Rapid communication
Outbreak of diarrhoeal shellfish poisoning associated with consumption of mussels, United Kingdom, May to June 2019
Nick Young, Charlotte Robin, Rachel Kwiatkowska, Charles Beck, Dominic Mellon, Penelope Edwards, Jonathan Turner, Paul Nicholls, Gavin Fearby, Debbie Lewis, Douglas Hallett, Tracy Bishop, Tracey Smith, Russell Hyndford, Lewis Coates and Andrew Turner

Surveillance
Epidemiology of Clostridioides difficile infections, France, 2010 to 2017
Mélanie Colomb-Cotnat, Laetitia Assouvie, Julien Durand, Côme Daniau, Lucie Leon, Sylvie Maugat, Sophan Soing-Altrach, Cécile Gateau, Jeanne Couturier, Isabelle Arnaud, Pascal Astagneau, Anne Berger-Carbonne and Frédéric Barbut

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Veronique Dermauw, Steven Van Den Broucke, Lieselotte Van Bockstal, Leon Luyten, Kim Luyckx, Emmanuel Bottieau and Pierre Dorny
We report on six cases of diarrhetic shellfish poisoning following consumption of mussels harvested in the United Kingdom. *Dinophysis* spp. in the water column was found to have increased rapidly at the production site resulting in high levels of okadaic acid-group lipophilic toxins in the flesh of consumed mussels. Clinicians and public health professionals should remain aware of algal-derived toxins being a potential cause of illness following seafood consumption.

Epidemiological investigation and findings

**Case finding**

An alert was sent to all health protection teams across England on day 2 asking about any reported cases of gastrointestinal illness following consumption of mussels. Local authorities in areas of product distribution were informed of the identified risk by email. Persons reporting illness who were identified by local authorities as having consumed the affected mussels were asked by PHE to complete a bespoke questionnaire on exposure and clinical data.

**Case definitions**

A probable case of DSP was defined as an individual with diarrhoea, three or more loose stools in 24 h, or vomiting or abdominal cramps or nausea, with date of onset from 7 days before to 1 day after notification of the outbreak, and time of onset 30 min to 24 h following consumption of mussels harvested from the affected site. Confirmed cases were as probable, but with an absence of pathogens in a stool sample that would otherwise explain illness.

**Results**

Thirteen individuals reported to have been unwell after consumption of mussels were contacted. Completed questionnaires were received from seven individuals, of which three were confirmed, and three probable cases. The cases ate at four separate venues. One
respondent did not meet the case definition as symptom onset was more than 24 h following consumption.

The epidemic curve for the outbreak is shown in Figure. The mean age of cases was 59 years (range: 37–76 years); three were male and three were female. All cases reported eating steamed mussels. Five cases ate mussels as a main course and one as a starter. Reported portion sizes ranged from 11 to 50 mussels.

The symptoms reported by cases are summarised in Table 1.

The mean incubation period was 11.3 h (range: 8–17 h) and the median duration of symptoms was 2.5 days. One case sought medical advice. There were no hospitalisations.

Faecal samples were tested from the three confirmed cases and the respondent not meeting the case definition. Samples were collected at a median of 3 days (range: 1–9 days) after resolution of symptoms. All four stool samples were microscopy and culture negative for Salmonella spp., Shigella spp., Escherichia coli O157, Campylobacter spp., Vibrio spp., Bacillus cereus and Clostridium perfringens. Anaerobic cultures were negative. Two stool samples yielded scanty growth of Staphylococcus aureus that was not considered commensurate with a root cause of food poisoning. Ova, cysts and parasites were not seen on concentration. Enzyme immunoassay was negative for Cryptosporidium and Giardia. Viral PCR testing was negative for rotavirus, sapovirus, astrovirus, adenovirus and norovirus.

Environmental investigation and findings
The mussels were produced in an offshore marine area. A routine shellfish monitoring programme is in place throughout England and Wales, including at the affected site. As a part of this programme, the water column is sampled every 2 weeks from April to September and cell counts of potentially harmful algal species are measured. Shellfish flesh samples are also tested for the presence of selected European Union (EU)-regulated biotoxins every 4 weeks during April to September each year unless phytoplankton counts and/or shellfish toxins are quantified above specified warning limits that require further precautions, including re-testing and closure.

Lipophilic toxin determination, including that for OA-group toxins, is routinely carried out using the method specified in in the EU-Harmonised Standard Operating Procedure for determination of lipophilic marine biotoxins in molluscs by LC-MS/MS [1]. Additional flesh and water samples were taken in advance of the planned sampling date following a report to the local authority from a local fisherman of a red-coloured algal bloom six miles offshore from the production site.

The local authority determined the source of the mussels by questioning venues linked to reports of illness.
Subsequently, the shellfish producer provided the outbreak control team with a complete list of all businesses who had received the affected mussels. Mussels from the site were harvested daily from 9 to 5 days before notification of the outbreak for commercial sale. The mussels were not tested by the producer for the presence of toxins. A large volume of mussels was distributed to seafood wholesalers, restaurants and pubs, and subject to the recall notice distributed by the producer 5 days before reports of illness to PHE. A limited number of businesses not linked to any known cases, including wholesalers, retailers, restaurants and pubs, responded to the recall stating they had sold some of the affected produce. No produce was found to still be in circulation at the time of the outbreak response.

Water column and shellfish flesh sampling results are summarised in Table 2. Measured densities of *Dinophysis* spp. in the water column increased rapidly from being undetectable 16 days before outbreak notification to 1,600 cells per litre 7 days before, coinciding with the time of harvesting of the affected batch and exceeding the England, Wales and Northern Ireland Food Standards Agency trigger level of 100 cells per litre. The level of total OA-group lipophilic toxins in mussel flesh was 338 µg OA equivalents (eq) per kg, following application of measurement uncertainty, 7 days before outbreak notification. This exceeded the maximum permitted limit (MPL) of 160 µg OA eq per kg defined by European Commission (EC) regulation 853/2004 [2]. Toxin concentrations quantified showed that an average of 94% of the OA-group toxins present in the mussels consisted of OA itself, with the remainder being dinophysistoxin 2 (DTX2).

Water column sampling 7 days before outbreak notification did not detect other harmful algal species apart from *Pseudo-nitzschia* spp., the causative diatom for domoic acid responsible for amnesic shellfish poisoning, at 1,320 cells per litre. This is below the trigger level of 150,000 cells per litre for this species.

Routine shellfish sampling at the same site during the same time period did not detect paralytic shellfish poisoning toxins. Trace levels of yessotoxins were detected, but along with traces of azaspiracids, they were well below regulatory levels. Amnesic shellfish poisoning toxins were below the limit of quantitation (LOQ).

### Control measures

In response to the elevated toxin levels quantified and reported 5 days before outbreak notification, the shellfish bed was immediately closed for harvesting as per standard practice in England. The Food Standards Agency urgently contacted local authorities of places where the affected product had been distributed to ensure that wholesalers and venues had acted upon the recall. Venues were asked whether any product had been frozen, for example in the form of stock, as this would not deactivate the toxin, but there was no evidence this had been done.

### Discussion

We report on six cases of DSP associated with consumption of mussels harvested in the South West of England. Without an available validated test for relevant toxins in human samples, the diagnosis was made based on characteristic clinical symptoms, including diarrhoea, abdominal pain, nausea and fever/chills, elevated levels of OA-group toxins in the flesh of mussels from the same batch as those consumed, the absence of faecal pathogens in stool of cases and epidemiological evidence of exposure to the produce.

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### Table 1

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhoea</td>
<td>6</td>
</tr>
<tr>
<td>Nausea</td>
<td>6</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>6</td>
</tr>
<tr>
<td>Chills</td>
<td>4</td>
</tr>
<tr>
<td>Fever</td>
<td>1</td>
</tr>
<tr>
<td>Vomiting</td>
<td>2</td>
</tr>
<tr>
<td>Other*</td>
<td>2</td>
</tr>
</tbody>
</table>

* Other includes lethargy, dizziness/fainting, heart palpitations and memory loss.

### Table 2

<table>
<thead>
<tr>
<th>Date of collection</th>
<th>Total OA-group toxicity in mussel flesh (µg OA eq/kg)</th>
<th>Dinophyceae cell counts (cells/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day −55</td>
<td>ND</td>
<td>40</td>
</tr>
<tr>
<td>Day −34</td>
<td>34</td>
<td>ND</td>
</tr>
<tr>
<td>Day −16</td>
<td>Not sampled</td>
<td>ND</td>
</tr>
<tr>
<td>Day −7</td>
<td>338</td>
<td>1,600</td>
</tr>
<tr>
<td>Day −1</td>
<td>499</td>
<td>200</td>
</tr>
<tr>
<td>Day 6</td>
<td>270</td>
<td>Not sampled</td>
</tr>
<tr>
<td>Day 12</td>
<td>121</td>
<td>Not sampled</td>
</tr>
<tr>
<td>Day 19</td>
<td>106</td>
<td>ND</td>
</tr>
</tbody>
</table>

Eq: equivalents; OA: okadaic acid; ND: not detected.

* In relation to day 0, when Public Health England South West was notified of three diners who were unwell following consumption of mussels in a restaurant.
DSP occurs following consumption of seafood containing high levels of the heat-stable OA-group toxins produced by dinoflagellates including Dinophysis spp., and is characterised by a rapid-onset of self-limiting gastrointestinal illness [3,4]. Recognised outbreaks of DSP are rare. Seventy cases were identified in 2013 following consumption of mussels harvested around the Shetland Islands [5] and 49 cases were identified in 1998 following consumption of UK-harvested mussels in London [6]. Outbreaks have been recorded in recent years in China, the United States, France and Canada [4,7-9].

The lowest-observed-adverse-effect level of OA is 45 to 50 µg OA eq per person [4,10]. In our study, an average main course portion of mussels (500 g in shell) would provide 41 µg OA eq., using a flesh weight yield of 24% [11]. This level of exposure is consistent with DSP as the cause of illness considering variability in portion sizes, flesh yield, body weight and toxin levels at the production site. Individual mussel sizes served were unavailable but would likely vary. Therefore, overall estimated portion weight was used to calculate the exposure dose. A limitation is that body weight (bw) was not recorded for cases and because of this, OA eq per kg bw could not be calculated.

A shellfish biotoxin programme monitoring the occurrence of harmful algal blooms and toxins in classified shellfish production areas in the UK, alongside food business operator checks, remains a robust system to protect population health. Nonetheless, a rapid increase in concentrations of Dinophysis spp. cells within the waters of the production site may have contributed to the outbreak, in tandem with shellfish harvesting occurring before official control results were reported and site closure. Whyte et al. (2014) demonstrated that a similar rapid increase in Dinophysis levels, resulting from a change in prevailing wind direction, occurred in the 2013 Shetland Islands origin outbreak [5]. Transdisciplinary research is required to predict future risk and inform monitoring, particularly given likely changes in the distribution of potentially-toxic species particularly if temperature of ocean water increases [12]. Our investigation suggested that affected produce may have been sold by restaurants and pubs with no known linked cases. Given that DSP is a self-limiting illness that may be under-reported by cases and has low awareness among clinicians, the actual number of persons affected in this outbreak is likely to be higher [13].

This outbreak highlights that clinicians and public health professionals should be aware of algal-derived toxins as a potential cause of illness following seafood consumption, and that the need for effective end-product testing of shellfish to ensure food safety remains.

Acknowledgments

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References


Conflict of interest

None declared.

Authors’ contributions

NY, CR, RK, CB, DM, PE, PN, GF, DL, DH, TB, TS, RH, LC, AT were members of the multi-agency team that that responded to initial reports of illness and subsequently managed the outbreak. All contributed to the collection of information required for this manuscript.

JT advised on, oversaw and reported on human microbiological testing.

