reference Centre of an infectious disease pathogen is on the one hand to confirm the diagnosis of the primary laboratory, yet more importantly, on the other hand to (sub)type the pathogen. Historically, this (sub)typing was performed by biochemical and serological tests and often remain the first methods of choice for primary screening. Over the past two decades several molecular subtyping methods (PCR, PFGE, MLVA, MLST, …) have been developed for more in-depth analysis. Some of these methods were sequencing-based, targeting specific regions of the bacterial genome. Although very useful, these methods are very linear and therefore limited to a relatively low number of different regions. In recent years new techniques for sequencing became available allowing us to sequence the entire bacterial genome in one single experiment, Whole Genome Sequencing (WGS). This promising technique extends molecular surveillance to a whole new level on much higher resolution. Several possibilities (and difficulties) for the use of WGS in enhanced molecular surveillance at a National Reference Centre will be discussed.
KATRIEN LAGROU

BIOGRAPHY

Katrien Lagrou is Head of the Microbiology Laboratory at the University Hospitals of Leuven and the coordinator of the Belgian National Reference Center for Mycosis. She is professor at the Faculty of Medicine of the University of Leuven (KU Leuven), Leuven, Belgium. Professor Lagrou obtained her Master’s degree in Pharmaceutical Sciences from the University of Leuven in 1992, and remained there to specialise in Laboratory Medicine between 1992 and 1997. During this period, she received a degree in Mycology from the Institute of Tropical Medicine in Antwerp, Belgium and completed her PhD in 2002.

Professor Lagrou’s main interest is the diagnosis and treatment of infections in severely immunocompromised patients, with a focus on invasive pulmonary aspergillosis. She is president of the Belgian Society of Human and Animal Mycology, Chair of the European Confederation of Medical Mycology (ECMM) Committee Academy and board member of the Belgian Society of Infectiology and Clinical Microbiology. Professor Lagrou is also a member of the Editorial Board of the journals Mycoses, Medical Mycology Case Reports and the Journal of Clinical Microbiology.

AZOLE RESISTANCE IN ASPERGILLOSIS

The environmental mold Aspergillus fumigatus (A. fumigatus), causes a broad spectrum of diseases varying from acute invasive aspergillosis (IA) to allergic and chronic infections. IA is the most serious entity among aspergillus-related diseases and is a common infectious complication in severely immunocompromised hosts like cancer or transplant patients.

Despite earlier diagnosis and more effective and/or less toxic antifungal drugs, mortality of invasive aspergillosis remains high fluctuating between 20 to 30% at best. Therapeutic options to treat aspergillus related diseases are limited, with triazole antifungals, like voriconazole or isavuconazole, as the recommended first line agents. However, current treatment is at risk as the numbers of triazole-resistance reports in A. fumigatus increase. Most triazole-resistant associated mutations are located in the encoding gene for the target protein of antifungal azoles, the 14-alfa-demethylase enzyme (Cyp51A gene) which converts lanosterol to ergosterol, a key component of the fungal cell membrane. Resistance may develop during triazole therapy (patient route) but may also develop in the environment (environmental route). The most frequently reported resistance mechanisms are the mutations TR34/L98H and TR46/Y121F/T289A, which are present in resistant A. fumigatus in the environment and commonly confer resistance to all triazoles. Hotspots for triazole resistance in A. fumigatus were identified in the Netherlands, namely compost from flower bulb waste, green compost and wood chippings.
Prevalence of triazole-resistance differs between countries, centers and underlying conditions (3.2%-36.3%), hence extrapolation of resistance prevalence data from other countries might not be representative of the current situation in our centers. In Belgium, triazole resistance prevalence in A. fumigatus is around 5% but in a recent retrospective study of all clinical records of haematological patients from the University Hospitals Leuven from 2012 to 2017, triazole resistance prevalence was 17.1% (6/35 culture positive IA cases, A. Resendiz Sharpe, 14th Fungal Update meeting, London, 15th & 16th March 2019).

REFERENCES

1. Mortality rates as high as 50 to 100% have been reported among triazole-resistant IA patients; and recently voriconazole-resistance was associated with increased mortality (62%) compared to voriconazole-susceptible (37%) in a cohort study of culture-positive IA cases with diverse underlying conditions (Lestrade PP et al. Clin Infect Dis 2018 Epub ahead of print). It is therefore essential to detect triazole resistance rapidly in patient samples and to conduct continuous surveillance for patient’s treatment recommendations.
YVES VAN LAETHEM

VACCINES IN THE PIPELINE
GEERT LEROUX-ROELS

BIOGRAPHY

Geert Leroux-Roels, graduated as MD at the Ghent University in 1976. As a medical student and during specialist training in internal medicine, he conducted doctoral research in clinical pathology and immunology. After obtaining a board certification in internal medicine and a PhD degree in biomedical sciences in 1981, he worked as a postdoc in the Scripps Research Institute (La Jolla, CA) and the Laboratory of Molecular Biology at the Ghent University. He was appointed professor of medicine and director of the laboratory of clinical pathology in 1989. Over the past 30 years, Geert Leroux-Roels and his team have studied the human immune response to HBV, HCV, HIV and influenza. A small animal model (human liver in uPA-SCID mouse) was developed to study of hepatotropic pathogens. He founded the Center for Vaccinology (CEVAC - Ghent University and University Hospital) and directed this unit for three decades. During this period, more than 250 clinical vaccine trials have been conducted. Numerous candidate vaccines against a large variety of pathogens and new adjuvant systems have been evaluated.

Geert Leroux-Roels has authored over 270 peer-reviewed articles and was a member of several international societies and scien

EFFECT OF ANNUAL INFLUENZA VACCINATION ON VACCINE EFFECTIVENESS

Annual vaccination against influenza is considered the most efficacious manner to prevent influenza infections and the morbidity and mortality they inflict. Annual vaccination is therefore recommended by many national health authorities, initially prioritizing populations at high risk for complications but progressively evolving towards a recommendation in the US and Canada for anyone over the age of 6 months.

A growing body of evidence suggests that consecutive influenza vaccination may blunt or reduce the protective effectiveness of a vaccine given in a current year. This observation was first made in the 1970s in an English boarding school were boys who received consecutive vaccinations were more at risk of infection during outbreaks than boys vaccinated for the first time (1). Several observational studies using a “test negative design” have confirmed this finding and triggered concern about the policy of annual influenza vaccination.

A meta-analysis of vaccine effectiveness studies from 2010-2011 through 2014-2015 (2) showed substantial heterogeneity in repeat vaccination effects. Most pronounced negative effects were observed for H3N2 in 2014-2015 (3). Observations across multiple seasons suggest that vaccine effectiveness may be influenced by more than one prior season.
Several immunological phenomena may be at play in reducing the effectiveness of influenza vaccines when given repeatedly. The “antigenic distance hypothesis” put forward by Smith et al (4) is the most useful theoretical framework to understand the variable effects of repeat vaccination. However, this explanation and several others that have been proposed are solely based on the antibody response to the hemagglutinin. It is not impossible that other components of the virus and different arms of the immune response are involved.

Further epidemiological studies across multiple seasons and more insight into the underlying immunological mechanisms are needed to understand repeat vaccination effects and guide future recommendations (5).

