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First human case of tick-borne encephalitis virus infection acquired in the Netherlands, July 2016

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In July 2016, the first autochthonous case of tick-borne encephalitis was diagnosed in the Netherlands, five days after a report that tick-borne encephalitis virus (TBEV) had been found in Dutch ticks. A person in their 60s without recent travel history suffered from neurological symptoms after a tick bite. TBEV serology was positive and the tick was positive in TBEV qRT-PCR. TBEV infection should be considered in patients with compatible symptoms in the Netherlands.

Until recently, tick-borne encephalitis virus (TBEV) was thought to be absent in the Netherlands and all cases of tick-borne encephalitis (TBE) were considered imported from endemic regions [1,2]. On 30 June 2016, the Dutch National Institute for Public Health and the Environment (RIVM) reported that Dutch *Ixodes ricinus* ticks were RT-PCR positive for TBEV-Eu, but no autochthonous cases had been diagnosed at that point [3]. This is the first report of an autochthonous case of TBE in the Netherlands.

Case description

In June 2016, a person in their 60s presented at a hospital in the middle of the Netherlands with complaints of malaise, fatigue, headache, nausea and a subfebrile temperature (37.9 °C) after a tick bite. The malaise and fatigue had started earlier that month (day 0), the other symptoms started two days later. On day 4, the general practitioner discovered a tick on the patient's left leg, removed it and started antibiotic treatment with doxycycline for 10 days. In retrospect, the bite is most likely to have occurred two days before onset of symptoms in a forested area between Driebergen en Maarn. Initially, the patient improved after antibiotic treatment and the symptoms disappeared. However, on day 12 the patient suffered from tremors, slow speech, weakness and fatigue. Subsequently, these symptoms progressed and fever (40.0 °C), nausea and vomiting developed on day 21. The patient was referred to the hospital on day 24. Neurological and general physical examination

revealed no other abnormalities, especially no signs of meningism. Laboratory blood tests showed no specific abnormalities (Table 1), nor did a computed tomography scan of the brain. Serum and cerebrospinal fluid (CSF) tested negative for Lyme borreliosis (Table 2), however, CSF showed a mononuclear cell reaction (Table 1).

Additional diagnostic tests were conducted to exclude other infectious diseases (Table 2). Although TBE was not considered endemic in the Netherlands, it was added to the differential diagnosis after the RIVM reported that TBEV had been detected in ticks in the eastern part of the country (Sallandse Heuvelrug), 100 km from Driebergen [3]. In 2016, the patient had not travelled to the Sallandse Heuvelrug or any other regions known to be endemic for TBE. Their last stay abroad had been in October 2015, in Paderborn, Germany, which is not a region endemic for TBEV [4]. They had not visited other places abroad in the past five years. The patient was not vaccinated against TBEV, but had received a vaccination against yellow fever virus in 2005.

Serum taken on day 24 and 36 was positive for anti-TBEV IgM (452 and 162 Vienna units (VIEU)/mL, respectively; cut-off: 63 VIEU/mL) and IgG (>650 and >650 VIEU/mL, respectively; cut-off: 100 VIEU/mL) (Progen Biotechnik). CSF was negative for IgM but IgG-positive. In addition, both sera were positive in a TBEV neutralisation assay (1/640). A TBEV-specific qRT-PCR on CSF, blood and urine was negative.

Fortunately, the patient had saved the dead tick, which was positive for TBEV by qRT-PCR with a Ct value of 21. Interestingly, based on comparison of partial NS5 sequences of the PCR products, TBEV in the patient's tick showed 93% homology with those found in Sallandse Heuvelrug, but 99% homology with a prototype TBEV-Eu Neudörfl strain.

TABLE 1

Clinical, chemical and haematological tests on blood and cerebrospinal fluid at hospital admission, tick-borne encephalitis case, the Netherlands, July 2016*

	Patient	Reference value
Blood		
Haemoglobin (mmol/L)	7.6	8.5–11.0
Leukocyte count (10^9 /L)	6.9	4.0–10.0
Thrombocyte count (10^9 /L)	211	150–400
Erythrocyte sedimentation rate (mm/hour)	60	0–19
Sodium (mmol/L)	131	135–145
Potassium (mmol/L)	3.9	3.6–5.1
Glucose (mmol/L)	6.1	4.0–7.0
C-reactive protein (mg/L)	12	0–10
Cerebrospinal fluid		
Polynuclear cells (cells/ μ L)	2	Not applicable
Mononuclear cells (cells/ μ L)	61	Not applicable
Erythrocytes (cells/ μ L)	128	Not applicable
Glucose (mmol/L)	3.5	2.0–4.0
Protein (g/L)	0.89	0.15–0.45

TABLE 2

Performed tests for infectious diseases, tick-borne encephalitis case, the Netherlands, July 2016*

	Blood	Cerebrospinal fluid
<i>Bartonella henselae</i>	IgM negative	ND
<i>Borrelia burgdorferi</i>	C6 IgG negative IgG negative IgM negative	PCR negative serum/liquor index IgM and IgG negative
<i>Treponema pallidum</i>	Serological screening negative	ND
Tuberculosis	IGRA negative	ND
HIV	Serological screening negative	ND
Enterovirus	ND	PCR negative
Parechovirus	ND	PCR negative
Herpes simplex virus 1 and 2	ND	PCR negative
Varicella zoster virus	ND	PCR negative

HIV: human immunodeficiency virus; IGRA: interferon gamma release assay; ND: Not done; PCR: polymerase chain reaction.

During clinical observation, the patient gradually improved. At discharge on day 37, no focal neurological deficits were present, but fatigue and mild subjective cognitive complaints (Montreal Cognitive Assessment 26/30) remained.

Discussion

This is the first report of a case of TBE in a patient infected in the Netherlands. Although liquor was negative for anti-TBEV IgM antibodies, the high serum IgM and IgG levels in an unvaccinated patient, combined with a typical biphasic clinical presentation and TBEV detected in the tick collected from the patient, confirmed the diagnosis of TBE [5]. Since the patient had not travelled abroad in the previous seven months, they must have been infected in the Netherlands, as the incubation period for TBE is no longer than a month

and *Ixodes* species only feed for several days per host [1,6].

TBE is considered an emerging disease due to its rising incidence and the expansion in new, previously uninfected, areas but until now, autochthonous human TBEV infection had not been reported in the Netherlands [3,7]. The presence of TBEV in ticks collected in the Netherlands was recently confirmed [3]. Interestingly, preliminary sequence data suggest that the TBEV detected in the tick from our patient had a higher homology to the prototype TBEV-Eu strain Neudörf1 than to those found in the Sallandse Heuvelrug. The Neudörf1 strain and related TBEV strains have been found throughout Europe, including Germany. Although it is highly likely that the TBEV-infected tick that bit our patient was acquired between Driebergen and Maarn, the exact origin of the tick requires further

investigation. Further studies are needed to determine the geographic spread and genetic diversity of TBEV in ticks in the Netherlands.

This case is an excellent example of the importance of tenacity and persistence in difficult diagnostic cases. Looking beyond guidelines and current evidence can lead to new findings, which can be beneficial not only for the individual patient but also for public health. Surveillance and widespread messages by public health institutes can be of great value to the diagnostic process, as they can provide clinicians with clues for the diagnosis of disease in individual patients.

This case has important implications. On a patient level, clinicians in the Netherlands need to add TBE to the differential diagnosis for patients hospitalised with (meningo)encephalitis or meningitis who may have been exposed to tick bites. On a public health level, further studies are needed to determine the extent of TBEV infections in humans in the Netherlands. These studies include surveillance of TBEV in humans, animals and ticks, as well as determining the risk of acquiring TBEV infection by serosurveillance studies in the general population, patient populations with unknown neurological disease and for professions at high risk for tick bites.

*Erratum

This article was originally published with an incorrectly ordered reference list. This was corrected on 19 August 2016. We apologise for this mistake.

On request of the authors, the date in the Table titles was corrected from June 2016 to July 2016. This change was made on 19 August 2016.

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Conflict of interest

None declared.

Authors' contributions

Joris de Graaf and Vishal Hira wrote the first draft of the manuscript. All other authors critically read and revised the manuscript. Joris de Graaf, Alie Schuitemaker, Paul Voorn, Liesbeth bij de Vaate and Vishal Hira contributed to the clinical management of the patient. Paul Voorn, Johan Reimerink, Ankje de Vries, Barry Rockx and Vishal Hira were responsible for laboratory testing.

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The health and economic burden of bloodstream infections caused by antimicrobial-susceptible and non-susceptible Enterobacteriaceae and *Staphylococcus aureus* in European hospitals, 2010 and 2011: a multicentre retrospective cohort study

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We performed a multicentre retrospective cohort study including 606,649 acute inpatient episodes at 10 European hospitals in 2010 and 2011 to estimate the impact of antimicrobial resistance on hospital mortality, excess length of stay (LOS) and cost. Bloodstream infections (BSI) caused by third-generation cephalosporin-resistant Enterobacteriaceae (3GCSE), methicillin-susceptible (MSSA) and -resistant *Staphylococcus aureus* (MRSA) increased the daily risk of hospital death (adjusted hazard ratio (HR) = 1.80; 95% confidence interval (CI): 1.34–2.42, HR = 1.81; 95% CI: 1.49–2.20 and HR = 2.42; 95% CI: 1.66–3.51, respectively) and prolonged LOS (9.3 days; 95% CI: 9.2–9.4, 11.5 days; 95% CI: 11.5–11.6 and 13.3 days; 95% CI: 13.2–13.4, respectively). BSI with third-generation cephalosporin-susceptible Enterobacteriaceae (3GCSE) significantly increased LOS (5.9 days; 95% CI: 5.8–5.9) but not hazard of death (1.16; 95% CI: 0.98–1.36). 3GCSE significantly increased the hazard of death (1.63; 95%

CI: 1.13–2.35), excess LOS (4.9 days; 95% CI: 1.1–8.7) and cost compared with susceptible strains, whereas methicillin resistance did not. The annual cost of 3GCSE BSI was higher than of MRSA BSI. While BSI with *S. aureus* had greater impact on mortality, excess LOS and cost than Enterobacteriaceae per infection, the impact of antimicrobial resistance was greater for Enterobacteriaceae.