AT and LC advised on, conducted and reported on algal and shellfish flesh testing.

NY, CR, RK, CB, DM, PE, JT, PN, GF, DL, DH, TB, TS, RH, LC, AT were involved in preparing the manuscript and gave final approval for the draft.

CR, RK, CB led on the epidemiological investigation.

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Epidemiology of *Clostridioides difficile* infections, France, 2010 to 2017

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**Background:** *Clostridioides difficile* is a leading cause of healthcare-associated diarrhoea in middle and high-income countries. Up to 2018, there has been no systematic, annual surveillance for *C. difficile* infections (CDI) in France. **Aims:** To provide an updated overview of the epidemiology of CDI in France between 2010 and 2017 based on five different data sources. **Methods:** This is a descriptive study of retrospective surveillance and alerts data. Incidence of CDI cases was estimated through the CDI incidence survey (2016) and data from the French National Uniform Hospital Discharge Database (PMSI; 2010–16). Testing frequency for CDI was estimated through the CDI incidence survey and point prevalence studies on healthcare-associated infections (HAI; 2012 and 2017). The national early warning response system for HAI (HAI-EWRS, 2012–17) and National Reference Laboratory data (2012–17) were used to follow the number of severe CDI cases and/or outbreaks. **Results:** In 2016, CDI incidence in acute care was 3.6 cases per 10,000 patient days (PD). There was a statistically significant increase in CDI incidence between 2010 and 2016 (+14% annually) and testing frequency was 47.4 per 10,000 PD. The number of CDI HAI-EWRS notifications decreased between 2015 and 2017 with only a few large outbreaks reported. **Conclusion:** The CDI incidence estimate increased from 2010, but remained below the European average of 7 per 10,000 PD in 2014; there were fewer severe cases or clusters reported in France. The consistency between PMSI and laboratory-based estimated CDI incidence could allow for more routine monitoring of CDI incidence.

**Introduction**

*Clostridium difficile*, officially renamed *Clostridioides difficile* in 2016, is responsible for 15–25% of antibiotic-associated diarrhoea cases [1,2] and is considered the leading cause of healthcare-associated diarrhoea in developed countries. *C. difficile* infections (CDI) can be severe (toxic megacolon, septic shock) and represent one of the most expensive nosocomial infections [1-5]. In more than 95% of CDI cases, the patient receives antibiotics just before the onset of diarrhoea [2].

The epidemiology of CDI has changed over the past 20 years with the emergence of a hypervirulent clone NAP1/027/BI implicated in large outbreaks of severe CDI worldwide with high mortality rates [6-11] in the beginning of the 2000s. Between 2006 and 2007, this clone was responsible for outbreaks of severe CDI in the north of France before spreading gradually throughout the territory [9]; these outbreaks were controlled by the end of 2007. In 2009, following the epidemic period, a national prospective, multicentric survey to assess CDI incidence and to characterise CDI strains was launched in France (ICD-Raisin study). It showed an incidence of 2.28 CDI per 10,000 patient days (PD) in acute healthcare facilities (HCF) and 1.15 per 10,000 PD in long-term facilities [12]. The European, multicentric, prospective, point prevalence study (PPS) of CDI in hospitalised patients with diarrhoea (EUCLID) study, conducted in 2014 [13], found an incidence of 3.9 per 10,000 PD in 2011-12 in France and an estimated average incidence of 7 per 10,000 PD (range 0.7–28.7) in Europe.

Up to 2018, there has been no systematic, annual surveillance for CDI in France. Since 2007, routine surveillance of CDI is based on two data sources: (i) notifications by HCF through the national healthcare-associated infections early warning and response system (HAI-EWRS) [14], which are only mandatory for severe CDI presentations and/or outbreaks. Since 2012, the HAI-EWRS has been accessible via an online application (e-SIN), (ii) microbiological data from the national reference laboratory for *C. difficile* (NRL, Paris). NRL data do not reflect the overall epidemiology of *C. difficile* in France, however, as the strains usually

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come from severe cases or outbreaks and sending of the strains to NRL is not mandatory.

To complement and update available data on CDI in France, a laboratory-based CDI incidence survey was conducted in 2016 through the pre-existing national multidrug-resistant bacteria surveillance programme (French acronym BMR-Raisin) [15]. The survey was offered as an optional module for laboratories within acute HCF to complete. Two national PPS of HAI and antimicrobial use were also conducted in 2012 and 2017, which included data on CDI prevalence and the number of samples tested for *C. difficile*. In addition, information on inpatient hospital stays with CDI was collected from the French national hospital stays database (French acronym PMSI), for the 2010–16 period. The aim of this study is to provide an updated overview of the epidemiology of CDI in France based on these five different data sources: (i) the 2016 CDI incidence survey, (ii) the 2012 and 2017 PPS, (iii) PMSI data, 2010–16, (iv) HAI-EWRS notifications, 2012–17 and, (v) NRL data, 2012–17. These five data sources have never been compared to each other, and only data from the PPS have been published elsewhere.

The aim of this study is to provide an updated overview of the epidemiology of CDI in France based on these five different data sources: (i) the 2016 CDI incidence survey, (ii) the 2012 and 2017 PPS, (iii) PMSI data, 2010–16, (iv) HAI-EWRS notifications, 2012–17 and, (v) NRL data, 2012–17. These five data sources have never been compared to each other, and only data from the PPS have been published elsewhere.

Of the five data sources, two have the purpose of alert: (i) notification to HAI-EWRS allows for a rapid real-time alert, communication between local team and regional or national support to help implementation of control measures, (ii) microbiological data from the NRL are used to follow the epidemic clone 027 and the potential emergence of more epidemic-prone or more virulent clones.

The purpose of the other three data sources are surveillance of CDI: (i) the CDI incidence survey, launched in 2016, was useful in providing a point estimation of the CDI incidence in acute HCF, (ii) PPSs are done every 5 years in France and provide a point estimation on CDI prevalence and testing frequency in a representative sample of HCF in France, (iii) PMSI data were analysed for the first time at national level to estimate CDI incidence in acute HCF.

### Methods

#### The 2016 *Clostridioides difficile* infections incidence survey

**Source of information**

Between April and June 2016, all laboratories within acute HCF in France were asked to complete an optional questionnaire including questions on: (i) algorithms used for CDI diagnosis, (ii) number of stool specimens tested for *C. difficile*, (iii) number of stool specimens that tested positive for CDI (there can be several for the same patient), and (iv) number of CDI cases and number of hospital-acquired CDI cases (HA CDI cases) for acute care wards within acute HCF. The questionnaire was in accordance with the European Centre for Disease Prevention and Control’s (ECDC) technical document for minimal *C. difficile* surveillance [16]. Participation was voluntary.

A CDI case was defined as per European Society of Clinical Microbiology and Infectious Diseases (ESCMID) recommendations [9] i.e. diarrhoeal stools or toxic megacolon and a positive laboratory assay for *C. difficile* TcdA and/or TcdB in stools or a toxin-producing *C. difficile* organism detected in stools via culture or PCR. A HA CDI case was defined as a positive sample at least 48 hours following admission, with no manifest CDI infection in the 6 months before admission. Patients hospitalised for less than 24 hours and dialysis patients were excluded. The reference algorithms for the detection of a CDI were those recommended by the ESCMID [17].

**Analysis**

The testing frequency for CDI was estimated using the ratio between the number of stools tested for *C. difficile* to the number of PD in acute HCF over the study period (data source were the annual administrative

### Table 1

<table>
<thead>
<tr>
<th>Hospital type</th>
<th>Number of participating HCF</th>
<th>Testing frequency per 10,000 PD</th>
<th>Stools that tested positive for CDI per 10,000 PD</th>
<th>CDI cases per 10,000 PD</th>
<th>HA CDI cases per 10,000 PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tertiary</td>
<td>12</td>
<td>52.8</td>
<td>6.1</td>
<td>4.7</td>
<td>2.7</td>
</tr>
<tr>
<td>Secondary</td>
<td>100</td>
<td>51.8</td>
<td>4.6</td>
<td>3.6</td>
<td>2.0</td>
</tr>
<tr>
<td>Primary</td>
<td>82</td>
<td>36.0</td>
<td>3.8</td>
<td>2.6</td>
<td>1.2</td>
</tr>
<tr>
<td>Specialised*</td>
<td>9</td>
<td>78.7</td>
<td>6.4</td>
<td>6.4</td>
<td>3.7</td>
</tr>
<tr>
<td>Total</td>
<td>203</td>
<td>47.4</td>
<td>4.7</td>
<td>3.6</td>
<td>1.9</td>
</tr>
</tbody>
</table>

CDI: *Clostridioides difficile* infection; HA: hospital acquired; HCF: healthcare facilities; PD: patient days.

* Including oncology centres only.
survey of HCF statistics for 2016 [18]). The incidence of stools that tested positive for CDI and the incidence of HA CDI cases per 10,000 PD was estimated using the ratio between the number of stools that tested positive for CDI or HA CDI to the number of PD in acute HCF over the study period. We also calculated the proportion of HCF performing CDI diagnosis using one of the reference algorithms.

The 2012 and 2017 point prevalence surveys

Source of information
In the 2012 and 2017 PPS, data on all HAI (including CDI), treatments and risk factors were collected for all patients present on the hospital ward on the day of the survey. In 2012, the PPS was sent to all HCF in France and the participation rate was 75% (1,938/2,594). In 2017, the PPS was conducted on a representative sample of 449 HCF (stratified by region and hospital type), with 403 (91%) participating. The methodology of these PPS has been described elsewhere [19]. A patient infected with *C. difficile* was defined as a compatible clinical presentation (diarrhoeal stools or megacolon) with detection of toxins A and B in the stools or pseudomembranous colitis diagnosed after coloscopy or compatible histology at endoscopy or autopsy.

Analysis
The prevalence of patients infected with *C. difficile* (in 2012 and 2017) and the testing frequency (i.e. the number of stools samples tested for *C. difficile* in acute HCF per 10,000 PD, variable retrospectively collected in 2017 only, based on 2016 data) were analysed. As the 2017 PPS was only conducted on a representative sample of HCF, 95% confidence intervals (CI) were calculated for the prevalence of patients with CDI. The prevalence of patients with CDI in 2012 and 2017 was compared using multilevel models (patient, HCF and region) with a Poisson regression (adjusting for age, sex, McCabe score, immunosuppression, urinary catheter, central venous catheter, peripheral venous catheter, respiratory assistance and hospital ward). Analyses were performed with Stata version 14.1 (StataCorp, College Station, Texas (TX), United States (US)).

The French national uniform hospital discharge database data, 2010–2016

Source of information
PMSI is a standardised national database describing all inpatient hospital stays [20] and is used for the production of standardised healthcare billing information and medical information concerning patients (comorbidities, age and sex). Pathologies are coded by principal diagnosis and optional associated diagnoses, by a clinician using the French version of the international classification of diseases 10th Revision (ICD-10) [21]. The ICD-10 code A04.7 ‘*C. difficile* enterocolitis’ must be used for every patient with a CDI, left to the appreciation of the physician that coded the stays.