REFERENCES

MARC ARBYN

BIOGRAPHY

M Arbyn has diplomas of MD, MSc, Dr in tropical medicine and PhD in medicine & health Science. After a career in Africa with Médecins Sans Frontières, he got involved in cancer research in Europe. MA coordinated the evaluation of new screening methods in the framework of the EU Network of Cervical Cancer Screening and is editor- of the European Guidelines for Cervical Cancer Screening and associated Supplements on HPV screening. His main activity deals with systematic reviews, Cochrane reviews and meta-analyses regarding cervical cancer screening, diagnosis and treatment of screen detected cervical cancer precursors and HPV vaccination. MA is involved in several international networks dealing with biobank-based cancer research (addressing etiological questions and possible application of biomarkers in screening), clinical validation of tests applied in screening, diagnosis and prognosis prediction and offers epidemiological support to health authorities regarding implementation of cervical cancer prevention.

MA is author of >200 papers, published in peer-reviewed journals, related to cancer prevention. MA is also specialised in trend analysis and age-cohort-period modelling of the incidence and mortality from cancer.

MA is coordinator of the Unit of Cancer Epidemiology, which is part of the Belgian Cancer Centre at the Scientific Institute of Public Health in Brussels.

IMPACT OF HPV VACCINATION ON THE INCIDENCE OF CERVICAL CANCER
Alethia™ CMV provides early detection of congenital CMV which is vital in guiding appropriate treatment for newborns.

- Report results with confidence with the First FDA cleared test for cCMV
- Utilizes saliva, an easy to collect and preferred sample type for congenital CMV testing
- Obtain results in less than one hour with minimal hands on time
- High NPV provides confidence to physicians and piece of mind to parents before their baby goes home

Your lab can improve outcomes for newborns.

- How would an FDA cleared cCMV test with a simple procedure and results in less than one hour help improve turnaround time and overall lab workflow?
- Knowing cCMV is the leading cause of deafness in newborns, how would you implement a test in your health system that can impact patient care and reduce costs associated with potential disease complications?
Results That Matter

- The simple procedure of Alethia CMV provides flexibility to best fit the laboratory’s workflow when compared to other testing technologies.
- Saliva is easy to collect and contains high viral load, allowing optimal detection and same day reporting for physicians and families.

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- Implementing newborn testing strategies for cCMV have been increasingly recognized for their potential medical benefits and improved patient outcomes.5
- Congenital CMV testing empowers physicians and families with the full understanding of their newborn’s health prior to leaving the hospital.

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The Alethia CMV Assay is an in vitro diagnostic molecular test for the detection of CMV from the saliva of newborns under 21 days. The test will provide an actionable result to enable the clinical team to develop an appropriate treatment plan.

Turnaround Time
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Sample Type
Saliva from newborns younger than 21 days

Sample Storage
- Saliva swabs can be stored at 19 - 30 C up to 48 hours.
- Refrigerated at 2 - 8 C up to 7 days.
- Frozen at ≤ -20C up to 14 days.

Kit Storage
Kit should be stored at 19 - 30 C

Performance
100% NPA 99.8% PPA

Catalog Number CPT Codes
Alethia CMV - 481325 87496
Alethia CMV
External Control - 479880

Sample Type
- Saliva swabs can be stored at 19 - 30 C up to 48 hours.
- Refrigerated at 2 - 8 C up to 7 days.
- Frozen at ≤ -20C up to 14 days.

Sample Storage

Performance

Catalog Number
CPT Codes

Alethia CMV - 481325 87496
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References

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WIM VAN BORTEL

BIOGRAPHY

Wim Van Bortel is a medical entomologist with more than 25 years of experience in research on vectors and vector-borne diseases in Europe, Africa and Asia. He obtained his Master degree in Biology at the University of Antwerp Belgium in 1990 after which he specialised in Medical Entomology at the Institut Pasteur Paris. In 2002, he obtained his PhD at the University of Antwerp Belgium. From 2010 till 2016 he was Senior Expert vector-borne diseases at the European Centre for Disease Prevention and Control where he was Deputy Head of ECDC’s Emerging and Vector-borne Diseases Programme from December 2013 till December 2016. Since 2017 he works at the Institute of Tropical Medicine Antwerp as senior Researcher in the Unit of Entomology and in the Outbreak Research Team. His research focuses on disentangling the role of arthropod vectors in transmission systems in order to improve vector-borne diseases prevention and control.

MOSQUITO MONITORING AND MOSQUITO-BORNE DISEASES RISK IN BELGIUM

Mosquito-borne diseases are a specific group of infections that represent emerging threats to Europe. They pose a challenge to national public health authorities due to their complex nature, i.e. their complex transmission systems driven by the interaction between the pathogen, the hosts, the vector, and the environment. Further, mosquito-borne diseases tend to globalize by the increasing mobility and travel which resulted in the fast and worldwide spread of vectors and the pathogens they transmit. Over the last ten years a series of local transmission events of so-called tropical mosquito-borne diseases, such as chikungunya and dengue fever, occurred in Europe. Other diseases re-emerge, like malaria in Greece, or spread to other countries, like West-Nile fever.

Between 2007 and 2010, Belgium set-up a nationwide inventory of mosquito biodiversity (MODIRISK project). This was an essential step towards understanding the risk of diseases transmitted by mosquitoes in Belgium. Between 2012 and 2016 surveillance of exotic mosquito species continued on an ad hoc basis. Since July 2017, a three-year active monitoring project of exotic mosquito species is implemented which provides essential information on the introduction and possible establishment of exotic mosquito species in Belgium.

In total four exotic mosquito species were collected between 2007 and 2018. Aedes koreicus and Aedes japonicus are locally established in Belgium. Aedes albopictus, intercepted for the first time in 2000, was found at several occasions between 2013 and 2016, and in 2018 we collected this species at five different points of entry. One individual of Anopheles pharoensis, a known vector of malaria, was intercepted in a cargo airport in 2018. Previous monitoring projects indicated that Aedes mosquitoes entered Belgium via import of lucky
bamboo plants and tyres. New for Belgium is the interception of Aedes albopictus and Aedes japonicus along the border with Luxembourg and Germany, respectively.

The presence of a competent vector is a necessary but not sufficient prerequisite in the emergence and dissemination of a mosquito-borne disease. The vector has to be well established and abundant, and the pathogen needs to be introduced into an area that is permissive for.
ELIZABETH MILLER & ANAIS BOTHY

BIOGRAPHY

Anaïs Bothy is a biological pharmacist specialized in the microbiology field. During a complete formation in clinical biology at UCL, she has developed a strong interest in microbiology and antibioresistance. She had completed her formation with inter-university certificates in hospital hygiene and antibiotherapy management. She’s now working as microbiologist and member of the infection control and prevention team at the hospital of ‘Centre de Santé des Fagnes’, Chimay, in south-east Belgium.

Elisabeth Miller is a medical doctor with an extensive clinical experience. Since mid 2018 she is working in the ‘National Surveillance of Infections in Hospitals’ service (NSIH) in Sciensano (Belgian Public Health Institute). She is responsible for the Outbreak Support Team in collaboration with the Belgian Federated Entities, which provide scientific support to help healthcare facilities to manage multidrug resistant organisms outbreaks. She is actually following the formation in hospital hygiene.