Introduction

Antimicrobial resistance (AMR) represents a significant global threat [1,2]. Response to this threat requires coordinated international interventions likely to involve commitment of substantial resources [3]. It is useful to obtain accurate estimates of the health and economic burden of AMR as these illustrate opportunities to improve health and reduce costs. Comprehensive data remain scarce; a recent World Health Organization (WHO) systematic review identified a “lack of properly

designed and conducted economic studies comparing the resource use associated with resistant versus non-resistant pathogens” [1].

Studies to determine health outcomes of infections with community and hospital onset must adequately account for confounding, the timing of infection (time dependency) and simultaneous impact on risk of death and discharge (competing risks), but also analyse a sample of sufficient size to produce precise estimates [4,5]. Furthermore, although the major determinant of the economic burden of such infections from the hospital perspective is the number of bed-days they consume, it is challenging to produce an appropriate economic valuation of each marginal bed-day [6].

Given the widespread dissemination of meticillin resistance among *Staphylococcus aureus* and resistance to third-generation cephalosporins among Enterobacteriaceae [7], we focused on these bacteria and resistance phenotypes. We examined bloodstream infections (BSI) because of their relatively high incidence, clinical impact and diagnostic certainty. We were interested in costs from the hospital perspective because this is the perspective from which decisions must be made to allocate resources to interventions such as antimicrobial stewardship and infection control.

Objectives

We sought to apply state-of-the-art methods to obtain unbiased and adjusted estimates of the excess length of stay (LOS), hospital mortality, and cost (from the hospital perspective) attributable to BSI caused by *S. aureus* and Enterobacteriaceae in European hospitals, and to compare the impact of antimicrobial non-susceptible versus susceptible strains.

Methods

Study design

We performed a multicentre, retrospective cohort study. The cohort consisted of all acute-care admissions at 10 European hospitals from 1 January 2010 to 31 December 2011. BSI were the time-varying exposure of interest and their impact on hospital mortality, LOS and cost was evaluated. Independent analyses were performed for BSI due to *S. aureus* and Enterobacteriaceae.

This report was formulated in accordance with the STROBE Statement [8].

Setting

A convenience sample of 10 European hospitals participated: three from Italy, two each from Germany and the United Kingdom, and one each from France, Spain and Switzerland. These participants were selected from a list of interested sites using a questionnaire addressing microbiological methods and clinical informatics. Hospitals were eligible if able to extract the required

data from institutional databases. All eligible hospitals were included.

Participants

We retrospectively identified all inpatient acute-care episodes lasting more than one calendar day that started during the study period. We excluded ambulatory, hospital-in-the-home and non-acute care episodes as well as emergency consultations without consequent hospital admission. There was no age limit. For patients with multiple admissions during the study period, only the first admission was included.

Exposures

We considered four exposures defined by causative bacteria and antimicrobial susceptibility. *Escherichia coli*, *Klebsiella* spp. or *Proteus* spp. strains causing BSI were classified as third-generation cephalosporin-susceptible Enterobacteriaceae (3GCSE) or third-generation cephalosporin-non-susceptible (3GCRE). Non-susceptibility to third-generation cephalosporins was defined as intermediate susceptibility or resistance to ceftazidime and/or one of cefotaxime, ceftriaxone or cefpodoxime. *S. aureus* strains causing BSI were classified as meticillin-susceptible (MSSA) or meticillin-resistant (MRSA). BSI was defined by one or more blood cultures with growth of the relevant bacteria.

Outcomes

The two primary outcomes were hospital mortality and excess LOS in hospital. Excess LOS was used to estimate costs from the hospital perspective.

Covariates

Baseline variables considered as potential confounders were age, sex, location prior to episode, elective/emergent admission, nights hospitalised in the previous 12 months in the same institution and 17 comorbidities [9]. The Charlson Comorbidity Index was computed for descriptive purposes, but comorbidities were included in the analyses as individual covariates. Two time-varying covariates were considered while patients were at risk for BSI: admission to an intensive care unit (ICU) and surgical procedure. To estimate the total impact of infection and avoid controlling for intermediates on the causal pathway, we did not adjust for events occurring after BSI onset, such as antibiotic exposure.

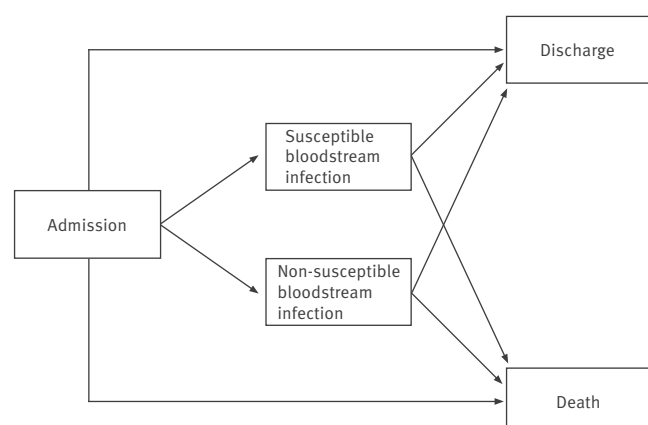
BSI were categorised as hospital-onset if detected after the first three inpatient calendar days [10], if the patient was transferred from a non-acute ward or another hospital, or if the patient was born during the current admission. All others were categorised as community-onset.

Data collection

One investigator from each site was trained in standardised data collection. Information technicians from each participating hospital extracted data from the hospital databases. Comorbidities were extracted using a validated algorithm based on ICD-9-CM and

FIGURE 1

Multistate model adopted for the analysis of the burden of bloodstream infections caused by antimicrobial resistance, 2010–2011



Patients entered the initial state on admission to acute care unless the infection date was before or equal to the admission date, in which case the patient was assigned directly to the appropriate intermediate infected state. Patients exited by entering one of two competing absorbing states (death or discharge alive), with or without passing through one of two intermediate states (bloodstream infection caused by susceptible or non-susceptible pathogens). *Escherichia coli*, *Klebsiella* spp. or *Proteus* spp. were classified as susceptible or non-susceptible to third-generation cephalosporins. *Staphylococcus aureus* was classified as susceptible or non-susceptible to methicillin.

ICD-10 codes [11]. Each dataset was reviewed for internal consistency and external plausibility by the central coordinating team, with potential errors triggering review by the local investigators.

Microbiological methods

Antimicrobial susceptibility testing was performed as per routine laboratory methods at each hospital. All laboratories participated in national or international quality assurance programmes and adhered to contemporary guidelines from the following bodies: Clinical and Laboratory Standards Institute (CLSI) for seven sites, European Committee on Antimicrobial Susceptibility Testing (EUCAST) for three sites, AntibioGram Committee of the French Microbiology Society (CA-SFM) for one site, British Society for Antimicrobial Chemotherapy (BSAC) for one site, and Deutsche Industrie Norm (DIN)-Medizinische Mikrobiologie for one site. Three sites used more than one guideline during the study period. Nine sites performed one or more MRSA confirmatory tests: oxacillin minimum inhibitory concentration (MIC) test ($n = 6$), *mecA* PCR ($n = 4$), and penicillin binding protein 2a (PBP2a) agglutination ($n = 4$). The site that did not perform these tests used disc diffusion (BSAC protocol) and the VITEK2 system with the AST-P578 panel (bioMérieux, Lyon, France). Confirmatory testing for extended-spectrum beta-lactamase (ESBL) production was performed by seven sites but not included in our definition of third-generation cephalosporin susceptibility.

Sample size

The sample size calculation was based on the estimated excess LOS for ESBL-positive BSI, informed by estimates from a pilot study [12]. We wished to find the number of infections such that, with a power of 80% and α equal to 5%, we could conclude that excess LOS was greater than excess LOS/2, an estimate of precision, i.e. to have sufficient power to detect a lower confidence limit of at least half of the point estimate. On the basis of incidence data from participating hospitals, we expected to include approximately 1,250 patients with BSI caused by 3GCRE, allowing estimates with good precision for an excess LOS of four days or more.

Statistical analysis

Descriptive statistics

Continuous variables are summarised as median with 25%–75% percentile, ordinal variables as count with percentage. BSI incidence density was computed by dividing the number of events by the number of patient-days at risk.