### Table 2

Mean rates of *Clostridioides difficile* testing frequency and prevalence of patients diagnosed with *C. difficile* by hospital type, France, 2012 and 2017 PPS

<table>
<thead>
<tr>
<th>Hospital type</th>
<th>Testing frequency per 10,000 PD</th>
<th>Prevalence of patients diagnosed with CDI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PPS 2017</td>
<td>PPS 2012</td>
</tr>
<tr>
<td></td>
<td>Rate</td>
<td>95% CI</td>
</tr>
<tr>
<td>Tertiary</td>
<td>68.11</td>
<td>41.77–94.45</td>
</tr>
<tr>
<td>Secondary</td>
<td>39.82</td>
<td>37.03–42.61</td>
</tr>
<tr>
<td>Primary</td>
<td>17.78</td>
<td>15.24–20.32</td>
</tr>
<tr>
<td>Specialised 2a</td>
<td>58.74</td>
<td>NA</td>
</tr>
<tr>
<td>Subtotal acute HCF</td>
<td>23.48</td>
<td>20.73–26.23</td>
</tr>
<tr>
<td>Specialised 1c</td>
<td>3.79</td>
<td>2.8–4.78</td>
</tr>
<tr>
<td>Total</td>
<td>15.86</td>
<td>4.13–17.59</td>
</tr>
</tbody>
</table>

CDI: Clostridioides difficile infection; CI: confidence interval; HCF: healthcare facilities; NA: not available; PD: patient days; PPS: point prevalence survey.

a Including oncology centres only.
b When there is only one primary sampling unit within a stratum, there is insufficient information to compute an estimate of that stratum’s variance.
c Including psychiatric care, rehabilitation centres and long-term facilities.
Procedures are coded using a standardised classification (Classification Commune des Actes Médicaux [22]). Patients can have more than one stay in the same year.

Analysis
The PMSI was analysed for the period 1 January 2010–31 December 2016. Stays with an ICD-10 code A04.7 for principal or associated diagnosis were extracted from the PMSI database for acute wards in acute HCF and stratified by type of facility, type of stay, age, sex and region. The variable ‘type of discharge’ was used to identify in-hospital deaths (attributability was unknown). Stays, where a colectomy was performed, were identified with the following CCAM codes: HHFA002, HHFA004, HHFA005, HHFA006, HHFA008, HHFA009, HHFA010, HHFA014, HHFA017, HHFA018, HHFA021, HHFA022, HHFA023, HHFA024, HHFA026, HHFA028, HHFA029, HHFA030 or HHFA031. To calculate incidences, the ratio between the number of cases and stays to the number of PD (source: SAE 2016) were calculated. Regional data were estimated using the variable ‘region of hospitalisation’. Incidences of stays with CDI between 2010 and 2016 were compared using Poisson regression with robust variance, adjusted for geographical region. Analyses were performed with Stata version 12 (StataCorp, College Station, TX, US).

Healthcare-associated infections early warning and response system notifications, 2012–2017

Source of information
The e-SIN database contains all healthcare-associated infections (HAI) notifications that have been reported since January 2012. Notifications report data on HCF (name and location) and data on date of detection of the first case, the number of cases and death, ward of hospitalisation, the microorganism responsible for infection and any control measures. A comment box for any additional information was available.

Analysis
HAI-EWRS notifications received between 1 January 2012–31 December 2017 were extracted from the e-SIN database. CDI notifications were identified based on the ‘microorganism’ item. The variables ‘region’, ‘number of cases’, ‘number of deaths’, ‘infectious site’ and ‘ward’ were extracted directly from the notifications. Ribotype of the strains was extracted from comments when necessary. An outbreak was defined as a notification reporting more than one case. Proportions of CDI notifications among HAI notifications between 2012 and 2017 were compared using a variance-weighted least-squares regression. Analyses were performed with Microsoft Excel 2013.

CDI: Clostridioides difficile infection; PD: patient days; PMSI: The French national uniform hospital discharge database.
National reference laboratory for *Clostridioides difficile* data, 2012–2017

Source of information
The NRL receives strains of *C. difficile* from voluntary French laboratories for characterisation. Most of these strains are linked to cases reported in the HAI-EWRS (clusters or severe forms of CDI). These strains are characterised by multiplex PCR which detects the main virulence factors (tcdA and tcdB genes encoding toxins A and B, respectively and cdtA and cdtB genes encoding the binary toxin) and by capillary gel-based electrophoresis PCR ribotyping, as described elsewhere [23]. The strains’ susceptibility patterns to antibiotics (metronidazole, vancomycin, erythromycin, tetracycline and moxifloxacin) are determined by the disk diffusion method. Antimicrobial susceptibility testing was performed according to the 2013 French CA-SFM (Comité de l’antibiogramme de la Société Française de Microbiologie) guidelines. Stain dilution (108 CFU/ml) is inoculated on Brucella Agar (Becton Dickinson) supplemented with vitamin K1 (1mg/ml) (Emprove, Merck), hemin (5 mg/L) (Applichem) and defibrinated horse blood (5%). Plates are incubated 48h at 35–37°C, and diameters are interpreted according to the criteria for anaerobic bacteria given by the CA-SFM. *C. difficile* ATCC 700057 is used as quality control. The method was the same throughout the 5 years.

Analysis
Number and characteristic of strains analysed by the NRL between 2012 and 2017 were described.

Ethical statement
Anonymous surveillance data were collected from patient charts only for the public interest mission of the French public health agency or its partners, in accordance with the French data protection authority. Analyses were only conducted on aggregated data and not on an individual level.

Results
The 2016 *Clostridioides difficile* infections incidence survey
In 2016, of more than 2,000 acute HCF in France, 203 participated in the CDI incidence survey, corresponding to 10% (3,056,445/30,854,819) of total PD for the same year. All hospital types (tertiary, secondary and primary) and all 17 regions in France was represented. The testing frequency for CDI was 47.4 per 10,000 PD, while the incidence of stools that tested positive for CDI was 4.7 per 10,000 PD (positivity rate of 10%). Diagnostic testing was performed using ESCMID-recommended algorithms in 65% of HCF. The incidence of CDI cases per 10,000 PD was 3.6, while the incidence of HA CDI cases was 1.9 (Table 1).

The 2012 and 2017 point prevalence surveys
In 2012, of 300,330 patients included in the study, 337 had CDI (prevalence = 0.11%). In 2017, of 80,988 patients included in the study, 83 had CDI (prevalence: 0.11%; 95% CI: 0.08–0.14). After adjusting for the indicators of severity, the prevalence of patients

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**Figure 2**
Regional incidence of CDI stays, France, PMSI data 2010 and 2016

CDI: Clostridioides difficile infection; PMSI: The French national uniform hospital discharge database.

Source: Geofila région 2016, Santé Publique France.
diagnosed with CDI remained stable between 2012 and 2017 (p > 0.05).

In 2017, the mean rate of C. difficile testing frequency was 15.9 per 10,000 PD. Large differences were observed across the HCF categories (Table 2), with tertiary care hospitals having the highest rates. Most patients (54/83; 65%) were hospitalised in secondary and tertiary hospitals (Table 2).

The French national uniform hospital discharge database data, 2010–2016
Between 2010 and 2016, 86,953 patients were hospitalised in acute HCF in France with a coded diagnosis of CDI, corresponding to 105,717 stays. A steady increase was observed from 2010 to 2015, followed by a slight decrease in 2016. The estimated incidence of stays with a CDI diagnosis significantly increased from 1.5 per 10,000 PD in 2010 to 3.4 per 10,000 PD in 2016 (+14% per year [95% CI: 13–16], Figure 1). This increase was observed in all French regions (Figure 2).

Of 86,953 patients hospitalised with CDI in acute HCF in France, 71,301 (82%) had only one stay, 11,304 (13%) had two and 4,348 (5%) had more than two. Patients aged 80 years or older accounted for 31,717 (36%) of patients, while those aged 14 years or less accounted for only 1,897 (2%). The sex-ratio was balanced in all age groups, except in patients aged over 80 years, where the proportion of women was higher (20,910/31,717; 66% women).

CDI was coded as the principal diagnosis in 39% (40,803/105,717) of stays. Of 64,914 stays where CDI was recorded as an associated diagnosis, the most frequent principal diagnoses were palliative care (2,281; 3.5%). In total, 3,746 different principal diagnoses were listed.

The proportion of stays where death occurred was 12% (12,614/105,717) and colectomies were performed in 1% of stays (1,250/105,717). These proportions calculated for each year are stable during the study period. These proportions were also stable when the analysis was restricted to stays where C. difficile infection was coded as the principal diagnosis: 2,571/40,803 (6%) patients died and 151/40,803 (0.4%) colectomies were performed.

Healthcare-associated infections early warning and response system notifications, 2012–2017
A total of 557 notifications with C. difficile were received between 2012 and 2017, involving 1,305 patients and including 159 deaths (attributable or not). A decrease in the number of notifications with C. difficile was observed from 2015 onwards (Figure 3). The trend in the number of cases over time is more variable. Proportions of CDI notifications among all HAI notifications decreased by 1% annually 2012–17 (p < 10−5, variance-weighted least-squares regression): CDI notifications account for 5% (80/1,551) of HAI notifications in 2012 and 2% (57/2,890) in 2017.

Of 557 notifications of CDI, 166 (30%) occurred in rehabilitation/long-term care units and 56 (10%) in geriatric units; a presumed or confirmed 027 strain of C. difficile was reported in 161 (29%) of the notifications.

Among all CDI notifications, 245/557 (44%) reported at least two cases. This proportion varied between 36% and 60% depending on the year. The median number of cases per episode was three (range: 2–21). A few outbreaks involving more than 10 cases were reported: two in 2012, one in 2013, one in 2014, three in 2015 and three in 2017. No large outbreaks were reported in 2016.

National reference laboratory for Clostridioides difficile data, 2012–2017
Between 2012 and 2017, the frequency of PCR ribotype 027 and PCR ribotype 078/126 significantly decreased from 21.7% to 9.56% (p < 0.0001) and 12.9% to 7.49% (p = 0.02), respectively (Table 3).

In 2017, of 387 C. difficile toxigenic strains, 25 (6.4%) were the ‘historical’ PCR-ribotype 027, susceptible to moxifloxacin and 99 (25.7%) produced binary toxin. All strains were susceptible to metronidazole and vancomycin. The other PCR ribotypes remained relatively stable, except PCR ribotype 002 (3.8% vs 8.01%) and PCR ribotype 106 (1% vs 4.65%) which slowly emerged from 2012 to 2017.
Discussion
This study has provided an updated overview of the epidemiological data available on CDI in acute HCF in France between 2010 and 2016, combining for the first time five different data sources. The CDI incidence survey, launched in 2016, was repeated in 2017 and 2018 but is time-consuming for laboratories and regional centres. The results of the 2017 and 2018 survey are not currently available (as at July 2019) and it will not be repeated after 2018. However, the CDI incidence in acute HCF in 2016 estimated using PMSI data were consistent with the incidence estimated from the CDI incidence survey conducted in the same year. A pilot study conducted in 2010 by the NRL [24] compared the sensitivity and specificity of a surveillance programme using PMSI data with laboratory-based surveillance data. It found that the PMSI data underestimated the incidence of CDI, compared with the laboratory results from the NRL (sensitivity 35.6%). This suggests the PMSI coding has improved since 2010 and that PMSI data in 2016 could closely represent the true incidence of CDI cases – opening up the possibility for a national surveillance system utilising routine monitoring of the incidence of stays with CDI (PMSI data), complemented with microbiological-based surveillance carried out by the NRL. The ICD-10 code A04.7 is specific for CDI, making easy the use of PMSI for surveillance purpose. In addition, HAI-EWRS is still needed to help monitoring alerts and repeated PPS will provide a point estimation on CDI prevalence and testing frequency in a representative sample of HCF in France.

The 2016 CDI incidence survey estimated the CDI incidence in acute HCF as 3.6 cases per 10,000 PD, which is an increase from the 2.3 cases per 10,000 PD estimated in 2009 by the ICD-Raisin study. This increase may be due to greater awareness of C. difficile infection among clinicians and to the development of more sensitive diagnostic tests for C. difficile such as PCR. In France, only diarrhoeic stool samples and patients aged 3 years and older are recommended to be tested for C. difficile. Test-of-cure testing after a treatment for CDI and routine screening for colonisation on non-diarrheic stools are not recommended. Only a minority of laboratories in France systematically test for C. difficile in all diarrhoeic stool samples, the majority only do it when requested by a physician. Therefore, the testing frequency depends on a physician’s awareness for C. difficile. CDI surveillance in England indicates that more than 75% of cases occur in patients aged 64 years and older [25]. Therefore, as the population is getting older and at a greater risk of CDI, an increase in incidence of CDI is possible [26].