MANAGEMENT OF OUTBREAKS OF RESISTANT BACTERIA IN HOSPITALS IN BELGIUM: THE EXPERIENCE OF THE OUTBREAK SUPPORT TEAM

Introduction: Carbapenemase-producing Enterobacteriaceae (CPE) are an emerging problem worldwide, especially in hospital settings where an increasing number of outbreaks are described. This study presents an outbreak with OXA-48 CPE occurred between August 2017 and November 2018 in the ‘Centre of Sante des Fagnes of Chimay’, a 144-beds hospital in south-east Belgium. We describe a step-by step approach of the measures implemented by the local Infection Control and Prevention team (ICP) to deal with the outbreak, in collaboration with the National Outbreak Support Team (OST).

Material and methods: Since the detection of the outbreak in June 2018, epidemiological investigations were made by collecting demographic data about the patients, the strains involved along with timing and geographical informations. Sporadic cases of OXA-48 CPE strains detected before June 2018 were also included to the analyse, to explore potential epidemiological link with the cases involved in the outbreak. Basal hypothesis were based on patient-to-patient cross-transmission via healthcare workers or contact with human excreta (particularly with stool). Several observations were made to identify critical points regarding these issues and to precise the need of intervention. When additional cases were detected despite application of ICP recommendations, environmental samples were collected (high-touch surfaces, shared medical devices and hospital water environment). During the whole process, OST has reviewed the situation and discussed the main issues encountered with our ICP team. They performed in-depth literature research aiming to identify
and recommend possible solutions/approaches. Both teams regularly exchanged information about how to best manage the outbreak until it was resolved.

**Results:** 29 patients were identified positive for one or more (max. 3) OXA-48 CPE strains between August 2017 and November 2018 in our institution, including 3 infections and 26 colonizations. The strains involved were: Klebsiella pneumoniae (15), *Escherichia coli* (10), Citrobacter freundii (8), Raoultella ornitholytica (4), Klebsiella oxytoca (2), Morganella morganii (3), Enterobacter cancerogenus (1). Surgery and geriatric units have been hardest hit by the outbreak with ten and six patients respectively, and temporarily closed to new admissions. Field observations spotted several mistakes in compliance of hygiene recommendations, with handling of human excreta - especially in bedpan management – and with patient rooms disinfection, leading in several local interventions. Environmental samples shown no grown of CPE strains on dry surfaces but sinks of four room previously occupied by colonized patients were found positive for various CPE strains (two Citrobacter freundii OXA-48 and two Klebsiella pneumoniae OXA-48), as well as water of one toilet bowl. Various sink and toilet decontamination efforts were undertaken including replacement of reservoirs and daily bleach use, along with emission of sink-contamination prevention guidelines. No additional cases were detected for six months despite active surveillance.

**Conclusion:** The outbreak eradication combined approach of classical infection prevention methods (strict compliance to hand-hygiene and review of disinfection procedures), together with active surveillance, improvement of bedpan management and consideration of potential environmental reservoirs. Further audits of practices still have to be set up in order to verify the long-term sustainability of our actions. Close collaborations between healthcare workers, ICP and OST are retained as a key element in the successful control of the outbreak.
NAIMA HAMMAMI

BIOGRAPHY

I’m a pediatrician with a background in public health. I’m currently working for the Agency for Care and Health as an advisor in infection control and prevention and outbreak management. I previously worked in Sciensano as an epidemiologist on hospital-based surveillances linking acquisition of nosocomial infections to quality of care. My previous work experiences are in global health and focused on addressing inequities in accessing health care (INGO, Institute of Tropical Medicine, consultant for WHO).

A SALMONELLA TYPHIMURIUM OUTBREAK IN PRIMARY SCHOOLS OF WEST AND EAST-FLANDERS

Background: On May 22nd and 23th 2018, the Flemish Agency for Care and Health received a notification from three schools in West- and East Flanders about a high number of children with gastro-intestinal complaints. These schools were supplied by the same caterer distributing around 10.000 meals/day among 102 schools in two provinces. We investigated the amplitude and source of this outbreak and present the measures taken to limit further transmission.

Methods: Probable cases were pupils from schools supplied by the caterer with symptom(s) of gastroenteritis between 18 May and 9 June. Confirmed cases were probable cases with Salmonella Typhimurium. To assess the amplitude of the outbreak and calculate attack rates per school, active case finding was organized via the school doctors, GP’s, hospitals and laboratories in the region. To estimate risk associated with school meals, an online survey in five schools was launched. To detect the causal micro-organism, human samples (children and employees from caterer) and 207 environmental and food samples were send to the national reference center.

Results: 546 cases were identified in 55 schools, including 399 confirmed cases in 50 schools, spread over 23 days. Measures linked with hygiene were taken and a temporary halt of the food distribution was imposed to decrease the risk of transmission. 423 students participated to the online survey, including 157 cases. Consumption of a meal provided by the caterer was significantly associated with illness (RR=8.1, 3.1-21.2). The school meal consumed on May 17th (turkey stew and salad) was the most likely source of transmission (RR=3.2, 1.5- 6.7). ARs among schools ranged 0-25%. Salmonella Typhimurium MLVA type 3-14-14-NA-0311 was detected in the stool samples from the children and in one stool sample from an employee (driver of catering service). Microbiological analyses in food and environmental samples were negative.
Conclusions: The school meal consumed 17 May was the most likely source of transmission. The range of ARs and the negative results of food samples may suggest a heterogenic spread. The epidemiological link with caterer and prolonged outbreak peak justified the halt of food distributions between 25 May and 9 June.
ROMAIN MAHIEU

BIOGRAPHY

Romain Mahieu is a medical doctor working as a public health officer for the Common Community Commission (COCOM) of Brussels-Capital. He is involved in the infectious disease surveillance, especially in the mandatory notifiable diseases, and in public health crisis management. Previously, he worked for the European Parliament to monitor and revise its emergency plans and in the Erasmus hospital.

HOT TOPIC: COPING WITH A MEASLES OUTBREAK IN BRUSSELS. WHY WILL THERE BE NO ELIMINATION BY 2020?

Vaccine-preventable diseases are still a global public health issue. Brussels-Capital is facing a measles outbreak since the beginning of 2019. Prophylactic measures were applied to control the spread in hospital and collectivity settings. Up-to-date epidemiological datas will be discussed in a worldwide and European context.
ABSTRACTS OF POSTERS
MYCOPLASMA PNEUMONIAE INFECTIONS ACROSS EUROPE AND ISRAEL (2011-2016)

Loens K¹, Bossuyt N², Lagrou K³, Beeton M L⁴, Zhang XS⁵, Uldum S A⁶, Bébéar C⁷, Dumke R⁸, leven G¹, Nir-Paz R⁹, Peyere S⁷, Spiller O B⁸, Chalker V⁵ on behalf of the European Study Group for Mycoplasma and Chlamydia

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Background

Mycoplasma pneumoniae is a leading cause of community acquired pneumonia, and is largely seen among young children, with large epidemics occurring every four to seven years. Here we looked to determine the diagnostic methods used to determine M. pneumoniae status, the seasonality in M. pneumoniae epidemics, availability of macrolide resistance data, association between age and prevalence and the effect of geographical location and timing of epidemics.

Materials/methods

A retrospective questionnaire was sent to 18 countries across Europe and Israel requesting details on the number of M. pneumoniae positive samples from January 2011 to April 2016. Information requested included: methods of detection, number of positives stratified by age group and week as well as macrolide resistance monitoring. The Moving Epicdemic Method was used to determine epidemic periods across the countries for the five periods under investigation.