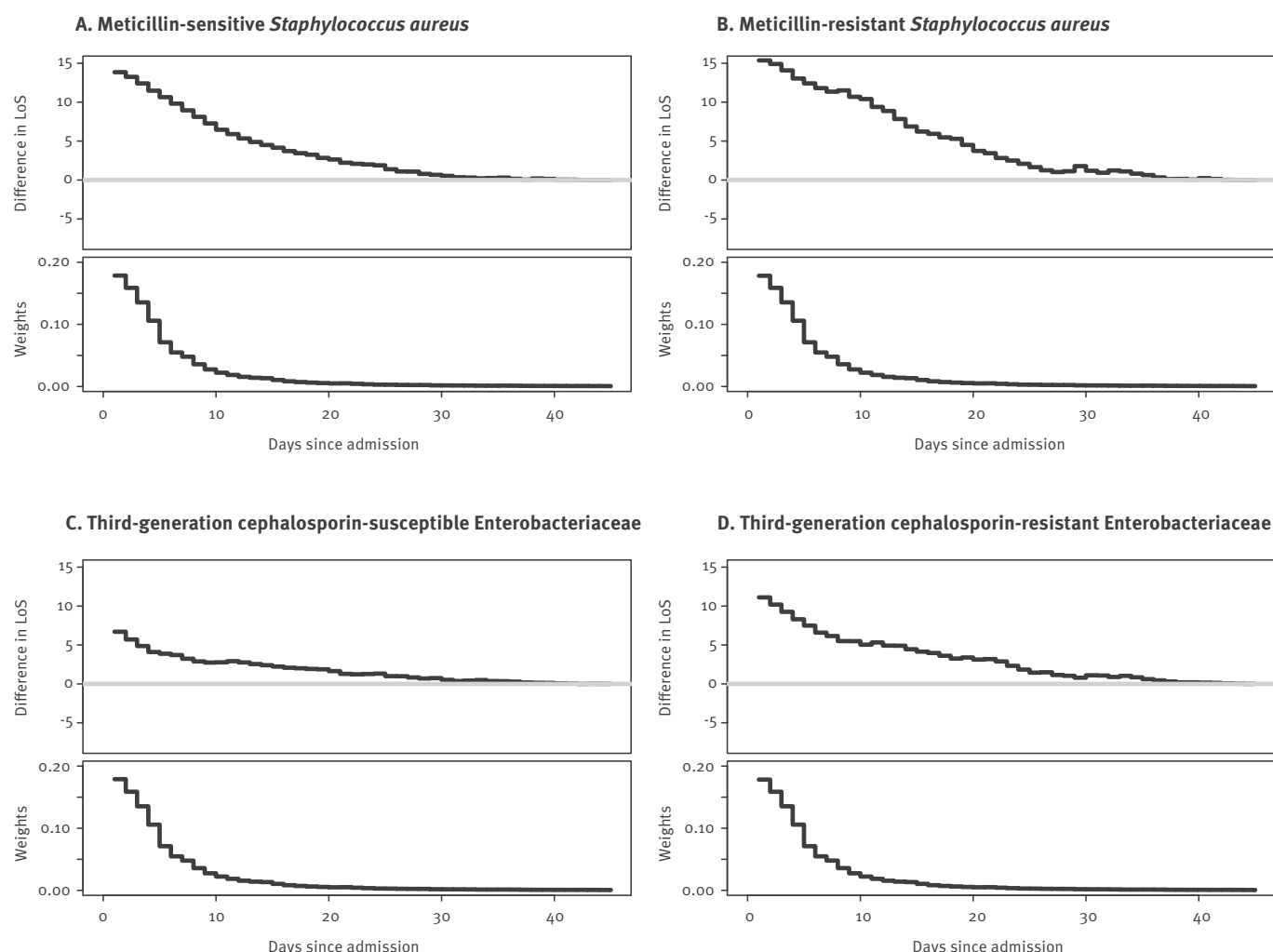
Estimation of mortality and excess length of stay

Two important characteristics of this dataset were the inclusion of time-varying exposures (BSI, surgery and ICU admission) and competing risks (death and discharge alive). We adopted the multistate model illustrated in Figure 1 to explicitly account for these characteristics [4]. Patients entered the initial state on admission to acute care and exited by entering one of two competing absorbing states (hospital death or discharge alive), with or without passing through one of two intermediate states (susceptible or non-susceptible BSI). Admissions were artificially right-censored at day 45 to reduce the influence of outliers. We reasoned that patients with such prolonged admissions were likely to remain hospitalised for other reasons not influenced by BSI.

Cox proportional hazards models were used to compare the *daily* risk (hazard) of reaching the endpoint, i.e. hospital death, discharge alive, and the combined end-of-stay endpoint (hospital death or discharge alive), between the three groups of patients (non-susceptible BSI, susceptible BSI and uninfected subjects). We fitted three models for each possible pairwise comparison between these three groups. Model 1 only included infection status as time-dependent variable. Model 2 adjusted for age, sex, emergent/elective admission, nights hospitalised in the previous 12 months and comorbidities. Age was centred at the cohort mean and divided by 10. Each comorbidity was included as a separate indicator variable. Model 3 additionally adjusted for two time-dependent variables while patients were at risk for BSI: ICU admission and surgical procedure. All Cox models were stratified by hospital to account for the multicentre nature of this study by allowing for clustering effects and site-specific heterogeneity in baseline hazards. The proportional

FIGURE 2

Results of multistate models to determine excess length of stay attributable to bloodstream infection caused by different combinations of bacteria and susceptibility, 10 European hospitals, 2010–2011 (n = 606,649)



LOS: length of stay; MRSA/MSSA: meticillin-resistant/susceptible *Staphylococcus aureus*; 3GCRE/3GCSE: third-generation cephalosporin-resistant/susceptible Enterobacteriaceae.

The upper half in each panel (A–D) illustrates the relationship between the expected change in LOS associated with a BSI (computed daily by subtracting the LOS of patients that had not experienced BSI on that day from those who had) and timing of BSI onset (in days from admission). The lower half of each panel presents the weights used to compute the summary excess LOS, calculated using the observed relative frequency BSI onset each day. For all types of infections, early BSI was associated with the greatest difference in LOS.

hazards assumption was checked by inspection of the Schoenfeld residuals. No major deviations were found.

Multistate models describe the *instantaneous* (in this case, daily) risk of transition between health states. The excess LOS associated with an infection was derived as a function of these transition probabilities [4]. We used the Aalen-Johansen estimators as a nonparametric estimator for the matrix of transition probabilities for all observed transition times [13]. The expected LOS (in days) was then computed by a function of the Aalen-Johansen estimator for the matrix of transition probabilities [4]. The expected change in LOS for each of the four BSI phenotypes was computed for each day of admission as the difference between the estimated

LOS, given that BSI (the intermediate state) had or had not occurred up to that day. The overall change in LOS was computed as a weighted average of these quantities, with weighting determined by the observed distribution of time to BSI onset. The expected difference in LOS between susceptible and resistant infections was produced similarly, by computing for each day the difference between the estimated LOS, given that the susceptible or resistant BSI had occurred up to that day, then computing a weighted average of these quantities determined by the observed distribution of day of BSI onset. Standard errors and confidence intervals were derived by bootstrap re-sampling runs.

TABLE 1

Characteristics of participating hospitals, analysis of the burden of bloodstream infections caused by antimicrobial resistance, 2010–2011 (n = 10)

Hospital	Country	Type	Acute beds	Study cohort	
				Admissions	Bed-days
1	France	Tertiary	1,200	64,071	424,361
2	Germany	Tertiary	1,200	67,094	456,564
3	Germany	Tertiary	1,332	59,517	431,384
4	Italy	Tertiary	1,100	89,401	677,788
5	Italy	Tertiary	1,050	53,947	373,665
6	Italy	Secondary	555	27,975	183,707
7	Switzerland	Tertiary	900	58,541	382,012
8	Spain	Tertiary	711	50,065	336,253
9	United Kingdom	Tertiary	1,050	92,569	423,534
10	United Kingdom	Tertiary	936	43,469	265,392
Total			10,034	606,649	3,954,660

Note: Admissions and bed-days relate to acute-care episodes from 2010 and 2011 included in the analysis. For patients with multiple admissions during the study period, only the first admission was included.

We adjusted excess LOS for the baseline covariates included in Model 2 using pseudo-observations [14]. Excess LOS was estimated for all possible subsets of the entire cohort created by removing a single patient. In each case, the excess LOS estimate was compared to the estimate derived from the full cohort; this difference or pseudo-observation contained information on the way in which patient-level covariates affected the LOS estimate. The pseudo-observations were then included in a generalised linear model with identity link and independent working covariance matrix to model the effect of covariates on the excess LOS. In practice, the regression coefficients were estimated using the generalized estimating equations approach with robust variance estimator to account for hospital-level clustering [15]. Time-dependent covariates (Model 3) were not included because this would have been difficult to implement and interpret. To reduce the influence of outliers, the original pseudo-observations were transformed using the cubic root function, similar to the common log transformation of LOS data but allowing for negative excess LOS.

Cost estimation

For each combination of bacterium and susceptibility, we computed the attributable cost of a single BSI from the hospital perspective as the product of excess LOS and the value of a bed-day [6]. We performed a Monte Carlo simulation with 10,000 samples to account for parameter uncertainty [16]. We used gamma probability distributions to represent the excess LOS associated with each BSI, fitting these distributions to the unadjusted estimate from the current study (Model 1) to best reflect our patient mix. We used log-normal distributions for two contrasting bed-day values, both obtained from the study hospitals as previously reported: an economic estimate of the opportunity cost

of a bed-day obtained by contingent valuation and the accounting cost derived by dividing the total annual hospital budget by the number of bed-days supplied during the same period [17].

To estimate the annual hospital costs of each BSI, these marginal costs were multiplied by the expected number of BSI cases per year, as estimated for a hospital with 450,000 bed-days using incidence densities calculated in the analysis here below. Results are presented as median with 95% credible interval, to two significant figures. A full description of data sources and probability distributions can be obtained from the corresponding author.

The cost estimation was implemented in OpenBUGS, version 3.2.3. Other statistical analyses were performed using R, version 3.1.0 (R Foundation for Statistical Computing) including the *etm*, *mvna*, and *survival* packages [18].

Ethics statement

This study was approved, with a waiver for individual informed consent, by the human research ethics committee at each institution.

Results

Participants

Ten public hospitals provided a cohort of 867,977 acute-care episodes involving 606,649 patients (Table 1). Each patient's first episode was included in the analysis.