Despite the increase of CDI in France (2010–16), the incidence of CDI in 2016 remains below the European

<table>
<thead>
<tr>
<th>Table 3: Number of Clostridioides difficile strains analysed by the NRL, by ribotype, France, 2012–2017</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR-ribotype</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>n %</td>
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<tr>
<td>----------------</td>
</tr>
<tr>
<td>27</td>
</tr>
<tr>
<td>014/020/077</td>
</tr>
<tr>
<td>078/126</td>
</tr>
<tr>
<td>002</td>
</tr>
<tr>
<td>001</td>
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<td>005</td>
</tr>
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<td>15</td>
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<tr>
<td>17</td>
</tr>
<tr>
<td>106</td>
</tr>
<tr>
<td>53</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

NRL: national reference laboratory.
average in 2014 (7/10,000 PD) [13], which could be explained by differences in CDI testing policies. The CDI incidence survey has estimated this *C. difficile* testing frequency at 47.4 stools tested per 10,000 PD in acute HCF in 2016, with differences regarding hospital type, while it was estimated at 62–69 stools tested per 10,000 PD on average in Europe [13]. This suggests that the proportion of stools tested for *C. difficile* is lower in France than in other European countries. The testing frequency estimated from PPS data were slightly different (23.48 stools tested/10,000 PD) and may be due to differences in participating acute HCF. In addition, data for the PPS also include long-term wards within acute HCF (lower testing frequency) whereas the CDI-incidence survey excluded data from long-term wards. Further, participation in the CDI-incidence survey was voluntary so participating HCF may have been more aware of CDI as a result of outbreaks; this potential self-selection bias could explain the higher testing frequency, compared to the testing frequency estimated by the 2017 PPS, done on a representative sample of HCF.

In parallel, the number of CDI HAI-EWRS notifications reached a peak in 2013-14 (118 notifications/year) before decreasing from 2015 onwards. There was also a decrease in the number of 027 strains identified at the NRL. The proportion of HAI-EWRS notifications involving 027 strains varied widely from year to year, but this proportion also took into account possible 027 strains not confirmed by the NRL. In addition, some laboratories use the GeneXpert method, which does not have high specificity for the 027 strain leading to an overestimation. NRL microbiology data show the emergence of PCR ribotype 002 and PCR ribotype 106 from 2012 to 2017. PCR ribotype 002 has been shown endemic in some nursing homes in Hong-Kong [27] and PCR ribotype 106 has been responsible for outbreaks in vascular surgery [28].

The low number of CDI outbreak notifications in France is less worrying than in other countries such as Germany, where the epidemic clone accounts for 40% of *C. difficile* strains [29]. The prevention and control of CDI are based primarily on appropriate microbiological testing practices, antibiotics stewardship policy and prevention of cross-transmission by implementing contact precaution [30,31].

According to the PMSI data, the proportion of severe forms (i.e. leading to death or colectomy) remained stable over time, but we do not know whether death is attributable to CDI. Two studies have already shown excess mortality related to CDI compared with age-matched and comorbidity-matched patients who did not have CDI [4,32].

The majority of CDI cases reported in our survey are HA, which is not surprising as the survey targeted only hospitalised patients and *C. difficile* is one of the most common HA pathogen [33]. For example, the 2016 European epidemiological report of CDI [34] found that HA CDI made up 74.6% of cases. The increase observed in CDI-related hospital stays differed between regions in France. Regional incidence disparities should be studied in the future and maybe partly explained by disparities in healthcare organisation, especially in overseas regions (HCF activities, specialities etc.). Incidence of CDI-related hospital stays differed with the age of the patient. It would be interesting to estimate incidence in different age groups, and that will be the subject of further work.

A limitation of our study is the difference between CDI definitions between the data sources: (i) in the CDI incidence survey, definition is based on clinical elements and microbiological confirmation as recommended per the ESCMID, (ii) in the PSSs, definition is based on clinical or histological elements +/− detection of toxins in the stools, (iii) in the PMSI and HAI-EWRS, CDI case definition is decided by the physician coding the stays or making the notification, but should normally match the ESCMID definition as recommended in France.

**Conclusion**

This study which combines five different data sources on CDI epidemiology in France for the first time shows that despite an increase of CDI incidence between 2010 and 2016, the incidence of CDI cases in France in 2016 remains below the European average. There is a low number of CDI outbreak notifications and there is a decrease in the number of 027 strains analysed by the NRL. Surveillance and alert for CDI remains however essential and thanks to this study, we have opened up the possibility for a national surveillance system utilising routine monitoring of the incidence of stays with CDI (PMSI data), complemented with microbiological-based surveillance carried out by the NRL. In addition, further studies will be needed to estimate incidence in different age groups and to explore the difference in regional incidences.

**Acknowledgments**

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**Conflict of interest**

None declared.

**Authors’ contributions**

Mélanie Colomb-Cotinat conceived and designed the study, contributed to the interpretation of the results and wrote the manuscript; Laetitia Assouvie conducted the PMSI and...
References


3. Czepiel J, Dróżdż M, Pituch H, Kuijper EJ, Perucki W, Sophan Soing-Altrach was involved in the HAI-EWRS data analysis and contributed to wrote the manuscript; Isabelle Arnaud and Pascal Astagneau provided results; Julien Durand provided PSMI data and was involved in their analysis; Frédéric Barbut, Cécile Gateau and Jeanne Couturier provided and analysed LNR data, contributed to their interpretation; Frédéric Barbut, Cécile Gateau and Jeanne Couturier provided and analysed PPS data and contributed to the interpretation of the results; Lucie Leon and Sylvie Maugat were involved in the PMSI data analysis; Sophie Soing-Altrach was involved in the HAI-EWRS data analysis.


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35. Czepiel J, Dróżdż M, Pituch H, Kuijper EJ, Perucki W, Sophan Soing-Altrach was involved in the HAI-EWRS data analysis and contributed to wrote the manuscript; Isabelle Arnaud and Pascal Astagneau provided results; Julien Durand provided PSMI data and was involved in their analysis; Frédéric Barbut, Cécile Gateau and Jeanne Couturier provided and analysed LNR data, contributed to their interpretation; Frédéric Barbut, Cécile Gateau and Jeanne Couturier provided and analysed PPS data and contributed to the interpretation of the results; Lucie Leon and Sylvie Maugat were involved in the PMSI data analysis; Sophie Soing-Altrach was involved in the HAI-EWRS data analysis.

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Enterovirus D68 serosurvey: evidence for endemic circulation in the Netherlands, 2006 to 2016

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Background: Enterovirus D68 (EV-D68) has caused major outbreaks of severe respiratory illness worldwide since 2010. Aim: Our aim was to evaluate EV-D68 circulation in the Netherlands by conducting a serosurvey of EV-D68 neutralising antibodies (nAb) among the Dutch general population. Methods: We screened 280 sera from children and adults in the Netherlands and used two independent sets of samples collected in the years 2006 and 2007 and in the years 2015 and 2016, time points before and after the first EV-D68 upsurge in 2010. Neutralisation capacity of the sera was tested against the prototype Fermon EV-D68 strain isolated in 1962 and against a recent EV-D68 strain (genotype B3) isolated in France in 2016. Results: Regardless of the time of serum collection, we found remarkably high overall seropositivity (94.3–98.3%) for nAb against both EV-D68 strains. Geometric mean titres increased in an age-dependent manner. Conclusions: Our data suggest that EV-D68 has been circulating in the Netherlands for decades and that the enterovirus surveillance does not accurately capture the prevalence of this clinically relevant pathogen.

Introduction
Enterovirus D68 (EV-D68), belonging to the Enterovirus D species within the Picornaviridae family, was first isolated in 1962 but not frequently detected before 2010 when it started causing large outbreaks of severe respiratory illness worldwide [1-5]. Clinical symptoms commonly associated with EV-D68 infection include fever, wheezing, cough and dyspnoea [1]. Young children and individuals with underlying conditions are at high risk of developing severe lower respiratory tract disease requiring admission to an intensive care unit (ICU) and mechanical ventilation [1,2,5]. The characteristics of EV-D68, such as acid lability of the virions, the respiratory transmission route and symptomatology in patients, resemble those described for the related rhinoviruses [6]. However, similar to poliovirus (PV) and enterovirus A71 (EV-A71), EV-D68 has the potential to spread to the central nervous system (CNS) causing neurological complications [2]. Acute flaccid myelitis (AFM) in children has been associated with EV-D68 infection [7-12].

Based on the viral capsid protein VP1 nucleotide sequence, EV-D68 isolates are classified into three clades A to C, all of which co-circulate globally [3]. In the Netherlands, EV-D68 has been detected sporadically since 1996 and the first upsurge of EV-D68 cases was reported in 2010 [4]. Continuous circulation has been observed from 2011 to 2016, with severe outbreaks in 2014 and 2016 [13-15]. Surveillance of enteroviruses (EV) occurs via the national public health networks in the context of the World Health Organization (WHO) polio surveillance, by detection of viruses from patients [16]. However, as most EV infections are asymptomatic or cause mild disease and since EV diagnostic testing is performed primarily on stool samples, detection rates are likely to account for only a minority of the true EV-D68 incidence [17].

Presence of neutralising antibodies (nAb) in serum is a widely accepted correlate of immunity and protection against severe disease associated with EV infection [18]. Thus, age-stratified serosurveys of nAb are a valuable method of understanding the prevalence of EV-D68 and evaluating the risk of an outbreak among the general population. As a part of the European Non-Polio Enterovirus Network (ENPEN) [19], we aimed to characterise the seroprevalence of nAb against EV-D68 among children and adults in the Netherlands.

Methods
We screened sera collected from the population in the Netherlands before and after the 2010 EV-D68 upsurge against two strains of EV-D68: the prototype Fermon strain so that data would be comparable to previous
Enterovirus D68 viruses and cell lines

The EV-D68 Fermon prototype strain (isolated in 1962) was obtained from the National Institute for Public Health and the Environment (RIVM, Bilthoven, the Netherlands). The EV-D68 genotype B3 clinical strain was isolated from a patient in 2016 in France and was a kind gift from Dr Bailly (Université Clermont Auvergne, Clermont-Ferrand, France). Both virus strains were cultured at 37°C, 5% CO₂ in rhabdomyosarcoma cell line (RD99; American Type Culture Collection, Manassas, United States (US)). Cells were maintained in Eagle’s minimum essential medium (EMEM; Lonza, Basel, Switzerland) supplemented with 8% heat-inactivated fetal bovine serum (FBS; Sigma-Aldrich, St. Louis, US), streptomycin (100 µg/mL; Lonza Bio Whittaker), penicillin (100 U/mL; Lonza Bio Whittaker), non-essential amino acids (NEAA; ScienCell Research Laboratories, Carlsbad, US) and L-glutamine (200 mM; Lonza, Basel, Switzerland). Chloroform treatment of the virus stocks was performed as described in the WHO Polio Manual [21]. Briefly, 10% (v/v) chloroform (Sigma-Aldrich, St. Louis, US) was added to each virus culture and vortexed vigorously for 5 min. Chloroform was removed by centrifugation for 10 min at 3000 rpm. The 50% tissue culture infective dose (TCID₅₀) of virus stocks was determined by means of end-point dilution using the Reed and Muench method [22].

Serum samples

We screened 280 anonymised serum samples from Dutch individuals aged 0–79 years. We used two independent sets of samples collected at time points before and after the 2010 EV-D68 upsurge in the Netherlands. Sera from 2006 and 2007 were obtained from the RIVM as part of the PIENTER2 study (Dutch acronym for the survey on the immunisation effect in the Netherlands for evaluation of the national immunisation programme: Pelling Immunisatie Effect Nederland Ter Evaluatie van het Rijksvacincatieprogramma [23]).