Results

F12/18 countries supplied data on M. pneumoniae infections accounting for 95,666 positive samples with NAAT being the most commonly used method for detecting M. pneumoniae status (10/12 countries). Routine macrolide resistance monitoring was not systematically in place. Combined data from 12 countries identified three epidemic periods during 2011/12, 2014/15 and 2015/16. During the year of 2011/12 numbers of positive samples were greater during the epidemic period than in the pre-epidemic period (average 17%, range 9% - 30%).
Examining the age distribution of *M. pneumoniae* positive samples in each country three patterns were apparent with positive samples skewed to younger age groups, positive samples skewed to older age groups and a bimodal distribution. Intriguingly during epidemic years there was an association between country latitude and week number in which epidemic periods commenced in a wave from Northern countries through to the South.

**Conclusions**

This study represents the largest collection of *M. pneumoniae* data to date detailing the methods for detection, the lack of macrolide resistance monitoring, trends in age distribution as well as an association between epidemics and latitude. Guidelines for testing may be beneficial by highlighting infections in all age-groups and a systematic process for case monitoring and macrolide resistance may be of future benefit to identify epidemic waves and resistance across Europe.

Keywords: *Mycoplasma pneumoniae*, epidemiology, diagnostics
MANUAL FOR THE ORGANIZATION OF PERFORMANT AND SUCCESSFUL VACCINATION CAMPAIGNS IN ORDER TO INCREASE INFLUENZA VACCINATION UPTAKE IN HEALTHCARE WORKERS

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Introduction

Healthcare workers (HCWs) can play an important role in transmission of influenza during nosocomial outbreaks in patients and residents of long-term care facilities (LTCFs). Despite the known advantages of immunizing HCWs, coverage rates are generally low, and range from 14% to 45.6% in Europe [1]. The Belgian National Immunization Technical Advisory Group (NITAG) recommends influenza vaccination for all HCWs and the Flemish government set a target of 80% coverage in HCW by 2020. We previously conducted a study to identify factors that influence uptake of flu vaccination in HCWs [2]. In follow-up of this study, we developed an instruction manual, scientific brochure and promotional materials, which were made available for flu coordinators of all Flemish hospitals and LTCFs. In the current survey we studied the implementation and impact of this manual and related documents on the vaccination coverage and change in attitude regarding influenza vaccination among HCWs.

Methods

The instruction manual, scientific brochure and promotional materials were developed based on the results of our previous study on attitudes towards influenza and influenza vaccination uptake in 5141 HCWs from 13 hospitals and 14 LTCFs [2]. Additionally, literature on interventions to increase vaccination uptake, and documents from the world health organization (WHO), Centers for Disease Control and Prevention (CDC) and European Centre for Disease Prevention and Control (ECDC) were consulted to develop a manual intended to help healthcare institutions increase the vaccination rate among staff members. The manual included 24 interventions addressing topics like vaccination without prior enrolment, mobile teams, involvement of supervisors, education, and extensive communication with staff members. The implementation of these interventions during the 2017 vaccination campaign was evaluated in 11 LTCFs. HCWs of the participating LTCFs were surveyed before and after the campaign to study potential changes in beliefs and attitudes regarding influenza vaccination. Furthermore, the local coordinators of the vaccination campaigns were interviewed about the utility of the manual. Change in vaccination coverage and perception of the vaccination campaign were described and linear mixed-effects models were used to determine change in attitudes of HCWs.
Results and conclusion
The mean vaccination coverage reported by the 11 LTCFs during the 2016 and 2017 influenza season was 53.6% and 67.9% respectively. The coverage increased 10-20% in all 9 LTCF that implemented 7 or more new interventions described in the manual, reaching a mean coverage of 71%. Two LTCFs who did not see an increase in vaccination coverage confessed that they had barely changed their campaign strategy and implemented few measures from the manual. In total 645 HCWs (response rate: 51.4%) completed the baseline survey and 524 (response rate: 41.8%) the evaluation survey, of whom 340 answered both surveys. The self-reported coverage was 58% in the 2016 campaign (measured at baseline), and 71.5% in 2017 (measured at follow-up). Several beliefs regarding influenza vaccination in HCWs changed significantly after the vaccination campaign in LTCFs that implemented multiple interventions. For example the seriousness of influenza was less underestimated (p=0.001) and the less HCW expected to have side effects after vaccination (p=0.03). Overall 68.2% of the respondents found the campaign informative and the majority indicated to be well informed about the risks of influenza (67.2%), the risk of transmitting influenza to the residents (70.4%) and the safety and efficacy of the vaccine (63.3%). The manual was considered a useful tool by the organizers of the vaccination campaigns.

Implementation of the measures described in the manual and use of related materials was associated with a change in attitudes towards influenza vaccination and with an increased vaccination uptake.


IMPLEMENTATION OF WHOLE-GENOME SEQUENCING FOR CHARACTERIZATION OF SHIGA TOXIN-PRODUCING ESCHERICHIA COLI AT THE BELGIAN NATIONAL REFERENCE CENTRE

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Introduction

Presently, conventional PCR is used at the Belgian national reference centre (NRC) for virulence characterisation (Siga toxin [stx] subtype, eaeA, ehxA, aaiC and aggR) of Shiga toxin-producing Escherichia coli (STEC), which is needed in the context of molecular surveillance. Yet, some reference laboratories are using whole-genome sequencing (WGS) to that end (Dallman et al. 2015; Lindsey et al. 2016). The sequencing data obtained after performing WGS enables to fully characterize STEC isolates, including O:H type, virulence genes and wgMLST, which facilitates epidemiologic surveillance and enables data comparison in case of multi-country outbreak. We compared the results obtained by traditional methods for O:H typing and virulence typing to those obtained using the E. coli genotyping plugin tool of BioNumerics in order to validate the implementation of WGS at the Belgian NRC.

Methods

Whole genome sequencing was performed using the HiSeq instrument (Illumina) on a selection of 40 Belgian human STEC strains isolated in 2018. The sequencing data was analysed using the E. coli genotyping plugin tool of BioNumerics (Applied Maths). In the context of surveillance, traditional methods were used for O:H serotyping and virulence typing as described on the website of the national reference centres for human microbiology (NRC website).

Results

There was 100% agreement between the O-types obtained via the traditional method and WGS for 26 out of the 40 isolates, which belong to the “Big 6” serotypes. Out of the remaining fourteen non-O157 isolates, five O-types were determined correctly, six could not be determined by both methods and three were only determined by one of these methods. FlicH7, eaeA and ehxA genes were detected correctly by the in silico PCR tool of BioNumerics in, respectively, 10, 31 and 36 isolates. Twenty-four out of the 40 isolates were positive for stx1. All of these were detected by in silico PCR. There was 100% agreement with stx1 subtyping. No isolates were positive for stx1c and stx1d. All thirty-one stx2 positive isolates were correctly determined by the in silico PCR tool. There was a good agreement with stx2 subtyping with the exception of 4 strains carrying...
both stx2a and stx2c, a well-described discrepancy encountered with the *E. coli* genotyping plugin tool. No isolates were positive for stx2g.

**Conclusions**

A good agreement was found between the results obtained by both, the traditional methods and WGS, for the O:H types and the virulence genes. Though some additional work is necessary to unravel the discrepancies encountered in some isolates, from now on, we will use the WGS technology at the NRC for molecular surveillance.