Median patient age at admission was 49 years (interquartile range (IQR): 28–69); 53% were female. Median LOS was four days (IQR: 2–7), and 588,118 (97%)

TABLE 2A

Characteristics of patients in *Staphylococcus aureus* and Enterobacteriaceae analyses, stratified by exposure to bloodstream infection in 10 European hospitals, 2010–2011 (n = 606,649^a)

Characteristic	Staphylococcus aureus analysis						Enterobacteriaceae analysis					
	MRSA BSI		MSSA BSI		Non-infected		3GCRE BSI		3GCSE BSI		Non-infected	
	n = 163		n = 885		n = 604,797		n = 360		n = 2,100		n = 603,972	
	n	%	n	%	n	%	n	%	n	%	n	%
Demographics												
Male sex	98	60.1	529	59.8	286,857	47.4	206	57.2	1,051	50.0	286,492	47.4
Median age at enrolment (IQR)	71 (59–81)		63 (45–76)		49 (28–69)		70 (58–78)		70 (56–80)		49 (27–69)	
Hospitalisation in the previous 12 months												
Two or more admissions	15	9.2	40	4.5	15,708	2.6	23	6.4	112	5.3	15,660	2.6
Two or more nights hospitalised	24	14.7	72	8.1	37,468	6.2	46	12.8	229	10.9	37,354	6.2
Admission details												
Emergent admission	111	68.1	639	72.2	282,382	46.7	217	60.3	1,588	75.6	281,844	46.7
Provenance												
Home	134	82.2	734	82.9	500,693	82.8	303	84.2	1,848	88.0	499,942	82.8
Transfer from other hospital	16	9.8	73	8.2	16,930	2.8	21	5.8	75	3.6	16,927	2.8
Transfer from non-acute ward	2	1.2	11	1.2	2,722	0.5	1	0.3	26	1.2	2,713	0.4
Born this episode	3	1.8	7	0.8	47,414	7.8	3	0.8	29	1.4	47,397	7.8
Comorbidities												
Cardiovascular disease	8	4.9	50	5.6	15,192	2.5	10	2.8	97	4.6	15,160	2.5
Congestive heart failure	29	17.8	109	12.3	22,935	3.8	32	8.9	195	9.3	22,886	3.8
Peripheral vascular disease	17	10.4	65	7.3	16,515	2.7	9	2.5	89	4.2	16,509	2.7
Cerebrovascular disease	14	8.6	77	8.7	22,908	3.8	27	7.5	165	7.9	22,836	3.8
Dementia	9	5.5	14	1.6	5,759	1.0	7	1.9	64	3.0	5,743	1.0
COPD	7	4.3	40	4.5	25,701	4.2	16	4.4	112	5.3	25,647	4.2
Connective tissue disease	2	1.2	19	2.1	5,123	0.8	2	0.6	31	1.5	5,124	0.8
Peptic ulcer disease	1	0.6	15	1.7	2,527	0.4	7	1.9	27	1.3	2,513	0.4
Mild liver disease	9	5.5	76	8.6	12,541	2.1	31	8.6	150	7.1	12,478	2.1
Diabetes without end-organ damage	23	14.1	127	14.4	38,004	6.3	28	7.8	255	12.1	37,937	6.3
Diabetes with end-organ damage	7	4.3	37	4.2	7,901	1.3	7	1.9	60	2.9	7,886	1.3
Haemiplegia or paraplegia	6	3.7	44	5.0	7,374	1.2	12	3.3	55	2.6	7,363	1.2
Renal disease	26	16.0	110	12.4	21,309	3.5	30	8.3	233	11.1	21,231	3.5
Neoplasia	13	8.0	79	8.9	43,830	7.2	45	12.5	277	13.2	43,641	7.2
Metastatic cancer	2	1.2	35	4.0	16,393	2.7	24	6.7	119	5.7	16,302	2.7
Liver diseases	2	1.2	31	3.5	3,047	0.5	11	3.1	49	2.3	3,030	0.5
HIV	0	0.0	12	1.4	1,277	0.2	1	0.3	17	0.8	1,271	0.2
Age-adjusted Charlson comorbidity index, median (IQR)	4 (3–5)		3 (1–5)		1 (0–3)		4 (2–5)		4 (2–5)		1 (0–3)	
Primary diagnosis category												
Certain infectious and parasitic diseases	20	12.3	132	14.9	13,216	2.2	53	14.7	411	19.6	13,060	2.2
Neoplasms	10	6.1	61	6.9	56,345	9.3	41	11.4	225	10.7	56,165	9.3
Blood and blood-forming organs and certain disorders involving the immune mechanism	1	0.6	6	0.7	4,400	0.7	4	1.1	9	0.4	4,399	0.7
Endocrine, nutritional and metabolic diseases	7	4.3	16	1.8	14,320	2.4	4	1.1	23	1.1	14,320	2.4
Mental and behavioural disorders	1	0.6	5	0.6	6,270	1.0	7	1.9	17	0.8	6,255	1.0
Nervous system, eye and adnexa, ear and mastoid process	3	1.8	34	3.8	40,844	6.8	5	1.4	24	1.1	40,848	6.8

BSI: bloodstream infection; COPD: chronic obstructive pulmonary disease; HIV: human immunodeficiency virus; ICU: intensive care unit; IQR: interquartile range; MRSA/MSSA: methicillin-resistant/susceptible *Staphylococcus aureus*; NA: not applicable; 3GCRE/3GCSE: third-generation cephalosporin-resistant/susceptible Enterobacteriaceae.

^a Patients experiencing BSI caused by Enterobacteriaceae were censored from the *S. aureus* analysis on the day of the Enterobacteriaceae BSI. Patients experiencing BSI caused by Enterobacteriaceae on the day of admission were therefore excluded from the *S. aureus* analysis. The inverse applies for the Enterobacteriaceae analysis.

TABLE 2B

Characteristics of patients in *Staphylococcus aureus* and Enterobacteriaceae analyses, stratified by exposure to bloodstream infection in 10 European hospitals, 2010–2011 (n = 606,649^a)

Characteristic	Staphylococcus aureus analysis						Enterobacteriaceae analysis					
	MRSA BSI		MSSA BSI		Non-infected		3GCRE BSI		3GCSE BSI		Non-infected	
	n = 163		n = 885		n = 604,797		n = 360		n = 2,100		n = 603,972	
	n	%	n	%	n	%	n	%	n	%	n	%
Circulatory system	35	21.5	201	22.7	80,361	13.3	53	14.7	221	10.5	80,324	13.3
Respiratory system	5	3.1	34	3.8	33,426	5.5	21	5.8	88	4.2	33,377	5.5
Digestive system	13	8.0	51	5.8	44,350	7.3	45	12.5	322	15.3	44,175	7.3
Skin and subcutaneous tissue	3	1.8	29	3.3	9,597	1.6	1	0.3	18	0.9	9,607	1.6
Musculoskeletal system and connective tissue	14	8.6	91	10.3	33,452	5.5	7	1.9	21	1.0	33,513	5.5
Genitourinary system	9	5.5	28	3.2	30,257	5.0	37	10.3	365	17.4	30,107	5.0
Pregnancy, childbirth and the puerperium	0	0.0	8	0.9	54,785	9.1	3	0.8	42	2.0	54,758	9.1
Certain conditions originating in the perinatal period	2	1.2	6	0.7	18,641	3.1	2	0.6	28	1.3	18,624	3.1
Congenital malformations, deformations and chromosomal abnormalities	2	1.2	7	0.8	12,357	2.0	1	0.3	16	0.8	12,350	2.0
Symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified	3	1.8	33	3.7	23,985	4.0	9	2.5	80	3.8	23,963	4.0
Injury, poisoning and certain other consequences of external causes	34	20.9	127	14.4	79,622	13.2	63	17.5	169	8.0	79,565	13.2
External causes of morbidity and mortality	0	0.0	3	0.3	85	0.0	0	0.0	1	0.0	87	0.0
Factors influencing health status and contact with health services	1	0.6	2	0.2	47,551	7.9	4	1.1	15	0.7	47,536	7.9
Epidemiological classification of BSI												
Hospital onset	101	62.0	434	49.0	NA		214	59.4	780	37.1	NA	
Community onset	62	38.0	451	51.0	NA		146	40.6	1,320	62.9	NA	
Interventions prior to BSI												
Surgical procedure	39	23.9	243	27.5	246,485	40.8	112	31.1	462	22.0	246,180	40.8
ICU admission	52	31.9	258	29.2	43,307	7.2	112	31.1	434	20.7	43,068	7.1

BSI: bloodstream infection; COPD: chronic obstructive pulmonary disease; HIV: human immunodeficiency virus; ICU: intensive care unit; IQR: interquartile range; MRSA/MSSA: methicillin-resistant/susceptible *Staphylococcus aureus*; NA: not applicable; 3GCRE/3GCSE: third-generation cephalosporin-resistant/susceptible Enterobacteriaceae.

^a Patients experiencing BSI caused by Enterobacteriaceae were censored from the *S. aureus* analysis on the day of the Enterobacteriaceae BSI. Patients experiencing BSI caused by Enterobacteriaceae on the day of admission were therefore excluded from the *S. aureus* analysis. The inverse applies for the Enterobacteriaceae analysis.

patients were discharged alive. Of the remaining cohort, 10,419 (1.7%) died and 8,112 (1.3%) remained in hospital at the end of the study period (and underwent administrative censoring). Baseline characteristics are presented in Table 2.

BSI incidence

Of the 1,048 admissions during which *S. aureus* BSI were detected, 885 (84%) and 163 (16%) were due to MSSA and MRSA, respectively. The incidence density of *S. aureus* BSI was 0.269 episodes per 1,000 patient-days at risk: 0.227 and 0.042 episodes per 1,000 patient-days at risk for MSSA and MRSA BSI, respectively.

Of the 2,460 admissions during which Enterobacteriaceae BSI were detected, 2,100 (85%) and 360 (15%) were due to 3GCSE and 3GCRE, respectively. The incidence density of BSI due to Enterobacteriaceae was 0.631 episodes per 1,000 patient-days at risk: 0.538 and 0.092 episodes per 1,000 patient-days at risk for 3GCSE and 3GCRE BSI, respectively.

Hospital mortality and discharge alive

In the *S. aureus* analysis, 149 (16.8%) and 36 (22.1%) patients with MSSA and MRSA BSI died in hospital, respectively, compared with 10,161 (1.7%) non-infected patients. In the Enterobacteriaceae analysis, 212 (10.1%) and 58 (16.1%) patients with 3GCSE and 3GCRE died in hospital, respectively, compared with 10,105 (1.7%) non-infected patients.