Neutralisation assay

The sera were tested using a previously described neutralisation assay [24]. Heat-inactivated sera were serially diluted in 96-well microtitre plates in a volume of 50 μL per well and incubated with 100 TCID₅₀ per 50 μL per well of EV-D68. Subsequently, 100 μL of RD99 cells were added and incubated for 7 days. Neutralising titres were calculated based on cytopathogenic effect using the Reed and Muench method and reported as the reciprocal titres of serum dilutions exhibiting 50% neutralisation [22]. An nAb titre of ≥1:8 was considered positive. In agreement with previous publications [20], we defined titres 8–64 as ‘low’, 64–128 as moderate, 128–512 as ‘high’ and >512 as ‘very high’.

Statistical analysis

Data were grouped in categories based on the following: the EV-D68 virus strain used in the assay (prototype Fermon or genotype B3 clinical isolate), serum collection time point (2006–07 or 2015–16), serum donor sex (male or female) and serum donor age. The overall EV-D68 nAb seroprevalences between different groups were compared using chi-squared tests. Kruskal–Wallis test with Dunn’s post hoc analysis was used to compare the overall and the age-stratified geometric mean titres between the prototype Fermon strain and the genotype B3 clinical isolate. One-way ANOVA with Tukey’s multiple comparisons test was used to compare the geometric mean titres between the age groups. Children younger than 1 year were excluded from the overall seroprevalence and overall geometric mean titre analyses because of the potential presence of maternal antibodies against EV-D68. Data were analysed using IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp., Armonk, US) and GraphPad Prism 7 (GraphPad Software Inc., La Jolla, US) with a significance level of p<0.05.

Ethical statement

The sera had been collected by population-based sampling approved by the Medical Ethics Testing Committee of the Foundation of Therapeutic Evaluation of Medicines (ISRCTN 20164309) [25]. Sera collected between 2015 and 2016 were residual samples from hospitalised patients and staff at the University Medical Centers (Amsterdam, the Netherlands). No
Results

High overall seropositivity for enterovirus D68 neutralising antibodies

As depicted in Figure 1, the overall EV-D68 nAb seroprevalence rates and geometric mean titres (GMT) were high, with no statistically significant differences between time points before and after the 2010 EV-D68 upsurge in the Netherlands or between the virus strains. The overall nAb seroprevalence in the 2006 and 2007 sera against the prototype Fermon strain was 94.3% (95% confidence interval (CI): 88.0–97.7) with a GMT of 123.7 (standard deviation (SD): 5.4) and in the 2015 and 2016 sera, it was 98.3% (95% CI: 94.0–99.8) with a GMT of 193.5 (SD: 3.9). No differences in the nAb seropositivity rates were found between female and male cases (data not shown).

Age-associated increase in enterovirus D68 neutralising antibody titres

Age-stratified analysis showed that in children below the age of 1 year the EV-D68 nAb seroprevalence was 95.1% (95% CI: 89.0–98.2) with a GMT of 199.7 (SD: 4.6) and in the 2015 and 2016 sera, it was 98.3% (95% CI: 94.0–99.8) with a GMT of 193.5 (SD: 3.9). No differences in the nAb seropositivity rates were found between female and male cases (data not shown).

ethical approval is required for anonymous use of residual serum in the Netherlands.
and adults (age groups 11–20, 21–30, 31–40, 41–50 and above 50 years) were 85–100% positive for EV-D68 nAb (Figure 2). Children and young adults in age groups 1–10 and 11–20 years had significantly lower GMT of nAb against the Fermon strain than against the genotype B3 clinical EV-D68 isolate (Table). Most adult age groups had high GMTs against both virus strains with no statistically significant differences between the virus strains (Table). Statistical pairwise GMT comparisons between age groups indicated that children younger than 1 year and children between 1 and 10 years of age had significantly lower GMT of nAb against both the Fermon and the genotype B3 clinical isolate when compared with the GMTs in the adult age groups (adjusted p values < 0.0001; Supplementary Tables S1 and S2).

**Enterovirus D68 clinical surveillance in the Netherlands, 1996–2017**

We extracted the EV-D68 case numbers reported during 1996 to 2017 in the Netherlands from the national Clinical Enterovirus Surveillance (CEVS) database (Figure 3) [26]. From 1996 to 2010, enterovirus testing was performed primarily on stool samples and few cases were observed. Because the 2010 EV-D68 outbreak was discovered via primary care surveillance done by Nivel, the Dutch Institute for Health Care Research, in respiratory samples that were not included in the CEVS, this outbreak is not visible in Figure 3 [4]. EV-D68 testing in respiratory samples has been gradually implemented following the 2010 outbreak. After 2010, 146 cases have been confirmed, most of them during an outbreak in 2016 [13].

**Table**

Geometric mean titres of neutralising antibodies against enterovirus D68, serosurvey, the Netherlands, 2006–07 and 2015–16 (n = 280)

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Number</th>
<th>M/F</th>
<th>Mean age in years (SD)</th>
<th>GMT (SD) EV-D68 Fermon</th>
<th>GMT (SD) EV-D68 genotype B3</th>
<th>Adjusted p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2006 and 2007 sera</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1</td>
<td>18</td>
<td>9/9</td>
<td>0.5 (0.3)</td>
<td>18.2 (2.6)</td>
<td>10.5 (3.2)</td>
<td>&gt;0.9999</td>
</tr>
<tr>
<td>1–10</td>
<td>22</td>
<td>11/11</td>
<td>5.2 (3.1)</td>
<td>19.6 (3.1)</td>
<td>55.6 (7.4)</td>
<td>0.0241</td>
</tr>
<tr>
<td>11–20</td>
<td>20</td>
<td>10/10</td>
<td>15.6 (3.0)</td>
<td>32.0 (3.8)</td>
<td>130.3 (3.7)</td>
<td>0.0013</td>
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<td>21–30</td>
<td>20</td>
<td>10/10</td>
<td>25.7 (3.0)</td>
<td>66.3 (1.9)</td>
<td>160.3 (3.6)</td>
<td>0.1263</td>
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<td>31–40</td>
<td>20</td>
<td>10/10</td>
<td>35.7 (3.0)</td>
<td>369.6 (2.5)</td>
<td>453.8 (2.7)</td>
<td>&gt;0.9999</td>
</tr>
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<td>41–50</td>
<td>20</td>
<td>10/10</td>
<td>45.8 (3.0)</td>
<td>530.1 (2.4)</td>
<td>304.4 (2.4)</td>
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<td>&gt;50</td>
<td>20</td>
<td>10/10</td>
<td>65.8 (8.9)</td>
<td>530.1 (2.7)</td>
<td>449.4 (3.2)</td>
<td>&gt;0.9999</td>
</tr>
<tr>
<td><strong>2015 and 2016 sera</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1</td>
<td>20</td>
<td>10/10</td>
<td>0.5 (0.3)</td>
<td>24.7 (2.0)</td>
<td>13.6 (3.1)</td>
<td>0.6094</td>
</tr>
<tr>
<td>1–10</td>
<td>20</td>
<td>10/10</td>
<td>5.5 (3.1)</td>
<td>23.8 (2.2)</td>
<td>49.3 (4.7)</td>
<td>0.2558</td>
</tr>
<tr>
<td>11–20</td>
<td>20</td>
<td>10/10</td>
<td>16.1 (2.9)</td>
<td>43.8 (2.8)</td>
<td>219.2 (3.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>21–30</td>
<td>20</td>
<td>10/10</td>
<td>26.1 (3.0)</td>
<td>66.3 (1.7)</td>
<td>339.4 (2.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>31–40</td>
<td>20</td>
<td>10/10</td>
<td>35.9 (3.0)</td>
<td>121.5 (2.8)</td>
<td>265.2 (3.3)</td>
<td>0.1742</td>
</tr>
<tr>
<td>41–50</td>
<td>20</td>
<td>10/10</td>
<td>46.0 (3.0)</td>
<td>230.7 (3.8)</td>
<td>301.7 (3.2)</td>
<td>&gt;0.9999</td>
</tr>
<tr>
<td>&gt;50</td>
<td>20</td>
<td>10/10</td>
<td>64.2 (8.2)</td>
<td>260.7 (3.2)</td>
<td>178.3 (3.6)</td>
<td>&gt;0.9999</td>
</tr>
</tbody>
</table>

EV: enterovirus; GMT: geometric mean titre; M/F: male/female; SD: standard deviation.

*Population-based sampling, National Institute for Public Health and the Environment (RIVM), Bilthoven.

*Residual sera from hospitalised patients and staff, Academic Medical Center, Amsterdam.

Numbers in bold indicate statistical significance.
From 1996 to 2010, enterovirus testing was performed primarily on stool samples, showing low numbers of cases in 2010, the first outbreak year in the Netherlands (highlighted in red). Since 2010, also respiratory samples have been increasingly tested for EV-D68.

lower respiratory infections and polio-like illness worldwide, but particularly in North America [1,4,5,9]. Concerns were raised that EV-D68 was developing from an infrequent cause of mild disease to a major human pathogen with neurovirulent properties [1]. This study is the first serological investigation into the prevalence of EV-D68 among the Dutch population.

In line with previous sero-epidemiological studies from Finland and China, with seroprevalence rates from 90 to 100% [20,27], the overall nAb prevalence was remarkably high in sera collected both before and after the first reported EV-D68 upsurge in the Netherlands in 2010. The nAb were specific to both the prototype Fermon EV-D68 strain and a recent genotype B3 clinical isolate from France. Age-stratified analyses indicated that the overall EV-D68 nAb seroprevalence was approaching 90% or more already in 1–10 year-old children. The higher GMT in the older age groups is most likely explained by frequent boosting. Our data suggest that EV-D68 circulation has been endemic in the Netherlands for decades.

Antigenic drift has been proposed as a mechanism to explain the sudden EV-D68 emergence [4,28,29]. We found that Dutch children and young adults had higher nAb titres against the recent genotype B3 clinical isolate EV-D68 than against the prototype strain. However, overall the sera from all time points and age groups could efficiently neutralise both EV-D68 strains with minimal differences between GMT. As we used anonymous serum collections, we were unable to relate the exposure histories of EV-D68 sample donors to our seroprevalence data. This is a limitation of our study. Cross-neutralisation by nAb elicited against other prevalent enteroviruses may be a confounding factor in our study. However, evidence of cross-neutralisation among different enterovirus serotypes is scarce [18,30]. Previously it was reported that EV-D68 could not be neutralised with the reference EV-D70 antiserum [29]. In the same report, it was suggested that a small antigenic variation between the 2014 outbreak viruses and the Fermon strain could explain differences in neutralisation titres.

We hypothesise that EV-D68 incidence in the Netherlands is underestimated based on the following: (i) in general, the majority of enterovirus infections are not reported as most infections are subclinical or cause only mild illness in healthy individuals [18]; (ii) standard molecular diagnostics cannot distinguish between rhinovirus and EV infection, and EV type-specific testing is predominantly based on stool sampling since EV are not perceived as relevant respiratory pathogens [17]; (iii) as reported previously in other countries [20,27], we observed a nearly universal prevalence of EV-D68 neutralising antibodies among the Dutch general population.

Conclusion
We report a high level of population immunity against EV-D68 and conclude that EV-D68 has been endemic and circulating in the Netherlands for decades. Our results suggest that the current EV surveillance does not accurately capture the EV-D68 prevalence in the Netherlands. In order to fully understand the EV-D68 disease burden, we propose monitoring and routine EV-D68 testing of nasopharyngeal aspirate or throat swab specimens for patients with acute respiratory presentations. Further research on antigenic variation and pathogenicity of the emerging EV-D68 variants is necessary to elucidate the factors underlying disease severity and outbreak dynamics.