**References**


RSV INFECTION IN HOSPITALIZED ADULTS WITH SEVERE ACUTE RESPIRATORY INFECTION DURING FOUR INFLUENZA SEASONS IN BELGIUM: PREVALENCE, SUBTYPE DISTRIBUTION, RISK FACTORS & OUTCOME

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Background
Respiratory syncytial virus (RSV) infection is recognized as an important cause of hospitalization in the elderly and adults with risk factors. Various RSV vaccines are in clinical phase evaluation. However, the required prevaccination data about risk groups, burden of disease and baseline epidemiology in adults are scarce in Europe. We aim to provide these data from an already existing surveillance of severe acute respiratory infections (SARI).

Materials/methods
We used data of influenza seasons 2012-2013, 2015-2016, 2016-2017 and 2017-2018 from the 6 sentinel hospitals of the Belgian SARI surveillance, to assess RSV prevalence, subtype distribution, risk factors and outcome among adults hospitalised because of SARI (fever >38°C or history of fever, dyspnoea and/or cough). Naso-pharyngeal swabs from all subjects were screened for Influenza and RSV by multiplex PCR. Socio-demographics, risk factors and complications were prospectively collected.

Results
The overall prevalence of RSV infection among SARI cases for the 4 seasons was 5.5% (165/3001). RSV prevalence varied highly between seasons (8.25%, 2.05%, 9.15% and 3.01% in 2012-2013, 2015-2016, 2016-2017 and 2017-2018 respectively). RSV-A was predominant in 2012-2013 and 2015-2016 and RSV-B in 2016-2017 season while both strains circulated in 2017-2018. RSV-infected subjects were older as compared to non-RSV SARI cases (71.3 vs 68.8 years, p=0.036). As compared to influenza infected subjects, RSV-infected subjects reported less frequently a history of fever (70.3% vs 82.6%, p<0.001) but more frequently complained of dyspnea (74.3% vs 64.2%, p=0.01). Presence of heart disease was more frequent in RSV patients as compared to influenza patients (39.9% vs 31.1%, p=0.04) and was the only risk factor associated with RSV among all SARI cases (OR 1.45, 95% CI 1.03-2.04). Complication rates (ICU admission, ARDS,
pneumonia) and mortality were similar between influenza and RSV-infected subjects but length of stay was longer in RSV-infected subjects (12.7 days vs 11.6 days, p= 0.04). RSV-infected subjects >80 years and with ICU admission had higher risk of death (OR 4.9, 95% CI 1.3-24 and OR 6.1, 95% 1-37.3, respectively).

Conclusions
If timed appropriately, surveillance networks using the SARI case definition allow surveillance of RSV seasonality, subtype distribution, severity and burden in an adult population.
The Belgian national reference center for BCC and other Gram negative non fermenters (GNNF) is a consortium of the laboratory of microbiology of UZ Brussel and the laboratory of microbiology of the Faculty of Sciences of UGENT. The main task of the consortium is the surveillance of BCC and other GNNF microorganisms in CF patients. Belgian laboratories send each year up to two BCC and GNNF isolates (excluding *P. aeruginosa* and *Acinetobacter* spp.) from each colonized patient. Each strain is first identified to the genus level by MALDI-TOF mass spectrometry at LM-UZ Brussel. Antibiotic susceptibility tests are performed on all submitted isolates. Secondly, RAPD is used if an earlier isolate of BCC and GNNF isolates from the same patient has been previously identified at the NRC, in order to check if it has to be further identified. All first isolates, isolates that do not match with previous ones from the same genus and each first isolate within a period of 12 months, are sent to LM-UGent for further characterization. This consists in the identification to the species level by gene sequencing of appropriate genes (*recA*, *nrdA*, *rpoD* or 16SrRNA) and MLST typing based on WGS data. This MLST characterization of the isolates is performed in order to further investigate the possible association between some subtypes and pulmonary unfavorable disease evolution in CF patients.

In 2018 we received 128 BCC-GNNF isolates from 103 patients. This number is comparable with previous years (average 126 cases from 2012 to 2017). Among the patients, 93 were CF patients, 8 had other lung diseases and 2 were affected by other diseases. The mean age of the patients was 27 years (median: 23), mean age for cases from 2012 to 2017 was 25 years (median: 22).

A total of 30 BCC and other *Burkholderia* species were referred to our NRC, 67% of which were *B. multivorans*, followed by *B. cenocepacia* (13%) and *B. vietnamiensis* (13%).

A total of 98 GNNF were referred to our NRC, 49% of which were *Achromobacter* spp., followed by *Stenotrophomonas maltophilia* (23%) and other GNNF at lower proportions.
A total of 48 Achromobacter spp. were referred to the NRC, 71% of which were A. xylosoxidans followed by A. insuavis (15%) and other Achromobacter spp. at lower proportions.

A total of 22 different BCC ST types were found among BCC isolates. ST-603 and ST-739 were the most frequent types among B. multivorans; each of these types was found in 3 different patients respectively. These types were also among the frequent ST’s in the past 6 years. A total of 35 different Achromobacter ST types were found. Just like previous years, ST-137 and ST-175 were the most frequent ST’s among A. xylosoxidans isolates in CF patients. These types were found in 5 and 4 patients, respectively. These 2 ST clones should be further investigated to study whether they are sharing the same source or if they are only representing the most frequent community acquired types. It is also interesting to mention that the ST-137 was also recently isolated from CF patients in France in 5 centers spread over the country.

The main conclusions based on the data of 2018 as well as data since 2012:

- The proportion of Burkholderia spp. isolates has been declining over the years while the proportion of other GNNF isolates has been increasing. The reason for these trends should be further investigated. Optimal measures and/or cures have been given against Burkholderia spp. while less optimal for other GNNF in CF patients?
- Three Belgian centers are contributing to a large proportion of the referred isolates. Other Belgian centers should include more cases in order to obtain a better representation of the distribution of cases over the whole country.
WHOLE GENOME SEQUENCING AS MOLECULAR TYPING METHOD FOR LEGIONELLA PNEUMOPHILA


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Background
Legionella pneumophila (L.pn) is a gram-negative facultative intracellular pathogenic bacterium that can cause Pontiac fever and legionellosis. Pontiac fever is a mild disease that can cure spontaneously while legionellosis is pneumonia with mortality rate ranging from less than 1% and up to 80% depending on several factors mainly the immune system of the host. The bacterium is found in air conditioning systems, cooling towers, showers, fountains and other devices that make aerosols. People become infected by inhalation of aerosols contaminated by Legionella. Typing of L.pn is until now mainly performed using the gold standard Sequence based typing (SBT) method. A standardized and accurate epidemiological typing method is of high need when an outbreak occurs. The SBT is very discriminatory and reliable; however, when dealing with very frequent sequence types, it is often very difficult to conclude whether environment and patients isolates belonging to the same frequent ST are related. Other methods, like Pulsed-field Gel Electrophoresis (PFGE), are applied to further discriminate such isolates. But it has a limited value because its discriminatory power is also not very high. The recent advances in whole genome sequencing (WGS) for outbreak investigations, both for nosocomial as for community outbreaks of several public health important microorganisms like L.pn are currently making big revolution. In order to implement WGS, as alternative method for SBT in routine use, we evaluated the new available WGS tools for the typing of strains from old outbreaks, small clusters and unrelated isolates..