TABLE 3

Results of proportional hazards models for hospital mortality and discharge alive, 10 European hospitals, 2010–2011 (n = 606,649)

Comparison	Exposure	Population	Mortality HR (95% CI)			Discharge alive HR (95% CI)		
			Model 1	Model 2	Model 3	Model 1	Model 2	Model 3
MSSA BSI vs non-infected	MSSA BSI	Hospitalised patients	2.58 (2.19–3.04)	2.41 (2.05–2.84)	1.81 (1.49–2.20)	0.34 (0.31–0.37)	0.38 (0.35–0.41)	0.54 (0.50–0.60)
MRSA BSI vs non-infected	MRSA BSI	Hospitalised patients	3.18 (2.29–4.42)	2.61 (1.88–3.63)	2.42 (1.66–3.51)	0.25 (0.20–0.32)	0.30 (0.24–0.38)	0.45 (0.36–0.58)
MRSA BSI vs MSSA BSI	Meticillin resistance	Patients with <i>S. aureus</i> BSI	1.19 (0.81–1.75)	1.20 (0.82–1.76)	1.26 (0.82–1.94)	0.74 (0.58–0.94)	0.73 (0.57, 0.94)	0.80 (0.61, 1.05)
3GCSE BSI vs non-infected	3GCSE BSI	Hospitalised patients	2.25 (1.96–2.58)	1.74 (1.51–1.99)	1.16 (0.98–1.36)	0.52 (0.49–0.54)	0.61 (0.58–0.64)	0.80 (0.75–0.84)
3GCRE BSI vs non-infected	3GCRE BSI	Hospitalised patients	2.88 (2.22–3.74)	2.25 (1.73–2.92)	1.80 (1.34–2.42)	0.37 (0.32–0.43)	0.43 (0.38–0.50)	0.57 (0.49–0.67)
3GCRE BSI vs 3GCSE BSI	3GC resistance	Patients with Enterobacteriaceae BSI	1.39 (1.02–1.90)	1.43 (1.05–1.96)	1.63 (1.13–2.35)	0.63 (0.55–0.73)	0.65 (0.56–0.75)	0.68 (0.57–0.81)

BSI: bloodstream infection; CI: confidence interval; HR: hazard ratio; MRSA/MSSA: methicillin-resistant/susceptible *Staphylococcus aureus*; 3GC: third-generation cephalosporins; 3GCRE/3GCSE: third-generation cephalosporin-resistant/susceptible Enterobacteriaceae.

Model 1: Susceptible and resistant BSI as time-dependent covariates (univariable analysis).

Model 2: As model 1 plus adjustment for age, sex, emergent/elective admission, nights hospitalised in the previous 12 months and comorbidities.

Model 3: As model 2 plus admission to intensive care and surgical procedures as time-dependent covariates.

Results from the Cox proportional hazards analyses for death and discharge alive should be interpreted together (Table 3) [19].

When adjusted for potential confounders, all BSI except 3GCSE significantly increased the hazard of hospital death compared with non-infected patients. This effect was greater for BSI due to *S. aureus* than BSI due to Enterobacteriaceae. Moreover, all BSI strongly reduced the hazard of discharge alive after adjustment for confounders, meaning that patients with BSI stayed longer in hospital (discharge alive HR less than 1) and were exposed to an increased daily risk of death throughout this prolonged period (mortality HR greater than 1).

Among patients with BSI due to Enterobacteriaceae, third-generation cephalosporin resistance significantly increased the hazard of death compared with third-generation cephalosporin susceptibility (adjusted hazard ratio (aHR): 1.63; 95%CI: 1.13–2.35). In contrast, the trend for methicillin resistance to increase hazard of death amongst patients with *S. aureus* BSI did not reach statistical significance (aHR: 1.26; 95%CI: 0.82–1.94). Similarly, while third-generation cephalosporin resistance significantly decreased the hazard of discharge alive among patients with BSI due to Enterobacteriaceae, methicillin resistance showed only a trend in this direction among patients with BSI due to *S. aureus*.

Excess length of stay

Table 4 presents the impact of BSI on the combined end-of-stay endpoint (end-LOS HR) and excess LOS (in days) when compared with patients without BSI.

All BSI reduced the daily all-cause hazard of discharge or death, i.e. led to prolonged hospital stay. This prolonging effect was greater for BSI due to *S. aureus* than for BSI due to Enterobacteriaceae, regardless of antimicrobial susceptibility status. For all types of BSI, diagnosis early during admission was associated with the greatest difference in LOS (Figure 2).

Table 4 also presents the end-LOS HR and excess LOS for BSI caused by resistant versus susceptible pathogens. While third-generation cephalosporin resistance significantly prolonged LOS amongst patients with BSI due to Enterobacteriaceae, methicillin resistance did not for the cohort of patients with *S. aureus* BSI.

The adjusted excess LOS estimate (Model 2) was taken from the model intercept, and should therefore be interpreted as the excess LOS caused by infection in a female patient with age equal to the mean age in the cohort, who has no comorbidities, has not been in hospital for the previous year, and was admitted electively. Increasing age, emergency admission, male sex, and all comorbidities except myocardial infarction decreased the excess length of stay associated with all four BSI types.

TABLE 4

Results of proportional hazards analysis for all-cause end-length of stay and excess length of stay estimates from multistate models, 10 European hospitals, 2010–2011 (n = 606,649)

Comparison	Exposure	Population	All-cause end-LOS HR (95% CI)			Excess LOS days (95% CI)	
			Model 1	Model 2	Model 3	Model 1	Model 2
MSSA BSI vs non-infected	MSSA BSI	Hospitalised patients	0.42 (0.39–0.45)	0.46 (0.43–0.49)	0.64 (0.59–0.69)	10.35 (9.44–11.26)	11.54 (11.45–11.63)
MRSA BSI vs non-infected	MRSA BSI	Hospitalised patients	0.36 (0.30–0.44)	0.42 (0.35–0.51)	0.61 (0.50–0.75)	12.22 (9.89–14.55)	13.33 (13.23–13.42)
MRSA BSI vs MSSA BSI	Meticillin resistance	Patients with <i>S. aureus</i> BSI	0.84 (0.68–1.03)	0.83 (0.67–1.02)	0.89 (0.71–1.12)	1.77 (-0.51–4.05)	2.54 (-3.19–8.27)
3GCSE BSI vs non-infected	3GCSE BSI	Hospitalised patients	0.57 (0.54–0.60)	0.66 (0.63–0.69)	0.84 (0.80–0.89)	4.36 (3.91–4.81)	5.87 (5.82–5.93)
3GCRE BSI vs non-infected	3GCRE BSI	Hospitalised patients	0.46 (0.41–0.52)	0.53 (0.47–0.60)	0.69 (0.60–0.79)	7.91 (6.66–9.16)	9.28 (9.20–9.35)
3GCRE BSI vs 3GCSE BSI	3GC resistance	Patients with Enterobacteriaceae BSI	0.72 (0.63–0.82)	0.73 (0.64–0.83)	0.78 (0.67–0.90)	3.53 (2.08–4.98)	4.89 (1.11–8.68)

BSI: bloodstream infection; CI: confidence interval; LOS: length of stay; MRSA/MSSA: methicillin-resistant/susceptible *Staphylococcus aureus*; 3GC: third-generation cephalosporins; 3GCRE/3GCSE: third-generation cephalosporin-resistant/susceptible Enterobacteriaceae.

Model 1: Susceptible and resistant BSI as time-dependent covariates (univariable analysis).

Model 2: As model 1 plus adjustment for age, sex, emergent/elective admission, nights hospitalised in the previous 12 months and comorbidities.

Model 3: As model 2 plus admission to intensive care and surgical procedures as time-dependent covariates.

Cost

The cost, from a hospital perspective, of each BSI and its annual cumulative incidence is presented in Table 5. While 3GCSE BSI was associated with the lowest per-infection cost (EUR 320; 95% credible interval (CrI): 80–1,300; or EUR 4,000; 95% CrI 2,400–6,700, using economic and accounting valuations, respectively), their relative frequency resulted in equal highest annual cost with MSSA (EUR 77,000; 95% CrI: 19,000–300,000; or EUR 970,000; 95% CrI: 590,000–1,600,000, using economic and accounting valuations, respectively).

Discussion

Per infection, *S. aureus* BSI had a greater effect on mortality, LOS and cost than BSI due to Enterobacteriaceae. Methicillin resistance, however, did not significantly increase the hazard of death or further prolong the excess LOS amongst patients with *S. aureus* BSI. This contrasts with BSI due to Enterobacteriaceae, where third-generation-cephalosporin-resistance increased both the hazard of mortality and excess LOS compared with susceptible strains. Furthermore, the annual cost, from a hospital perspective, of BSI due to Enterobacteriaceae was equivalent to the cost of *S. aureus* BSI because the higher incidence of the former balanced the greater per-infection impact of the latter.

This study incorporated several novel methodological approaches to the recently described challenges when estimating the impact of AMR [5]. Multistate modelling is an extension of survival analysis that permits explicit modelling of time-varying exposures and competing outcomes [4], but previous applications to hospital

epidemiology have not addressed confounding. We employed the flexible pseudo-observation regression technique to adjust these estimates for time-invariant potential confounders [14]. We also formally computed the excess LOS due to infections caused by non-susceptible compared with susceptible pathogens [20] rather than heuristically extrapolating this as the difference between excess LOS associated with each infection type compared with non-infected controls. Inclusion of the entire cohort of acute inpatients from 10 hospitals over two years facilitated precise estimates and avoided selection bias at patient-level, a risk when using matched cohorts.