Acknowledgments
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Conflict of interest
None declared.

Authors’ contributions
Set-up of the study: EK, KW, DP, KB; executing the experiments: EK, Gk; samples and database data deliverance: KB, FvdK; writing of the manuscript: EK, KB, DP, KW; supervision of the project: DP, KW.

References


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Any supplementary material referenced in the article can be found in the online version.

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BACKGROUND: Few case reports on human infections with the beef tapeworm *Taenia saginata* and the pork tapeworm, *Taenia solium*, diagnosed in Belgium have been published, yet the grey literature suggests a higher number of cases. **Aim:** To identify and describe cases of taeniasis and cysticercosis diagnosed at two Belgian referral medical institutions from 1990 to 2015. **Methods:** In this observational study we retrospectively gathered data on taeniasis and cysticercosis cases by screening laboratory, medical record databases as well as uniform hospital discharge dataset. **Results:** A total of 221 confirmed taeniasis cases were identified. All cases for whom the causative species could be determined (170/221, 76.9%) were found to be *T. saginata* infections. Of those with available information, 40.0% were asymptomatic (26/65), 15.4% reported diarrhoea (10/65), 9.2% reported anal discomfort (6/65) and 15.7% acquired the infection in Belgium (11/70). Five definitive and six probable cases of neurocysticercosis (NCC), and two cases of non-central nervous system cysticercosis (non-CNS CC) were identified. Common symptoms and signs in five of the definitive and probable NCC cases were epilepsy, headaches and/or other neurological disorders. Travel information was available for 10 of the 13 NCC and non-CNS CC cases; two were Belgians travelling to and eight were immigrants or visitors travelling from endemic areas. **Conclusions:** The current study indicates that a non-negligible number of taeniasis cases visit Belgian medical facilities, and that cysticercosis is occasionally diagnosed in international travellers.

INTRODUCTION

*Taenia saginata* and *Taenia solium* are the two most common species of tapeworms causing infection in humans. Cattle and pigs are intermediate hosts of *T. saginata* and *T. solium*, respectively. They acquire muscular infection, cysticercosis, upon ingestion of *Taenia* spp. eggs shed with the stools of human tapeworm carriers, either through direct contact or indirectly via contaminated water or application of sewage sludge [1]. Taeniasis, an intestinal infection of humans with the adult tapeworm, is acquired by consuming undercooked infected meat and usually causes only mild clinical symptoms [1], with complications rarely occurring, e.g. intestinal obstruction [2]. In the case of *T. solium*, humans can also acquire cysticercosis upon accidental ingestion of eggs. In humans, the larval stage has a marked affinity for the central nervous system (CNS), causing a condition called neurocysticercosis (NCC). Epilepsy/seizures, chronic headaches and focal deficits are among the most common manifestations of NCC [3]. Globally, *T. solium* was ranked the first food-borne parasite of public health concern [4] and the leading cause of deaths from food-borne diseases [5]. In Europe, *T. solium* was ranked as 10th most important food-borne parasite of public health concern, whereas *T. saginata* was ranked as the 13th [6].

*T. saginata* carriers are common worldwide, including in Europe, yet their number is not well estimated, possibly because of the mild symptoms related to infection [7-11]. In Europe, the infection has been detected both in cattle and humans, suggesting ongoing transmission of the parasite [10-12]. Conversely, the presence of *T. solium* is considered to be restricted mainly to areas with poor sanitary conditions, inadequate hygiene, open defecation, the presence of free roaming pigs and poverty [13]. Human cysticercosis cases are found in vast areas of Africa, Asia and Latin America where *T. solium* is endemic [14]. However, cases have
also been reported in non-endemic areas, such as the United States (US), Canada and Europe. Recent reviews describe a total of 275 case reports for western Europe and 58 for eastern Europe for the period 1990 to 2015 [10,11]. Cysticercosis cases diagnosed in these areas often arise from returning travellers and immigrants from endemic areas [15], as well as from untreated *T. solium* tapeworm carriers who would pose a risk to themselves, family members and other contacts in non-endemic areas [16-18]. It is important to obtain accurate epidemiological data on cysticercosis cases in humans and pigs, as well on taeniasis cases caused by *T. solium* in humans. Currently, neither taeniasis nor human cysticercosis are notifiable diseases in the European Union (EU), which limits assessment of the epidemiology of *T. saginata* and *T. solium* in this area [10,11,19].

In Belgium, few reports are available on human *Taenia* spp. infections. Only two taeniasis cases [20,21] and two NCC cases, diagnosed in Belgium, have been published and described in scholarly publications [22,23]. When screening grey literature, however, there are indications that an additional number of taeniasis cases have occurred in Belgium [10]. For instance, between 1980 and 1989, the annual sales of niclosamide doses, a drug prescribed for tapeworm infection, i.e. taeniasis, diphyllobothriasis, hymenolepiasis, fluctuated between 35,000 and 60,000 [24]. In 2013, 11,350 niclosamide doses were sold [25]. Moreover, a review of grey literature indicated that hospital databases and national registries in Europe, including countries neighbouring Belgium, harbour information on a large number of cysticercosis cases diagnosed between 1990 and 2015 that have not been described in scholarly publications (4,901 in western Europe and 772 in eastern Europe) [10,11].

Given the lack of information on the occurrence of taeniasis and human cysticercosis in Belgium, the primary objective of this study was to identify and describe cases of taeniasis and cysticercosis diagnosed in two Belgian referral medical institutions from 1990 to 2015. More specifically, we aimed to summarise the number, socio-demographic information, clinical features, diagnostic test results and treatment of taeniasis and cysticercosis cases.

### Methods

#### Study design and setting

This observational study consists of a retrospective analysis of data on suspected and confirmed taeniasis and cysticercosis cases diagnosed between 1990 and 2015 in two Belgian referral medical institutions: the Institute of Tropical Medicine Antwerp (ITMA) and the Antwerp University Hospital (UZA). The ITMA is the national reference centre for tropical medicine and parasitic diseases in Belgium, and runs a large travel clinic. The UZA is a tertiary teaching hospital that hosts the hospitalisation unit of the ITMA. The institutions closely collaborate on the integrated care of patients with tropical diseases. Serology for cysticercosis is performed at the ITMA for patients of both institutions, while stool examination is performed at each institution separately.

#### Study population and data sources

The search strategy differed at the two institutes, but aimed for maximal data capture from available sources (Table 1). At the ITMA, the central laboratory database was searched for patients with serological and/or stool analyses positive for *Taenia* spp.. Following this, the medical files of patients positive for any of the tests

### Table 1

Characterisation of data sources for retrospective data collection of taeniasis and cysticercosis cases diagnosed at two referral medical institutions, Antwerp, Belgium, 1990–2015

<table>
<thead>
<tr>
<th>Data source</th>
<th>Type of data retrieved</th>
<th>Data availability period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central laboratory database</td>
<td>Patients with serology and/or stool examination positive for <em>Taenia</em> spp.</td>
<td>1994–2015</td>
</tr>
<tr>
<td>Antwerp University Hospital (UZA)</td>
<td>Central laboratory database</td>
<td>1990–2015</td>
</tr>
<tr>
<td>Central laboratory database</td>
<td>Patients with submitted samples for <em>Taenia</em> spp.-related serology</td>
<td>1990–2015</td>
</tr>
<tr>
<td>Central medical record database</td>
<td>Patients with stool examination positive for <em>Taenia</em> spp.</td>
<td>1990–2015</td>
</tr>
<tr>
<td>Uniform hospital discharge dataset</td>
<td>Patients with registered ICD-9 code for taeniasis or cysticercosis (1990–2014)</td>
<td>1990–2015</td>
</tr>
<tr>
<td></td>
<td>Patients with registered ICD-10 code for taeniasis or cysticercosis (2015)</td>
<td>1990–2015</td>
</tr>
</tbody>
</table>

ICD: International Classification of Disease.

* Tapeworm OR lintworm OR platworm OR taenias* OR tenias* OR (taenia AND (solium OR saginata)) OR (tenia AND (solium OR saginata)) OR neurocystic* OR cysticerc*.
mentioned were reviewed, additional relevant data were collected, and an ITMA database for suspected taeniasis and cysticercosis cases was created. Only electronic files were searched, which were available from 1994 onwards at ITMA.

At the UZA, the first step was to search the central laboratory database for patients with samples submitted for *Taenia* spp.-related serology and/or stool examination positive for *Taenia* spp. Additionally, the following keyword query was run for the period 2001 to 2015 in the central medical record database: tapeworm OR lintworm OR platworm OR taenias* OR tenias* OR (taenia AND (solium OR saginata)) OR (tenia AND (solium OR saginata)) OR neurocystic* OR cysticerc*. Hospitals in Belgium are also required to register clinical data of non-ambulatory patients in the government-run uniform hospital discharge dataset (UHDDS). The UZA UHDDS was searched for patients with taeniasis or cysticercosis related International Classification of Disease (ICD)-9 (1990–2014) and ICD-10 diagnosis codes (2015) (see Supplementary Table S1 for full list of codes and description). Medical records of all retrieved patients from these three databases at UZA were reviewed, relevant additional data were collected and a specific UZA database for suspected taeniasis and cysticercosis cases was created after excluding duplicates within each database. The databases of ITMA and UZA were then merged and checked for duplicates, which were excluded from further analysis.
**Case definition**

A taeniasis case was considered confirmed when *Taenia* spp. eggs or proglottids were identified upon stool examination, or where an ITMA/UZA physician witnessed an adult *Taenia* spp., e.g. during surgery. As eggs of *Taenia* spp. cannot be distinguished in stool, species identification, i.e. the differentiation of *T. saginata* vs *T. solium*, was based on the number of uterine branches in expelled proglottids when available.

Patients in the cysticercosis database were first listed based on the presence of cysticercosis-related signs or symptoms, being registered with an ICD code for cysticercosis or any mentioning of cysticercosis as part of a differential diagnosis. Subsequently, patients on the list were evaluated by two medical doctors experienced in tropical medicine, and ultimately classified as follows: definitive NCC case, probable NCC case (both based on the revised Del Brutto criteria [26]), definitive non-CNS CC case (based on anatomopathology results), unlikely NCC or non-CNS CC case (i.e. medical files indicate atypical symptoms and imaging results), patient with other definitive diagnosis (i.e. medical file mentions final diagnosis different from NCC or non-CNS CC diagnosis), or patient with insufficient information available to allow full evaluation.

**Diagnostic tools**

At both institutions, stool parasitological analyses are run for *Taenia* spp. detection, i.e. direct microscopic examination and parasitological examination after enrichment.

ITMA conducts the serological analyses for *T. solium* IgG antibody (Ab) test using the commercial Cysticercosis Serum Microwell ELISA kit (DRG International Inc., Springfield, New Jersey, US). The manufacturer reports a sensitivity (Se) of 87% (95%CI: 69.3–96.2%) and a specificity (Sp) of 96% (95%CI: 85.7–99.5%), and mentions the possibility of cross-reactions in case of *Echinococcus* spp. infections. Results are expressed as negative or positive. Furthermore, the ITMA performs a *T. solium* antigen (Ag) test using the commercial Cysticercosis Ag ELISA kit (apDia, Turnhout, Belgium), with a reported overall (both viable and dead cysts) Se of 94.0% (95%CI: 87.4–97.8%). In a panel of Peruvian samples from a non-endemic area, the Sp was 100.0% (95%CI: 83.2–100.0%), whereas in a group of Belgian blood donors, the Sp was 99.3% (97.6–99.9%), according to the manufacturer. Results are expressed as

---

**Figure 2**

Confirmed taeniasis cases by sex diagnosed at two referral medical institutions, Antwerp, Belgium, 1990–2015 (n = 216)

![Figure 2](https://www.eurosurveillance.org/)

*Of 221 confirmed taeniasis cases, there were five missing values.*
negative (optimal density (OD) ≤ 0.8), positive (OD ≥ 1.3) or inconclusive (0.8 < OD < 1.3).