Methods
A panel of 77 clinical and environmental L.pn isolates was selected for WGS typing evaluation. Out of these isolates, 7 and 12 (+4 as non-related controls) isolates belonging to 2 important old outbreaks respectively. The remaining isolates belonging to small clusters as well as non-related isolates of very diverse ST types. Genomic DNA was extracted and purified from pure cultures of the isolates using Qiagen dneasy blood & tissue kit (Qiagen). The proper amount of genomic DNA was then used for the library preparation using the Kappa HyperPlus Library Preparation Kit (Kapa Biosystems). Sequencing was perfor-
med on the MiSeq sequencing system (Illumina) using the MiSeq Reagent kit v2 (500 cycle): 2 x 250 bp read-length. Assembly of the reads was done with the SPAdes assembly software. Sequencing and assembly were performed at BRIGHTcore WGS platform of VUB-ULB. WGS data were further analysed using Core genome/whole genome MLST (cg/wgMLST) using the available schemes, namely the “Ridom SeqSphere+ software”, “Applied Maths-Bionumerics” software and the 50 loci cgMLST (CDC/ESGLI_ESCMID).

Results and conclusions

• Based on quality control results at each of the different steps of WGS, optimal results were obtained by using the above mentioned protocol.
• Based on the data interpretation schemes evaluated, concordant results were obtained by *Legionella pneumophila* cgMLST in comparison to SBT results for the tested isolates.
• Comparable results were also obtained by the different tested interpretations typing tools.
• The availability of public WGS interpretation tools allows for standardisation between different laboratories.
• The higher discriminatory power of WGS in comparison to previously described typing methods will allow for more accurate results and conclusions, especially for environment investigations in outbreak situations.
• The higher throughput of WGS is also very valuable for identifying a potential outbreak source, as several samples are generally taken from a big number of suspected areas.
EVALUATION OF TWO NOVEL IMMUNOCHROMATOGRAPHIC SCREENING ASSAYS FOR DETECTION OF CARBAPENEMASE PRODUCERS

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Background
Carbapenemase producers are frequently involved in nosocomial outbreaks, making early and reliable laboratory detection essential. We evaluated the performance of two recently introduced immunochromatographic screening tests for the detection of carbapenemase producers including the RESIST-4 O.K.N.V. K-set (Coris BioConcept, Gembloux, Belgium) and NG-Test CARBA 5 (NG Biotech, Guipry, France) tests.

Materials/methods
A collection of 15 selected Enterobacteriaceae and 2 Pseudomonas spp. strains producing carbapenemases type OXA-48/KPC/VIM/NDM, and 5 non-carbapenemase-producers were tested. The strains were well-characterized by our laboratory using multiplex PCR (N=9) or originated from our quality control collection (N=13). All isolates were cultured and subcultured (±24h at 35-38°C, 5% CO2) on Columbia agar + 5% sheep blood (Biomérieux). Using the RESIST-4 test, a solution of one bacterial colony in 12 drops of buffer was prepared of which 3 drops were added to the cassettes labeled (i) KPC and OXA-48 and (ii) NDM and VIM. For the NG-Test CARBA 5 test, one bacterial colony was dispensed in 5 drops of buffer and 100 µL of the mixture was pipetted on the test cassette of OXA-48/KPC/VIM/NDM/IMP. Diagnostic accuracy of both screenings tests were determined.

Results
All (non)-carbapenemase-producers were correctly detected by both screening tests (results are listed in Table 1). Both tests showed an excellent sensitivity and specificity of 100%. Results were available in less than 20 minutes.

Conclusion
Both RESIST-4 O.K.N.V. K-set and NG-test CARBA 5 are rapid, easy and accurate tests for screening of carbapenemase producers and less expensive than multiplex PCR.
<table>
<thead>
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<th>Resistance mechanism (reference method)</th>
<th>MIC meropenem (µg/mL)</th>
<th>Isolate</th>
<th>Result RESIST-4</th>
<th>Result CARBA 5</th>
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<td>VIM</td>
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FIRST BELGIAN HEPATITIS E SEROPREVALENCE STUDY SHOWS
LOW STABLE BIRTH-COHORT SPECIFIC SEROPREVALENCE UNTIL
2014, WITH RECENT 2016-2018 INCREASE IN SINGLE CENTRE
ESTIMATES

Ho E, Hutse V, Verburgh V, Jacques M, Theeten H, Litzroth A, Suin V, Van Gucht S, Blaizot S,

Background and Aims
Recent studies have shown rising seroprevalence of hepatitis E virus (HEV) in
younger age cohorts in Europe, but substantial regional differences are found.
We aimed to evaluate trends in time in birth cohort-specific HEV seropreva-

lence and regional differences in Belgium.

Methods
Firstly, we performed a retrospective analysis of HEV IgG seroprevalence on two
national serum banks obtained from sentinel laboratories in 2006 and 2014.
Five to ten-year age cohorts held equal amounts of samples and were equally
distributed in sex and regional origin. Secondly, a prospective, single centre HEV
IgG evaluation was performed of 1200 patients visiting the Hepatology depart-
ment between 2016 and 2018. Wantai anti-HEV IgG ELISA assays were perfor-
med. Results equal or above 1.1 OD/cutoff were considered positive, below 1.1
as negative. Statistical analysis with R included a one-sided power analysis to
specifically estimate required sampling for birth cohort-specific seropreva-

lence evaluation (1604 and 2087 samples respectively). Chi-square analysis or
Fisher’s Exact Test was performed in SPSS 25 to compare sex, region and birth
cohort proportions.

Results
Overall HEV IgG seroprevalences were 4.7% (76/1604, CI 3.2-6.3) and 5.8%
(121/2087, CI 4.5-7.1) in 2006 and 2014 (p=0.161), respectively. In the single
centre analysis, HEV IgG seroprevalences were 8.6% (43/499, CI 6.2-11.1) and
17.1% (120/701, CI 14.3-19.9) in 2016 and 2018 (p < 0.001), respectively. No sig-
nificant differences between sexes were found for any of the years (p=0.603,
p=0.942, p=0.944, p=0.657). Significant regional differences were found in
2014 (p=0.021) with a significant rise between 2006 and 2014 in two provin-
ces: Hainaut (1.5% to 6.0%, p=0.043) and Namur (3.7% to 14.7%, p=0.037).
We found no significant birth cohort-specific differences between 2006 and
2014, but in the single centre analysis we found a significant increase between
2016 and 2018 in the two oldest birth cohorts (born 1942-1947: 11.9% to 27.3%,
p=0.028 and born 1948-1953: 15.4% to 41%, p=0.002).

Conclusion
Compared to reported seroprevalences in surrounding countries, initial ana-
lysis of Belgian nationwide HEV IgG seroprevalence shows stable and rather
low rates. No birth cohort-specific increase in seroprevalence is seen between
2006 and 2014. A regional increase of seroprevalence was however found in
two south-western provinces. In addition, single centre analysis between 2016 and 2018 suggests a recent rising seroprevalence, especially in older birth cohorts.

**Figure:** HEV IgG birth cohort-specific seroprevalence in Belgium, between 2006 and 2014 and 2016 and 2018.
INCIDENCE-BASED DISEASE BURDEN OF FOOD- AND WATERBORNE INFECTIOUS AGENTS IN BELGIUM, 2013-2017

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Background
The total incidence of symptomatic infections among the general population and the related disease burden per pathogen are essential indicators that may guide public health policies. These indicators are not directly evident from health-care-based surveillance networks, because of under-ascertainment (symptomatic infections without pathogen-specific diagnosis) and underreporting (diagnoses not reported to the surveillance network). We aim to estimate the number of symptomatic cases among the general population and related burden of disease for eleven food- and waterborne pathogens in Belgium in 2013-2017.