We used a previously reported economic valuation of the opportunity cost of hospital bed-days to translate excess LOS to cost of BSI from the hospital perspective [17], employing a Monte Carlo simulation to preserve uncertainty in this estimation. Substantially higher cost estimates were produced using an accounting bed-day value in order to demonstrate the importance of the costing approach used. Accounting values are readily obtained but only show what has historically been spent on a bed-day. As the majority of hospital costs are fixed, this figure does not represent an amount that could be recouped should the infection be avoided. We contend that economic values, based on the opportunity cost of occupied bed-days, are appropriate for making decisions from the hospital perspective about future resource allocation for infection control programmes [21]. The lower cost of BSI, and also of AMR, obtained using the economic valuation provides insight into the financial challenges faced by hospital leadership when considering such

TABLE 5

Monte Carlo simulation results using economic and accounting bed-day values to estimate the cost of bloodstream infections, 10 European hospitals, 2010–2011 (n = 606,649)

Exposure	Population	Excess LOS per BSI days (95% CrI) ^a	Expected annual cumulative incidence per hospital ^b	Estimated cost per infection EUR (95% CrI)		Estimated cost per hospital-year EUR 1,000 (95% CrI)	
				Economic costing ^c	Accounting costing ^d	Economic costing ^c	Accounting costing ^d
MSSA BSI	Hospitalised patients	10.3 (9.3–11.5)	102	760 (190–3,000)	9,500 (5,800–16,000)	77 (19–300)	970 (590–1,600)
MRSA BSI	Hospitalised patients	12.2 (9.9–14.7)	19	890 (220–3,600)	11,000 (6,600–19,000)	17 (4.1–67)	210 (130–360)
Meticillin resistance	Patients with <i>S. aureus</i> BSI	1.9 (–0.7 TO 4.6)	NA	120 (–60 TO 740)	1,600 (–700 TO 5,000)	NA	NA
3GCSE BSI	Hospitalised patients	4.4 (3.9–4.9)	242	320 (80–1,300)	4,000 (2,400–6,700)	77 (19–300)	970 (590–1,600)
3GCRE BSI	Hospitalised patients	7.9 (6.6–9.4)	41	560 (140–2,300)	7,300 (4,300–12,000)	24 (5–94)	300 (180–510)
3GC resistance	Patients with Enterobacteriaceae BSI	3.5 (2.1–5.1)	NA	250 (60–1,100)	3,200 (1,600–6,000)	NA	NA

BSI: bloodstream infection; CrI: credible interval; LOS: length of stay; MRSA/MSSA: methicillin-resistant/susceptible *Staphylococcus aureus*; NA: not applicable; 3GC: third-generation cephalosporins; 3GCRE/3GCSE: third-generation cephalosporin-resistant/susceptible Enterobacteriaceae.

^a Output from probabilistic sensitivity analysis based on input distributions, reproduced to demonstrate consistency with estimates from the current study.

^b Estimated for a hospital with 450,000 bed-days annually (95% CrI not displayed because precision from the study cohort is such that no additional uncertainty is added to the model).

^c Employs the bed-day valuation derived from a contingent valuation survey that estimated the opportunity cost of each bed-day consumed by a patient with BSI.

^d Employs the bed-day valuation computed by dividing total hospital budget for one year by the number of bed-days supplied during the same period. Refer to [8] for further details. All costs are displayed at two significant figures.

interventions under existing funding arrangements. While we used the unadjusted excess LOS for this estimation to best reflect the patient mix in our cohort, the adjusted results and covariate coefficients could be used to transfer our excess LOS estimate to settings with different patient mix.

A recent WHO systematic review of the published scientific literature on the health and economic impact of AMR concluded that the quality of evidence on hospital LOS and mortality was ‘very low’ in most cases [1]. It also identified a paucity of studies comparing hospital costs incurred by infection with resistant versus susceptible isolates of *E. coli* and *K. pneumoniae*. Our results are consistent with this review’s finding that third-generation cephalosporin resistance is associated with increased risk of mortality among patients infected with *E. coli* or *K. pneumoniae*. We found that third-generation cephalosporin resistance increased the hospital LOS associated with BSI caused by Enterobacteriaceae, while previous reports were ‘inconsistent’ for *K. pneumoniae* BSI and found no excess LOS for *E. coli* BSI. Our results do not support the review’s finding that infection with MRSA is associated with increased mortality and LOS compared with MSSA. Potential explanations include more appropriate empiric antibiotic therapy during our study compared with older studies and inflated estimation of

excess LOS in previous studies due to time-dependent bias [5,6]. In addition, daily risk (or hazard) of death, as estimated here, can be expected to be smaller than the cumulative risks reported in the review. Although seemingly in contrast to older literature, our findings are consistent with another recent, large European multicentre study that found that methicillin resistance had no significant impact on mortality (adjusted hazard ratio (aHR), 1.1; 95% CI: 0.7–1.8) or excess LOS (0.6 days; 95% CI: –3.7 to 5.3), whereas third-generation cephalosporin resistance increased both risk of death (aHR: 2.9; 95% CI: 1.2–6.9) and excess LOS (5.0 days; 95% CI: 0.4–10.2) [22,23]. A similarly modest impact of AMR has been reported in the European ICU setting [24].

These data should be interpreted within the context of the study design. The dataset was extracted retrospectively from existing databases. Concerns regarding the quality of ICD coding data have been well described [25], although the Charlson comorbidity index derived from administrative databases has elsewhere proven superior to chart review [26]. We relied on routine antimicrobial susceptibility results performed by local laboratories using guidelines from five different organisations. However, for MRSA and 3GCRE, there should not be a major misclassification bias. We were unable to detect community-onset healthcare-acquired infection,

however our primary results do not depend on this distinction. In addition, we could not include antibiotic exposure data. However, we consider delayed appropriate antimicrobial therapy to be on the causal pathway between antimicrobial resistance and the outcomes of interest [27], so exclusion of this information from our analysis is appropriate. We were unable to follow up patients post discharge, thus cannot report 30-day mortality or longer-term sepsis outcomes [28]. As with any observational study, we cannot exclude residual confounding. Our research question, however, is not amenable to an experimental study, and by accounting for time-dependent bias and important confounders, these results add to the existing literature. Finally, our study was designed to evaluate cost from the hospital perspective and addressed neither societal costs, macroeconomic indicators, nor the global health-economic implications of a post-antibiotic future [29,30].

This multicentre study, conducted in 10 European hospitals, could cautiously be extrapolated to large hospitals in other high-income settings, although the burden of BSI will clearly vary depending on incidence, treatment and hospital funding schemes. However, the current study did not address the lack of data in this field from low- and middle-income countries, where limited diagnostic and therapeutic resources, combined with lower proportion of gross domestic product available for healthcare, are likely to translate to a greater burden of disease.

Our data demonstrate the substantial health and economic burden imposed by BSI in European hospitals. Per infection, BSI caused by non-susceptible strains were associated with higher mortality risk and cost than susceptible strains. Given that BSI due to non-susceptible *S. aureus* and Enterobacteriaceae strains are likely to add to rather than replace those due to susceptible strains [31,32], the additional impact of AMR is substantial. However, the higher incidence of BSI due to susceptible strains means that these currently represent a greater health and economic burden than non-susceptible strains, emphasising the importance of surveillance and infection control policies that target *infections* rather than *resistance*.

TIMBER Study group members

In addition to the named authors of this work, the TIMBER study group includes Cristina Masuet-Aumatell, PhD, and Marta Banqué Navarro, PhD (Hospital Universitari de Bellvitge, L'Hospitalet de Llobregat, Spain), and Chiara Falcone, MS (Papa Giovanni XXIII Hospital, Bergamo, Italy).

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was an investigator-initiated project supported by an unrestricted research grant provided by Pfizer Europe. The sponsor had no role in study design, in the collection, analysis, and interpretation of data, in the writing of the report or in the decision to submit the paper for publication. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Conflict of interest

HS is supported by research grants from The German Center for Infection Research (DZIF), the European Union (MagicBullet, Grant Agreement 278232), Novartis and Pfizer, has received speaking fees from Astellas, AstraZeneca, Gilead, MSD, Novartis, Oxoid and Pfizer, and is an advisory Board Member or consultant to AstraZeneca, Basilea, Cubist, FAB-Pharma, Novartis, SOBI, The Medicines Company, Theravance, and ThermoFischer. S. Hagel reports having received lecture fees from Pfizer, MSD, and Astra Zeneca. S. Harbarth reports having received investigator-initiated research grants funded by Pfizer and B. Braun; he is also a member of the advisory boards of Destiny Pharma, bioMerieux, Novartis and DaVolterra. Other authors: no conflicts to declare.

Authors' contributions

AS, AA, JB, NG and SH designed the study. AS, RM, ET, GDA, CF, FP, XB, HGH, JE, OT, JM, MAB, AP, AZ, CM, DN, HS, NH, S Hagel and MP obtained the data. AS cleaned the data. AA and AS analysed the data. AS wrote the first draft. All authors revised the report and approved the final report. SH raised the funding. SH, NG and JB supervised the study.

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Prevalence and incidence of toxoplasmosis: a retrospective analysis of mother-child examinations, Styria, Austria, 1995 to 2012

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In Austria, mandatory screening for the prevention of congenital toxoplasmosis stipulates a serological test for antibodies against *Toxoplasma gondii* as early as possible in pregnancy. In the case of a seronegative result, subsequent tests at intervals of 8 weeks are requested. We analysed serological data from Styria, an Austrian federal state, to determine the seroprevalence and incidence of *Toxoplasma* infections. The study included 353,599 tests from 103,316 women during 158,571 pregnancies from 1995 to 2012. The age-adjusted seroprevalence decreased from 43.3% in 1995 to 31.5% in 2012, with a yearly decline of 0.84% (95% confidence interval (CI): 0.79–0.88). The intergravid incidence showed an annual decrease of 4.2%. The average yearly incidence of intragravid and intergravid seroconversions was 0.52% (95% CI 0.45–0.61) and 0.72% (95% CI 0.67–0.77), respectively. If the difference between these rates ($p < 0.001$) can be explained by the effect of primary prevention such as avoiding raw meat and taking hygiene precautions when encountering cats or preparing vegetables, only ca two of seven (28%) infections were avoided by hygiene measures taken by pregnant women. Primary prevention may therefore have its limits.