Data retrieval
A total of 683 patients were retrieved from the UZA and ITMA databases (Figure 1). After duplicate removal within each institute, taeniasis and cysticercosis databases were created for each institution, and then merged per condition. The merged ITMA-UZA taeniasis database contained data for 290 patients, whereas the merged ITMA-UZA cysticercosis database contained data for 341 patients. Merged databases then underwent a final check for duplicates and critical review of medical files.

Merged taeniasis database
In the merged ITMA-UZA taeniasis database (290 patients), 69 patients were excluded on the basis of a negative stool test result (n = 20) or there being insufficient data to evaluate the case further (n = 49). The final dataset contained 221 confirmed taeniasis cases. Of the eight patients with a taeniasis-related ICD-9 or ICD-10 code registered, only two cases could be confirmed: one had a positive stool test result while an adult *Taenia* spp. was seen and removed during an unrelated laparoscopic procedure for the other. Both cases were added to the dataset. Of the remaining six, one had a negative stool sample analysed and five lacked information on why they were registered as taeniasis cases.

Merged cysticercosis database
The merged ITMA-UZA cysticercosis database contained 341 potential cysticercosis patients (Figure 1). Medical records of these patients were screened for relevance (i.e. cysticercosis-related symptoms or signs, mentioning of cysticercosis in medical record, or registered ICD code for cysticercosis), with a shortlist of 115 patients resulting. Of these, 102 were excluded after additional detailed review of medical records by two medical doctors with experience in tropical medicine: 28 were categorised as possible NCC cases; 51 as unlikely NCC cases; 19 with different final diagnoses established; and four with insufficient information to render evaluation possible. The final dataset contained five definitive cases of NCC, six probable NCC cases and two definitive cases of non-CNS CC.

Of the 19 patients in the database who had a relevant ICD-9 or ICD-10 code registered, three were definitive NCC cases, three were probable NCC cases, five were categorised as possible NCC cases, two as unlikely NCC cases, two as having different diagnosis and four

<table>
<thead>
<tr>
<th>Type of cysticercosis case</th>
<th>Age category (years)</th>
<th>Geographical area of origin</th>
<th>Travel/immigration</th>
<th>Geographical area of travel/immigration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definitive NCC</td>
<td>0–18</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>19–30</td>
<td>South America</td>
<td>Travel</td>
<td>Western Europe</td>
</tr>
<tr>
<td></td>
<td>19–30</td>
<td>Sub-Saharan Africa</td>
<td>Immigration</td>
<td>Sub-Saharan Africa</td>
</tr>
<tr>
<td></td>
<td>19–30</td>
<td>Western Europe</td>
<td>Travel</td>
<td>South America</td>
</tr>
<tr>
<td></td>
<td>31–49</td>
<td>Western Europe</td>
<td>Travel</td>
<td>Central/Eastern/Southern Asia</td>
</tr>
<tr>
<td>Probable NCC</td>
<td>19–30</td>
<td>Southern Asia</td>
<td>Immigration</td>
<td>Southern Asia</td>
</tr>
<tr>
<td></td>
<td>19–30</td>
<td>Southern Asia</td>
<td>Immigration</td>
<td>Southern Asia</td>
</tr>
<tr>
<td></td>
<td>19–30</td>
<td>Sub-Saharan Africa</td>
<td>Immigration</td>
<td>Sub-Saharan Africa</td>
</tr>
<tr>
<td></td>
<td>31–49</td>
<td>Eastern Asia</td>
<td>Immigration</td>
<td>Southern Asia</td>
</tr>
<tr>
<td></td>
<td>31–49</td>
<td>Southern Asia</td>
<td>Immigration</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>31–49</td>
<td>Sub-Saharan Africa</td>
<td>Immigration</td>
<td>NA</td>
</tr>
<tr>
<td>Definitive non-CNS CC</td>
<td>31–49</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>31–49</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

CC: cysticercosis; CNS: central nervous system; NA: not available; NCC: neurocysticercosis.

Cases were ordered by age category and then alphabetically by region of origin.

Table 2
Characteristics of definitive and probable cysticercosis cases diagnosed at two referral medical institutions, Antwerp, Belgium, 1990–2015

<table>
<thead>
<tr>
<th>Type of cysticercosis case</th>
<th>Age category (years)</th>
<th>Geographical area of origin</th>
<th>Travel/immigration</th>
<th>Geographical area of travel/immigration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definitive NCC</td>
<td>0–18</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>19–30</td>
<td>South America</td>
<td>Travel</td>
<td>Western Europe</td>
</tr>
<tr>
<td></td>
<td>19–30</td>
<td>Sub-Saharan Africa</td>
<td>Immigration</td>
<td>Sub-Saharan Africa</td>
</tr>
<tr>
<td></td>
<td>19–30</td>
<td>Western Europe</td>
<td>Travel</td>
<td>South America</td>
</tr>
<tr>
<td></td>
<td>31–49</td>
<td>Western Europe</td>
<td>Travel</td>
<td>Central/Eastern/Southern Asia</td>
</tr>
<tr>
<td>Probable NCC</td>
<td>19–30</td>
<td>Southern Asia</td>
<td>Immigration</td>
<td>Southern Asia</td>
</tr>
<tr>
<td></td>
<td>19–30</td>
<td>Southern Asia</td>
<td>Immigration</td>
<td>Southern Asia</td>
</tr>
<tr>
<td></td>
<td>19–30</td>
<td>Sub-Saharan Africa</td>
<td>Immigration</td>
<td>Sub-Saharan Africa</td>
</tr>
<tr>
<td></td>
<td>31–49</td>
<td>Eastern Asia</td>
<td>Immigration</td>
<td>Southern Asia</td>
</tr>
<tr>
<td></td>
<td>31–49</td>
<td>Southern Asia</td>
<td>Immigration</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>31–49</td>
<td>Sub-Saharan Africa</td>
<td>Immigration</td>
<td>NA</td>
</tr>
<tr>
<td>Definitive non-CNS CC</td>
<td>31–49</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>31–49</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>
medical files did not contain sufficient information to allow evaluation.

Statistical methods
Descriptive statistical analyses, i.e. the calculation of proportion, percentage, median and range were performed in R software version 3.5.1 (R Foundation, Vienna, Austria) [27].

Ethical statement
For this retrospective analysis, we obtained ethical approval from the Institutional Review Board of ITMA (1018/15) as well as from the UZA Ethics Committee (15/34/350). Both the UZA and the ITMA apply an opt-out strategy for the use of de-identified retrospective medical data. After checking the final databases for duplicates, all names were removed and a study-specific patient code was generated. Moreover, only the variables of interest were extracted for the study analyses and all other information was removed. Because of the low number of cysticercosis cases obtained through our search and to ensure non-identifiability of patients, clinical and laboratory data were presented separately from background variables such as demographic information, country of origin and travel history, and in a different order. Clinical and laboratory data were presented by year of diagnosis, whereas as background variables were presented by age group.

Results

Number of samples analysed
The annual number of stool samples analysed at ITMA/UZA for parasites, including Taenia spp., for the period 1994 to 2015 fluctuated (median: 5,108; range: 4,150–6,786), with a sharp increase from 4,183 in 2007 to 6,595 in 2008. This was when UZA also started running large numbers of stool examinations. The annual number of serum or cerebrospinal fluid samples analysed with the T. solium Ab ELISA gradually decreased over 1994 to 2015, with 1,623 samples analysed in 1994 versus 241 in 2015 (median: 492.5; range: 224–1,623). On the contrary, the annual number of such samples analysed with the T. solium Ag ELISA increased from 1998 (n = 46) to 2005 (n = 507), but decreased thereafter to 163 in 2015 (median: 237.5; range: 46–507).

Taeniasis cases
A total of 221 confirmed taeniasis cases were identified. The median number of confirmed taeniasis cases per year was 9, with peaks of 18 in 1998 and 24 in 2014 (Figure 2). The median percentage of confirmed taeniasis cases on the total number of samples analysed for parasites (1995–2015) was 0.21% (range: 0.06–0.35%). For 170 of 221 confirmed taeniasis cases (76.9%), the infection was reported to be caused by T. saginata, while for the other cases (23.1%), the species was not mentioned. No cases of taeniasis caused by T. solium were identified. The majority of taeniasis cases were male (141/216 (5 missing values (mv)); 65.3%), adult individuals (168/206 (15 mv); 81.6%).

### Table 3

<table>
<thead>
<tr>
<th>Year of diagnosis at ITMA or UZA</th>
<th>Clinical symptoms</th>
<th>Serology</th>
<th>Stool</th>
<th>Imaging</th>
<th>Additional information</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001</td>
<td>None</td>
<td>1 month before diagnosis and at diagnosis: Ab-ELISA: neg; Ag-ELISA: pos 1 month after diagnosis: Ag-ELISA: neg</td>
<td>NA</td>
<td>MRI: 20 lesions, with ring enhancement, no or slight oedema 2 months after diagnosis: MRI: one lesion, several patchy zones</td>
<td>NA</td>
</tr>
<tr>
<td>2004</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>No eosinophilia</td>
</tr>
<tr>
<td>2009</td>
<td>Epilepsy</td>
<td>Ab-ELISA: neg</td>
<td>Neg</td>
<td>MRI: 1 lesion temporo-occipital right, perilesional oedema 1 month after diagnosis: MRI: reduction in volume and perilesional oedema</td>
<td>No eosinophilia T. solium Ab/Ag-ELISA CSF: neg</td>
</tr>
<tr>
<td>2010</td>
<td>6 months before diagnosis: photopsia left eye, balance disorder, mild headache</td>
<td>Ab/Ag-ELISA: neg</td>
<td>NA</td>
<td>MRI: one cystic ring-enhancing lesion occipital horn right, perilesional oedema, one presumed vascular lesion frontal right</td>
<td>NA</td>
</tr>
<tr>
<td>2011</td>
<td>1 month before diagnosis: headache, dysesthesia, nausea</td>
<td>Ab/Ag-ELISA: neg</td>
<td>NA</td>
<td>CT, MRI: one cystic lesion fronto-parietal left, perilesional oedema</td>
<td>No eosinophilia</td>
</tr>
</tbody>
</table>

Ab: antibody; Ag: antigen; CSF: cerebrospinal fluid; CT: computed tomography; ITMA: Institute of Tropical Medicine Antwerp; MRI: magnetic resonance imaging; neg: negative; NA: not available; pos: positive; UZA: Antwerp University Hospital.
Ten individuals with confirmed taeniasis reported diarrhoea (10/65; 15.6%), six anal discomfort (6/65; 9.2%), five general itch (5/65; 7.7%), eight reported having abdominal pain (8/65; 12.3%) and 26 of 65 were asymptomatic at the time of diagnosis (40.0%) (156 mv).

Treatment information was available for 30 confirmed taeniasis cases. Most cases were treated with praziquantel (n = 19), while others were treated with niclosamide (n = 5), albendazole/mebendazole (n = 2) or a combination of drugs (n = 4), of which three were a combination of niclosamide and praziquantel.

For 70 confirmed taeniasis cases there was information on travel history. Of these, 11 (15.7%) were autochthonous cases who had not travelled outside Belgium in the past 10 years; seven (10.0%) were considered allochthonous cases as they were described as recent immigrants or children who were recently adopted. The remaining 52 cases (72.3%) had travelled but it could not be determined whether the infection was acquired abroad or in Belgium as the dates of travel were not specified.