Materials/methods
In order to translate cases captured by surveillance into total numbers of symptomatic infections among the general population, multiplication factors were estimated. These included underreporting of positive diagnoses, test sensitivity, proportion of samples analyzed, proportion of samples prescribed and submitted and finally proportion of medical care seeking. We used surveillance data, reimbursement data, clinical hospital data, literature and expert opinions. The burden of disease was quantified as ‘Disability Adjusted Life Years’ (DALYs), expressing the number of healthy life years lost due to morbidity and mortality associated with infection. Disease models were used to describe the various health states (morbidity and outcomes) and adapted from ECDC (BCoDE), WHO (FERG) or custom designed. Monte Carlo simulations were used to account for uncertainty around incidence and DALY estimates. Source attribution proportions were used from WHO expert elicitations.

Results
The average annual multiplication factors to translate surveillance-based numbers into total incidence ranged from 1.1 (Clostridium botulinum) to 171 (Cryptosporidium). Incidence estimates were highest for norovirus (21,384 symptomatic cases per 100,000 population). The average DALY per case was highest for C. botulinum (370 DALYs per 100 cases). The annual disease burden was highest for Campylobacter (5,148 DALYs annually). The annual disease burden attributable to foodborne transmission was highest for Campylobacter and Salmonella (3,905 and 2,018 DALYs annually).

Conclusion
Quantification of the disease burden related to food- and waterborne diseases indicated a considerable burden. Preventive measures aiming to increase food safety may focus on Campylobacter and Salmonella because of the highest associated food-attributable burden.
WEATHER DYNAMICS EXPLAIN PART OF THE INCREASE IN REPORTED DOMESTIC LEGIONELLOSIS CASES, BELGIUM 2010-2017

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Background
In many European countries, the number of reported domestic legionellosis cases is increasing. This might be linked to easier access to diagnostic methods and an increased awareness among physicians. There is increasing evidence that the number of legionellosis cases is associated with meteorological factors. In Belgium, the number of reported domestic legionellosis cases increased from 131 in 2010 towards 200 in 2017, with a peak of 217 cases in 2016. Over these 8 years, both the daily maximal temperature and the relative humidity showed an increasing trend. We aim to identify an association between the number of reported domestic legionellosis cases in Belgium and the selected meteorological variables.

Materials/methods
The case-based information concerning the reported legionellosis cases was obtained from a combination of three surveillance systems: the laboratory sentinel surveillance network, the national reference center and the mandatory notifications. As a date variable, date of onset was used. Meteorological data was obtained from the weather institute (KMI) in Uccle, Brussels. Daily data was available for the daily average relative humidity (RH) and for the daily maximum temperature (Tmax). The meteorological variables were averaged per week. Time series analyses were performed using Poisson regression, adjusted for annual seasonality. RH and Tmax were included in the model with multiple time lags and interaction terms were included.

Results
There was an increasing trend over time, with 0.0013 legionellosis cases per week during 2010-2017, representing an increase of 14 cases per year. When adjusting for the meteorological components RH and Tmax, we still identify an significant increase over time, with 0.0011 cases per week during 2010-2017, representing an increase of 12 cases per year. We can thus conclude that the overall observed increase over time in the number of reported legionellosis cases is partly explained by the dynamics of these two meteorological variables over time.

Conclusion
We show an association between selected meteorological conditions (RH and Tmax) and the occurrence of domestic cases of legionellosis. The overall increase of reported legionellosis cases is partly explained by an increase in RH and Tmax. Overall, these associations may be useful in predicting periods of high risk and targeting public health interventions.
EVIDENCE-BASED PRIORITISATION OF INFECTIOUS DISEASES FOR PUBLIC HEALTH AND SURVEILLANCE IN BELGIUM

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Background
Many infectious agents present public health threats in a continuously changing context, and therefore many public health programs struggle with priority setting and are in need of evidence-based guidance. The objective of this study was to prioritize multiple pathogens, according to their relative importance for public health and surveillance in Belgium during 2010-2016.

Materials/methods
The multi-criteria decision analysis (MCDA) approach is based on a balanced set of (semi-)quantitative criteria, that together quantify the relative impact of each pathogen. 101 pathogens and a set of 18 criteria were selected within a working group. Subsequently, the individual criteria were weighted according to their relative contribution to the overall risk, by a panel of experts (n=80) in an online survey. In a second online survey, 37 experts scored each pathogen against the set of criteria, guided by help data. The weighted sum of the average values per pathogen and per criteria composed the total score per pathogen.

Results
Among the five main criteria groups, the highest weights were assigned to ‘impact on the patient’, followed by ‘impact on public health’ and ‘incidence and trend’ was ranked thirdly, although differences in perception were identified between clinicians, microbiologists and public health workers. The pathogens with the highest importance for public health and surveillance were identified as *Bordetella pertussis*, HIV, HCV and HBV. The pathogens with the highest score for probability of increased risk in the next 10 years were: *Klebsiella* (invasive), *Enterococci* (invasive), *Pseudomonas* (including MDR) and *Neisseria gonorrhoea*. Among the pathogens for which improvement of the surveillance system was thought desirable, were: congenital CMV, congenital infections in general, avian influenza, *Pseudomonas*, MDR *N. gonorrhoea*, enterovirus, MRSA, dengue and tuberculosis.

Conclusion
This study identifies a ranking of pathogens based on context specific (semi) quantitative variables and expert perspectives. This approach is a valuable tool that guides priority setting in surveillance and public health, identifies possible gaps in current programs and reveals context specific directions for improved preparedness.
CLONAL SPREAD OF VANCOMYCIN RESISTANT E. FAECIUM ST80, ST117 AND ST203 ACROSS BELGIAN HOSPITALS

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Background
Since 2014, the Belgian National Reference Centre (NRC) receives an increasing number of vancomycin resistant E. faecium (VREfm) strains from infected or colonized patients. Many hospitals reported on outbreaks with VREfm (n=4 in 2012 to ≥20 in 2015-2017). Our aim was to study the molecular epidemiology of VREfm across Belgian hospitals based on whole genome sequencing (WGS).

Materials/methods
VREfm were submitted on a voluntary basis to the NRC. Species identification was confirmed by MALDItof (Bruker). Antibiotic susceptibility was determined according to CLSI before and to EUCAST since 2013. WGS was performed on a selection of 338 VREfm using Nextera XT (2 x250bp), MiSeq (Illumina Inc.), and followed by comparative genome analysis using Mauve and CLC Genomics Workbench v9.5.3 (clcbio, Denmark). Core genome phylogenetic analysis was performed using ParSNP followed by gene by gene analysis using Chewbbacca to identify the core genome MLST allelic differences.

Results
The majority of the sequenced strains belong to ST80 vanA (n= 80, 12 hospitals), ST117 vanA (n= 48, 17 hospitals), ST117 vanB (n= 78, 27 hospitals), and ST203 vanA (n= 45, 10 hospitals). Of these, 121 were isolated from an infection, 130 were screening isolates. WGS analysis revealed a polyclonal structure of VREfm outbreaks: 4 major ST80 clusters with several subclusters and 6 ST117 vanA clusters, were identified. A VREfm ST117 vanB cluster was found in 6 hospitals within the same region; a second cluster was detected in 6 hospitals in Brussels Capital region. Concentrating on ST80 vanA, clonal spread was found in 3 hospitals within the same area (27 genetically closely related isolates, separated by 29 core genome SNPs). The first strains were isolated in H2, followed by H1. H5 submitted 6 VREfm; 2/6 ST80 vanA. The postal code of 1 of them fell within the area of H1 and H2 and within the clade (Fig).