Introduction

Primary maternal infection with *Toxoplasma gondii* during pregnancy can lead to prenatal infection of the unborn child, and vertical diaplacental transmission of *Toxoplasma* can seriously damage the embryo. Some prenatally infected children, asymptomatic at birth, can develop retinochorioiditis and other sequelae months or years later [1]. Since 1975, Austria has run prenatal screening for the early detection and treatment of toxoplasmosis, with the first test for *T. gondii* as early as possible in pregnancy [2]. If antibodies against these parasites are detected, the sample is further tested

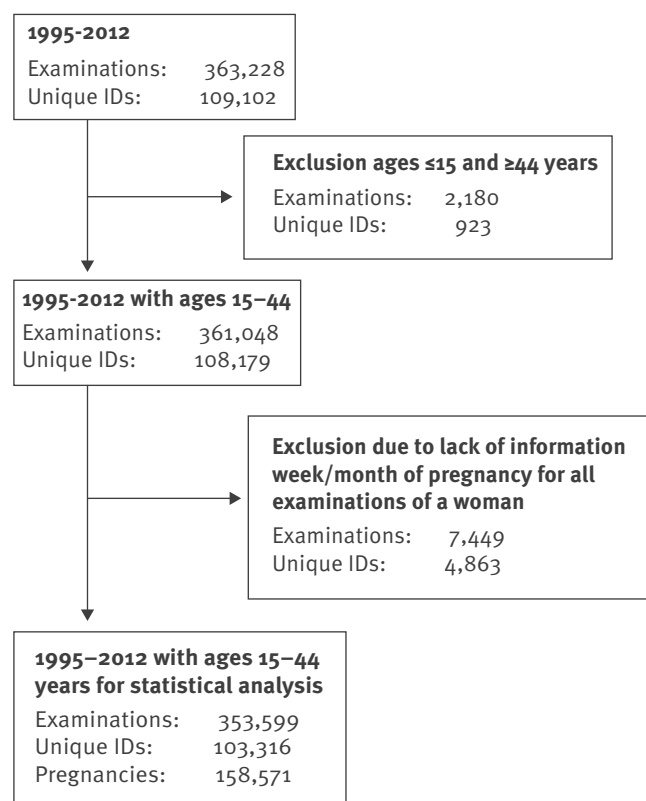
for specific IgM antibodies. A negative IgM report indicates a late latent infection that poses no threat for the current pregnancy. When a woman tests positive for IgM, the actual time of infection is determined as precisely as possible with special tests (avidity test, IgM immunosorbent agglutination assay, etc.). If there is still a suspicion of a primary infection in pregnancy, treatment according to the Austrian guideline is begun [2,3].

When the first test fails to show antibodies, the Austrian screening programme, which is part of the check-ups specified in the mother-child booklet (MCB), calls for further tests at 8-week intervals until the birth of the child. Development of specific antibodies to *T. gondii* in the further course of pregnancy is positive proof of a primary infection during pregnancy. Seroconversion is an indication for treatment. In recent years, a number of studies and meta-analyses have been undertaken to evaluate the effectiveness of antiparasitic treatment in pregnant women with *Toxoplasma* infections, but the results are inconclusive [4–6].

Evaluation of the screening programme for toxoplasmosis depends not only on the assessment of the effectiveness of treatment but also on a good understanding of the epidemiology of the disease. There are large variations in the seroprevalence and incidence of toxoplasmosis throughout the world. Countries and areas with low or very low incidence include the United States and northern European countries such as Norway, but also south-east Asia and the Sahel Zone [7]. In recent decades, there has been a clear decrease in the seroprevalence of latent infections, especially in industrialised countries [8]. A study in the United States of native-born inhabitants aged 12–49 years covering the years 2009–2010 produced an age-standardised

FIGURE 1

Flowchart explaining the selection of data for the analysis of prevalence and incidence of toxoplasmosis, mother-child examinations, Styria, Austria, 1995–2012



Unique ID: each woman had a unique code assigned, so that examinations could be identified as pertaining to that particular woman.

seroprevalence of 6.7%, compared with 9% in 1999–2004 and 14.1% in 1988–1994 [9]. Factors that influence the probability of a human infection with *T. gondii* include climatic conditions in the region or country, nutritional habits of the inhabitants, the degree of development and the infection rates of the local cat population. Cats as definite hosts of *T. gondii* are able to shed oocysts through faeces. A moderate seroprevalence of 30–50% of persons with a latent infection is assumed in middle and southern Europe [7]. In Austria, a local study covering 2000–2007 showed a moderate seroprevalence of 31% in pregnant women [10]. In France, the average seroprevalence of latent infections among pregnant women was calculated as 54% in 1995 and decreased to 44% in 2003 [11]. Seroprevalence is highest in the moist tropical countries of South America and in tropical Africa.

There are few longitudinal cohort studies on the epidemiology of *Toxoplasma* infections. In an area with an implemented screening programme and centralised laboratory diagnostics, as is the case in two of the federal states in Austria, large-scale data analysis is possible. Styria, one of the nine federal states in Austria, has a population of 1.2 million. In Styria, *Toxoplasma*

tests for pregnant women are usually processed in a central facility, the MCB service of the Styrian Health Insurance (Steiermärkische Gebietskrankenkasse or Stmk. GKK), where they record ca 9,000 pregnancies per year, representing 80–90% of all births in Styria.

The aim of this study is to determine the development of seroprevalence of latent *Toxoplasma* infections in pregnant women in Austria, a central European country, with direct calculation of the incidence of seroconversion during and between pregnancies in the period 1995–2012. It is assumed that differences between intragravid and intergravid seroconversion rates are due to the effects of primary prevention, such as avoiding raw meat and taking hygiene precautions when dealing with cats and vegetables. Since reliable data on adherence to the check-up schedule in the MCB are important for the evaluation of the screening programme, the number of seronegative women who had at least two follow-up tests will be determined to detect any changes that occurred over the years.

Methods

Our retrospective cohort study was approved by the Institutional Review Board of the Medical University of Graz; Austria (EK Nr.: 26–031 ex 13/14).

The analysis was based on results of *Toxoplasma* tests done by the MCB service in the period from 1 January 1995 to 31 December 2012 that were exported from Stmk. GKK's laboratory data system. Identifying fields that fell under data-privacy laws were pseudonymised. The dataset was rigorously tested for consistency and plausibility.

Antibody screening

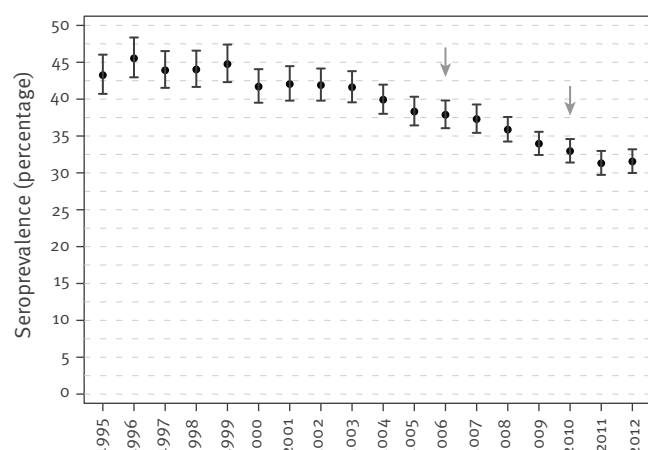
During the period covered by the study, three different test systems were used to screen for antibodies against *T. gondii*: from 1 January 1995 to 18 June 2006, the MCB service used slides coated with *T. gondii* for indirect immunofluorescence test (IIFT). From 19 June 2006 to 5 December 2010, the determinations were made with the automated Vidia Toxo IgG System from bioMérieux. Since 6 December 2010 *Toxoplasma* IgG has been determined automatically using the Architect System from Abbott Diagnostics. *Toxoplasma* IgM test to confirm seroconversion was determined with the following systems: from 1 January 1995 to 18 June 2006, the automated Vidas Toxo IgM System from bioMérieux; from 19 June 2006 to 5 December 2010, the automated Vidia IgM assay from bioMérieux; and since 6 December 2010, the automated Architect System IgM assay from Abbott Diagnostics.

Defining pregnancies

The dataset included information on week or month of gestation when examination took place. Since there was a unique ID for each woman, examinations could be identified as pertaining to a single woman. For 80% (82,446/103,316) of women and their examinations, complete and coherent information on gestational age

FIGURE 2

Annual age-adjusted seroprevalence of *Toxoplasma gondii* infections in pregnant women aged 15–44 years, Styria, Austria, 1995–2012 (n=158,571)



Age adjustment was done using direct standardisation with the 1995 female census population of Styria as the reference population. Arrows indicate the change from IIFT to Vidia (2006) and from Vidia to Architect (2010).

was available. Sometimes information on gestational age was not provided for each examination but from the time course (date of examinations) it was obvious that they pertained to one particular pregnancy (16% of women and their examinations). If there was no information for several examinations in sequence or contradictory information on gestational age, we classified them as belonging to the same pregnancy when they took place within a time window of 300 days. Women with no information at all about week or month of gestation for all their examinations were excluded from the dataset.

Statistical analysis

The first examinations per pregnancy for a woman were used to determine the seroprevalence of latent toxoplasmosis aged between 15 and 44 years. Positive and borderline results were considered evidence of a past infection. A random-sample follow-up study at a Competence Centre for Toxoplasmosis (Vienna General Hospital) comparing sera with borderline test results and the gold standard, the Sabin-Feldmann dye test, showed that of 30 borderline sera, only one showed no antibodies. Although none of the older results could be rechecked, it seemed acceptable to also classify them as antibody positive [7]. The proportions of latent infections calculated in this way are shown both as yearly age-corrected seroprevalence and per age group and year. Additionally, a separate analysis was made for the time periods when the IIFT assay and ELISA assays were used.

The yearly incidence of new infections was calculated in three ways. For better comparability, the incidence, when not stated otherwise, always refers to

seronegative women, i.e. the population of women at risk for infection, and not all women in the dataset.

(i) A frequently used procedure for estimating the incidence is based on the calculation of the increase in seroprevalence per year of life [7,12].