Cysticercosis cases

Our search identified five definitive cases of NCC, whereas another six were categorised as probable NCC cases. Two definitive cases of non-CNS CC were also

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### Table 4
Probable neurocysticercosis cases diagnosed at two referral medical institutions, Antwerp, Belgium, 1990–2015

<table>
<thead>
<tr>
<th>Year of diagnosis at ITMA or UZA</th>
<th>Clinical symptoms</th>
<th>Serology</th>
<th>Stool</th>
<th>Imaging</th>
<th>Additional information</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006</td>
<td>1 year before diagnosis and at diagnosis: epilepsy</td>
<td>NA</td>
<td>NA</td>
<td>MRI: several cystic lesions, frontal cyst with inflammation</td>
<td>NA</td>
</tr>
<tr>
<td>2011</td>
<td>3 years before diagnosis: intermittent headaches, insomnia</td>
<td>10 and 8 months before diagnosis: Ab-ELISA: pos; Ag-ELISA: neg 7 months before diagnosis: Ab-ELISA: neg At diagnosis: Ab-ELISA: pos</td>
<td>8 months before diagnosis: neg</td>
<td>MRI: cystic enhancing lesion in nucleus caudatus, perilesional oedema, superior two smaller cystic lesions 5 months after diagnosis: MRI: reduced oedema</td>
<td>10 and 9 months before diagnosis: Schistosoma ELISA: pos; Strongyloides-ELISA: pos; ELISA for filariae: pos 8 months before diagnosis: eosinophilia At diagnosis: Schistosoma-ELISA: pos; Strongyloides-ELISA: pos</td>
</tr>
<tr>
<td>2012</td>
<td>Prior to the diagnosis: epileptic seizure Around 4 months before diagnosis: sensory disorders</td>
<td>Ab-ELISA: neg, Ag-ELISA: pos</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>2012</td>
<td>3 years before diagnosis: four epilepsy events, thereafter no symptoms</td>
<td>Ab/Ag-ELISA: neg</td>
<td>Neg</td>
<td>2 months before diagnosis: CT: intracranial lesions At diagnosis: MRI: five supratentorial lesions: three with ring enhancement, two temporal right, one occipital right, one frontal right, one left parietal</td>
<td>Accidental finding ELISA for filariae: pos; Strongyloides-ELISA: pos</td>
</tr>
<tr>
<td>2012</td>
<td>8 and 6 years before confirmatory diagnosis at ITMA or UZAa: epilepsy At diagnosis: twitching feeling in foot, chronic headache</td>
<td>NA</td>
<td>NA</td>
<td>CT: normal; MRI: one lesion cortical frontal right</td>
<td>NA</td>
</tr>
<tr>
<td>2012</td>
<td>Since 3 years before diagnosis: chronic headache 3 months before diagnosis: paresis left leg</td>
<td>2 months before diagnosis: Ab/Ag-ELISA: neg</td>
<td>NA</td>
<td>MRI: two nodular lesions parietal cortex right</td>
<td>2 months before diagnosis: eosinophilia; Strongyloides PCR and ELISA: pos 1 month before diagnosis: Strongyloides PCR and ELISA: neg At diagnosis: no eosinophilia</td>
</tr>
</tbody>
</table>

Ab: antibody; Ag: antigen; CT: computed tomography; ITMA: Institute of Tropical Medicine Antwerp; MRI: magnetic resonance imaging; neg: negative; NA: not available; pos: positive; UZA: Antwerp University Hospital.

a Diagnosis earlier established outside of ITMA or UZA.
identified. Of the five definitive NCC cases, three were female and two were male, one was below 18 years old, three were between 19 and 30 years old and one was between 31 and 49 years old (Table 2). For the youngest definitive NCC case, no information apart from the anatomopathological result was available. Of the other four definitive cases, two were born in Belgium, one of whom travelled to South America 1 year before the diagnosis and one of whom had travelled to several countries in Asia 3 years before the diagnosis, one had immigrated from sub-Saharan Africa 13 years before the diagnosis was established and one was a visitor from South America. While three had a single lesion detected on magnetic resonance imaging (MRI), their symptoms and signs presented were diverse: one suffered from epilepsy, while the other two reported headaches in combination with different neurological disorders, e.g. balance disorder and dysesthesia (Table 3). The remaining definitive NCC case with imaging information available, in contrast, had 20 lesions, but exhibited no symptoms. The overall time between onset of symptoms and diagnosis for the definitive NCC cases ranged between 0 and 5 months. Of the four definitive NCC cases with serology available only one had positive *T. solium* Ab-ELISA or Ag-ELISA results (positive Ag-ELISA).

Of the four definitive NCC cases with treatment information available, two received anthelmintic treatment, one underwent surgery and one was treated with a combination of anthelmintics and surgery. Anthelmintic treatment consisted of albendazole for three cases, and both praziquantel and albendazole for one case.

In the group of probable NCC cases (n=6), one was female and five were male (Table 2). Two cases came from the Democratic Republic of the Congo, while the others were from India, China (Tibet), Nepal and Afghanistan.

One probable NCC case had a single lesion, one had two lesions, three had three or more lesions, and for one probable case, this information was not available (Table 4). Symptoms and signs present in the group of probable NCC cases included epilepsy, headache and other neurological disorders, e.g. partial paralysis. The time between onset of symptoms and diagnosis for the probable NCC cases ranged between 2 months and 16 years.

One probable NCC case had a positive *T. solium* Ag-ELISA result only, while another had a positive Ab-ELISA result only. No cases had eosinophilia, except for two with documented concurrent helminth infection.

No information on treatment was available for one probable NCC case, whereas four received an anthelmintic treatment with albendazole and one underwent surgery. One of the probable NCC cases was an accidental finding during imaging for an unrelated indication.

Both cases of definitive non-CNS CC, one diagnosed in 2005 and one in 2008, had a single nodule in the abdominal skin. For the latter, the nodule was reported to have developed 13 years earlier. No further information was available for these patients.

**Discussion**

We conducted a comprehensive retrospective investigation for taeniasis and cysticercosis cases in two referral medical institutions in Belgium. The total number of confirmed taeniasis cases retrieved for the study period was higher than those extracted from hospital/laboratory-based registries from other western European countries, e.g. France, Denmark, Portugal, but the median annual number of cases was lower than that reported in the epidemiological bulletins and national registries of the United Kingdom, Spain and Slovenia [10]. Confirmed taeniasis cases in our study reported rather mild symptoms, which is in line with the literature [1]. All taeniasis cases for whom the causative species could be identified were *T. saginata* carriers. Eleven of 70 taeniasis cases were acquired in Belgium. Cattle acquire bovine cysticercosis through ingestion of eggs shed by human *T. saginata* carriers, with research in Belgium pointing to wastewater contaminating pastures as a source of infection [28]. A recent study estimated that over 33% of Belgian cattle may be infected with *T. saginata* [29], suggesting the continued completion of the parasite’s lifecycle in the country. Given this and the habit of eating of raw or undercooked beef in Belgium, a certain risk of acquiring taeniasis remains. The continued transmission of *Taenia saginata* in Belgium has an economic impact: taeniasis has an estimated cost for the human health sector of up to EUR 795,858 per year, whereas bovine cysticercosis has an estimated cost for the meat industry of up to EUR 3,408,455 per year [30]. Furthermore, for 23.1% of taeniasis cases in our study, the causative species was not known and this group could thus potentially include some *T. solium* carriers. They would pose a risk to themselves, family members and other contacts with respect to cysticercosis development [16-18]. Unfortunately, classical methods to examine stool cannot always distinguish species and molecular differentiation, although recommended, is not routinely done in Europe [10,11].

The number of definitive and probable cysticercosis cases found in our study was in line with the number of cases reported at the hospital/laboratory level in Austria, Denmark and Sweden, but much lower than the number of cases for Portugal, Spain the Netherlands, France and Italy [10]. Most cases seemed to be detected closer towards the end of the study period, possibly because of an actual increase in cases or increased awareness of specialists at the study institutes about the condition. After 2015, cysticercosis continued to be diagnosed at both institutes, with another three definitive NCC cases reported (Supplementary Table S2, Supplementary Table S3). Overall, the definitive and probable cysticercosis cases identified in our
study had diverse travel, migration and age characteristics, indicating that identifying high-risk patients for NCC is difficult. For some cases, it took several months and even years before the diagnosis was established, possibly because of the often non-specific signs and symptoms presented by patients, e.g. chronic headaches, and limited experience of some physicians with tropical diseases. Furthermore, serological test results are not always conclusive. For instance, commercially available Ab-ELISA kits are reported to exhibit low sensitivity and frequent cross-reactions [31], and while the enzyme-linked immunoelectrotransfer blot (EITB) assay has a close to perfect performance in terms of sensitivity and specificity to detecting Ab, the test is expensive, cumbersome and not routinely used [32]. Good sensitivity and specificity were reported for the Ag-ELISA; but this test only detects the presence of viable cysticerci, the earliest stage of NCC [33]. NCC-associated epilepsy however, is thought to occur when cysticerci present in the CNS start to degenerate or have even calcified [34,35]. Current diagnostic guidelines for NCC therefore advise the combined evaluation of imaging results, clinical manifestations and exposure-related factors, e.g. serology, travel history, to establish the diagnosis and assess the degree of certainty [26].

This study has several limitations. At the ITMA, software did not allow searching the medical records for certain terms or ICD codes, which means that a certain number of true cases may have been missed. However, because UZA medical records were searched for specific terms and because the ITMA and UZA databases were merged, the risk of missing true cases was rather low. In contrast, relying on positive Ab-based serology as one of the search criteria may have led to the inclusion of false-positive cases because of cross-reactions. However, the thorough critical review of medical files allowed excluding irrelevant cases. It is noteworthy, that the use of ICD codes to retrieve taeniasis and cysticercosis cases was not faultless as some cases with relevant ICD codes could not be assigned undoubtedly to taeniasis or cysticercosis diagnosis and therefore had to be excluded. Furthermore, as is inherent to retrospective surveys of medical files, information was often incomplete and a critical review of imaging results was not possible when they were not electronically stored. Finally, as case identification requires the intensive review of medical files and related ethical clearance, our study was restricted to two Belgian referral medical institutions. Nevertheless, we expect most cysticercosis cases to have received a confirmatory diagnosis at ITMA, as it is the national reference national reference centre for infectious and tropical diseases. As for the taeniasis cases, apart from dedicated screening in adopted children and recent immigrants from endemic areas at ITMA and UZA, other large hospitals in Belgium are expected to have diagnosed a considerable number of cases as well during the study period.

Overall, the findings of the current study confirm that taeniasis and cysticercosis cases are consulting Belgian hospitals in larger numbers than reported in scholarly publications. As a proportion of taeniasis cases caused by *T. saginata* were acquired in Belgium, improved taeniasis case management, including correct treatment of cases and disposal of expelled tapeworms, as well as a multi-sectoral One Health approach are warranted to control the parasite’s transmission in the country. Furthermore, molecular differentiation of tapeworms is advised in order to detect *T. solium* carriers. Regarding cysticercosis, clinical awareness as well as serological testing of individuals at risk, such as travellers and immigrants, with suggestive symptoms are key. Also the complexity of management should be highlighted during medical training to ensure adequate referral to, or supervision by experts in the field such as those in tropical medicine.

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**Conflict of interest**

None declared.

**Authors’ contributions**

Veronique Dermauw: conceived the study, analysed the data, contributed to the data interpretation, drafted and reviewed the manuscript.

Steven Van Den Broucke: collected the data at ITMA, contributed to the data interpretation, critically revised the manuscript.

Lieselotte Van Bockstal: collected the data at UZA, critically revised the manuscript.

Emmanuel Bottieau: contributed to data collection at ITMA, critically revised the manuscript.

Kim Luycx: contributed to data collection at UZA, critically revised the manuscript.

Pierre Dorny: coordinated the study, contributed to the data collection at ITMA, critically revised the manuscript.

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