Conclusion
Based on the core genome data VREfm ST80, ST117 and ST203 can be divided into distinct clusters. Clonal spread of VREfm ST80 vanA and ST117 vanB within several hospitals is shown. Typing of VREfm is critical to prevent further spread of dominant clones.
Tree Belgian ST80 vanA positive E. faecium strains (WGS-based)
In Western Europe, the incidence of both respiratory and cutaneous diphtheria, caused by toxin-producing *Corynebacterium diphtheriae*, *C. ulcerans* or *C. pseudotuberculosis*, has been low over the past few decades thanks to the use of an effective vaccine and a high level of vaccination coverage. However, the disease still has not been eradicated and continues to occur in all of Europe.

In order to prevent sequelae or a fatal outcome, diphtheria antitoxin (DAT) should be administered to suspected diphtheria patients as soon as possible upon onset of symptoms, but economic factors and issues concerning regulations have led to poor availability of DAT in many countries. The European Centre for Disease Prevention and Control (ECDC) and World Health Organization (WHO) have called for EU-wide solutions to this DAT-shortage. Since 2017, DAT for clinical use has been available again in Belgium.

The Belgian National Reference Centre for toxigenic corynebacteria confirms the identification of potentially toxigenic corynebacteria and determines their toxigenicity. It collects and preserves all strains and performs further typing, including multi-locus sequence typing (MLST) and susceptibility testing for penicillin, erythromycin, clindamycin and rifampicin. Additional relevant data, such as patient vaccination status, travel and animal contacts, are collected on all confirmed diphtheria cases for surveillance purposes.

During the twenty-year period from 1990 to 2009, Belgium remained diphtheria-free. Since 2010 however, sporadic cases of infection by toxigenic corynebacteria have started to reoccur, along with an increase in incidence of non-toxigenic *Corynebacterium* infection. During the 2010–2017 period, fourteen diphtheria cases have occurred in human patients, both cutaneous and respiratory and caused by toxigenic *C. ulcerans* as well as *C. diphtheriae*. This includes one fatal case of respiratory diphtheria in an unvaccinated three-year-old child. A fifteenth toxigenic *C. ulcerans* isolate was found in a healthy carrier.
Our results show a potential re-emergence of diphtheria in Belgium, seemingly following a Europe-wide trend. A rise in C. ulcerans cases in particular has been noted in many European countries. At present, it is difficult to state an exact cause of this comeback. Possible causes could be waning immunity, decreased vaccination in certain populations, or import from other countries, as well as improved diagnosis due to better diagnostic techniques and heightened surveillance.

These results highlight the importance of continued and heightened efforts towards diphtheria surveillance and vaccination, as well as the necessity of quick access to a ready-to-use DAT stockpile.
Background & objective

The last report on pertussis seroprevalence in Belgium was based on samples collected during 2012. In the context of the EUPert-LabNet_2017 seroprevalence surveillance study, residual sera were collected in Belgium during the second half of 2017. In this study, the incidence of pertussis infections in the older age groups will be explored across Europe. In Belgium, sera were collected by six centres, equally distributed between Flanders, Wallonia and the Brussels Capital Region. A total of 750 samples (125/centre) were collected from subjects in the 40-49 years age group and 750 samples (125/centre) from subjects in the 50-59 years age group. Anti-PT IgG levels were measured using a fluorescent bead-based multiplex immunoassay and analysed using predefined cut-off levels. Overall, anti-PT IgG levels >50 IU/ml (representing pertussis infections in the last two years) and >100 IU/ml (representing recent pertussis infections) were found in 168 (11.4%) and 63 (4.3%) sera, respectively.

Care must be taken with the interpretation since anti-PT IgG induced after booster vaccination (e.g. in the context of the cocoon immunisation strategy) cannot be excluded. No difference between age groups was observed. For both cut-off levels, a predominance of anti-PT IgG antibodies was observed in Flanders as compared to Wallonia and the Brussels Capital region. These results highlight the presence of a *Bordetella pertussis* reservoir in the middle-aged Belgian population.
EPIDEMIOLOGY AND PHYLOTYPE DYNAMICS OF HEPATITIS E VIRAL DISEASE IN BELGIUM, 2010-2017

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Background
Reported cases of autochthonous hepatitis E viral disease have increased in the last decade in many European countries and concern mainly genotype 3 (HEV-3). Little is known about the distribution of hepatitis E viral subtypes among identified cases and the distribution of cases among age groups and regions in Belgium. We aim to identify epidemiological trends of hepatitis E cases since 2010 and to describe phylotype dynamics.

Materials and method
Molecular and epidemiological data was collected by the National Reference Centre (NRC). Suspected patients were patients for whom clinicians requested either hepatitis E serology and/or polymerase chain reaction (PCR) analyses. Confirmed cases were IgM and/or PCR positive individuals.

Results
Median age of hepatitis E cases was >50 years. The overall confirmation ratio (confirmed cases/suspected patients) dropped from 8.1% (2010) to 3.5% (2014) and increased to 4.6% (2015) and 4.4% (2017). Successful genotyping was performed on 223/263 PCR-positive samples (85%). Among those, 92% were of genotype HEV-3. Confirmed HEV-3 cases increased from 0.21 (2010) to 0.98 per 100,000 inhabitants (2017), whereas the reported number of cases per population was higher in the South of Belgium, with higher numbers of confirmed HEV-3 cases per population in Wallonia (1.22) and Brussels (1.17) compared to Flanders (0.81 per 100,000 inhabitants, 2017). Overall most common subtypes among the HEV-3 strains were 3f, 3c and 3e. Subtype 3c cases increased significantly from one (2010) to 36 cases (2017), whereas other HEV-3 subtypes remained stable or showed milder increase (3f).
Conclusion
The continuous increase in the number of hepatitis E confirmed cases between 2010-2017 indicates probably a rising awareness among physicians in Belgium. The stable to slightly increasing laboratory confirmation ratio between 2014 and 2017 indicates that infection pressure most likely did not drop between 2014 and 2017 in Belgium. Hepatitis E viral phylotypes identified from patients were similar to and interspaced with those identified from Belgian swines, suggesting transmission from pigs to humans. Studies to detect hepatitis E virus in food products will contribute to shape dietary recommendations for high risk groups (e.g. immunocompromised) and identify possible interventions in food production processes.
ACKNOWLEDGEMENTS

Our acknowledgements go to the speakers for their interesting presentations, and to the chairpersons for leading the discussions.

The members of the Scientific Committee of the seminar have, once again, selected a varied and attractive programme. Their input and suggestions have been very much appreciated.

We are also very grateful to the sentinel laboratories and the NRCs, as well as all other Sciensano partners, for their daily work in contributing to public health.

We thank our colleagues from the service Epidemiology of infectious diseases and the Finance and Communication departments for their support. Our special thanks go to Nathalie Verhocht and Ledia Jani for their enthusiasm and the efficient administrative assistance.

This seminar was financially supported by contributions of the private sector, of which several companies have been sponsoring us for many years.