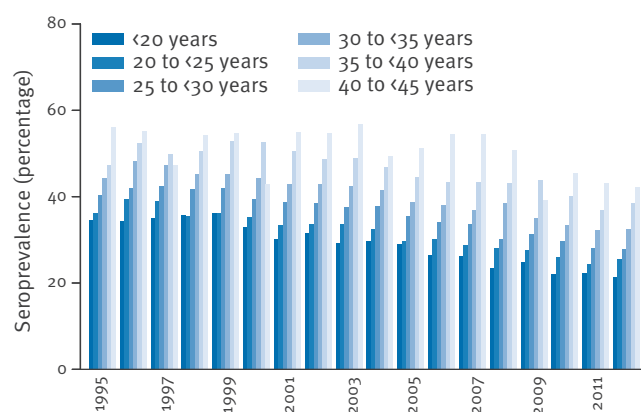
(ii) Seroconversions in pregnancies. These were women who did not have antibodies against *T. gondii* at the MCB check-up at the start of the pregnancy but at a later examination in the same pregnancy. Only those cases with a first negative examination after 1 January 1995 were considered. It is generally accepted that new *Toxoplasma* infections are associated with specific IgM antibodies [13], so that an intragravid seroconversion requires the presence of specific IgM antibodies. These intragravid seroconversions were related to the total risk period that was monitored on the basis of all MCB examinations at the MCB service within a pregnancy.

(iii) Infections that developed between pregnancies. Due to the extensive data available, it is possible to identify new infections (here as intergravid seroconversions as distinct from intragravid seroconversions, see above) between pregnancies. We analysed data from women who had two or more pregnancies and whose last examination in her first MCB-documented pregnancy failed to show antibodies to *Toxoplasma*. The woman's entire dataset was examined for later *Toxoplasma* tests during any subsequent pregnancy. If the first test of a pregnancy showed antibodies and the last test from the previous pregnancy did not, then the infection must have occurred in the period between these two tests. The time point of these infections was defined using the following sampling procedure with 10,000 replications: for each woman with an intergravid seroconversion, we uniformly sampled the date of seroconversion from the time period between the two tests. The total risk time between all test pairs (neg-neg, neg-pos) formed the basis for the incidence calculation. The median number of intergravid seroconversions per year over all replications were used to calculate the incidence for 3-year intervals and for the total incidence over all 18 years.

For seroprevalence and incidence, 95% confidence intervals (95% CI) were calculated based on the exact method under a binomial distribution. Age adjustment was done using direct standardisation with the 1995 female census population of Styria as reference population and using 5-year age groups from 15 to 44 years. Changes in seroprevalence over time were analysed with a logistic regression model. To estimate the incidence based on the increase of seroprevalence per year of age, a binomial regression model with identity link was used with the implicit assumption that force of infection did not change over the years. Poisson regression models were applied to estimate changes in incidence (based on intragravid and intergravid seroconversion) over time. Poisson rates were compared with an exact test. A p-value < 0.05 was considered

FIGURE 3

Yearly seroprevalences of pregnant women aged 15–44 years with antibodies to *Toxoplasma gondii*, by age groups, Styria, Austria, 1995–2012 (n=158,571)



The first test per pregnancy is considered.

statistically significant. All analyses were performed using the R statistical software (version 3.2.2) [14].

Results

For the study period of 1995–2012, there were 363,228 screening tests. After application of the inclusion and exclusion criteria, 353,599 screening tests were analysed from 103,316 women and their 158,571 pregnancies (Figure 1). The median age in all pregnancies (first examination) was 27.2 years (interquartile range (IQR) 23.9–30.7) in 1995 and 29.8 years (IQR 25.9–33.5) in 2012.

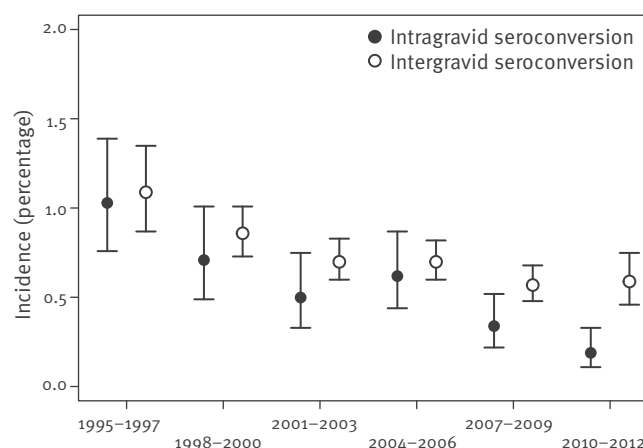
Seroprevalence of *Toxoplasma* infections in pregnant women

The annual age-adjusted seroprevalence is shown in Figure 2.

In 1995, 43.3% (95% CI: 40.7–46.0) of the pregnant women showed antibodies against *T. gondii* at the first examination per pregnancy; in 2012 the seroprevalence was 31.5% (95% CI: 30.0–33.2). In the 18 years studied, the seroprevalence of women with latent infections on average decreased by 0.84% yearly (95% CI: 0.79–0.88, $p < 0.001$). Furthermore, we made a separate analysis of the time periods for the IIFT assay and ELISA assays (Vidia and Architect). In the time period 1995–2006, there was a decline in seroprevalence of 0.56% (95% CI: 0.46–0.66, $p < 0.001$) yearly, while from 2006 to 2012, there was a 1.20% annual decline (95% CI: 1.00–1.40, $p < 0.001$). Figure 3 shows the yearly seroprevalence according to age groups of pregnant women who showed antibodies to *T. gondii* at their first examination in pregnancy. Seroprevalence is shown to increase with age ($p < 0.001$, linear test for trend).

FIGURE 4

Toxoplasma gondii infections: incidence of intra- and intergravid seroconversions in women aged 15–44 years at three-year intervals, Styria, Austria, 1995–2012



The incidence (%) of intra- and intergravid seroconversions with the corresponding 95% confidence intervals.

Incidence of new infections

Calculation of the age-dependent increase in seroprevalence during the 18-year study period showed an average estimated incidence of 0.85% new infections per year (95% CI: 0.81–0.89).

From 1995 to 2012, 167 intra-gravid seroconversions were registered. There was complete agreement between the cases extracted from the database and the MCB service's internal documentation. The observation period was 31,940 person-years, for an average yearly incidence of 0.52% (95% CI 0.45–0.61) for seronegative women, see Table 1 and Figure 4.

The general infection risk for a pregnant woman can be determined if the probability of infection is assumed to be the same throughout pregnancy. With an average seroprevalence for the eighteen years of latent infections of 39.0% (95% CI: 38.5–39.4) and duration of pregnancy of 0.75 years, a woman's risk, regardless of toxoplasmosis status, of acquiring a new infection with *T. gondii* is 0.24% per pregnancy (range: 0.20–0.28). This means that a new infection with *T. gondii* can be expected in one in 409 pregnancies.

Among 42,816 women studied for possible changes in *Toxoplasma* status between pregnancies, i.e. having two or more pregnancies, there were 732 cases of intergravid seroconversion (Table 1). The average annual total incidence was 0.72% (95% CI: 0.67–0.77) in seronegative women. The Poisson regression gave an average yearly reduction of 4.2% (95% CI: 2.5–5.8) of intergravid seroconversions during the period 1995–2012.

Comparison of the two incidences gave a significant difference between intergravid seroconversions (0.72%)

TABLE 1

Toxoplasma gondii infections: incidence of intra- and intergravid seroconversions for in women aged 15–44 years at three-year intervals, Styria, Austria, 1995–2012

Year	Intragravid seroconversion		Intergravid seroconversion	
	Incidence (%)	95% CI	Incidence (%)	95% CI
1995–1997	1.03	0.76–1.39	1.09	0.87–1.35
1998–2000	0.71	0.49–1.01	0.86	0.73–1.01
2001–2003	0.50	0.33–0.75	0.70	0.60–0.83
2004–2006	0.62	0.44–0.87	0.70	0.60–0.82
2007–2009	0.34	0.22–0.52	0.57	0.48–0.68
2010–2012	0.19	0.11–0.33	0.59	0.46–0.75
Total	0.52	0.45–0.61	0.72	0.67–0.77

CI: confidence interval.

and intragravid seroconversions (0.52%) ($p < 0.001$; comparison of Poisson rates).

Primarily seronegative pregnant women who presented for at least three examinations

Table 2 comprises the percentage of women attending the MCB service at Stmk. GKK who underwent at least two follow-up tests due to an initial negative test (i.e. at least three tests during a pregnancy). The classification by year is based on the date of the first examination during a pregnancy).

Discussion

Conforming to a trend in other industrialised countries, the proportion of women in the state of Styria, Austria, who already have a latent infection with *T. gondii* before pregnancy has been decreasing. In 1995, 43.3% (95% CI: 40.7–46.0) of pregnant women showed antibodies to *T. gondii* at the first examination per pregnancy; in 2012 the figure was 31.5% (95% CI: 30.0–33.2). In the 18-year study period, the seroprevalence of latent infections in pregnant women decreased by 0.84% (95% CI: 0.79–0.88) annually. When screening began in 1975, about half of pregnant women showed antibodies against *T. gondii* [15]. When the total seroprevalence of latent infections does not change over the years, the incidence of new infections can be estimated from the age-dependent increase in seroprevalence. Under the false assumption that seroprevalence stayed constant, an average new infection rate of 0.85% per year was calculated for the 18-year study period. In a paper published in 1994, Larsen and Lebech drew attention to the fact that with decreasing seropositive rates, the incidence calculation based on the age-dependent increase led to overestimation of the annual *Toxoplasma* infection rate [16]. If hypothetical human infections with *T. gondii* were to completely stop at a certain point in time, the age-dependent increase in seroprevalence would persist for many years. In this

hypothetical model the persisting increase would lead us mistakenly to suppose a still existing infection-rate.

The average yearly incidence of *Toxoplasma* infections in the 18-year study period in pregnancies (intragravid seroconversions) and between two pregnancies (intergravid seroconversions) was 0.52% (95% CI: 0.45–0.61) and 0.72% (95% CI: 0.67–0.77) respectively per year for seronegative women. There was an average decrease in annual intergravid incidence of 4.2% over the years of the study. There was a similar decrease in both the incidence during pregnancy and between pregnancies. At the end of the period of 2010–2012, the intergravid incidence was 0.59% (95% CI: 0.46–0.75). Therefore it is to be expected that the seroprevalence of latent *Toxoplasma* infections will continue to show a clear decrease. That means, however, that the number of women of childbearing age who are at risk of infection will increase. The net effect is that although primary infections in pregnancy are decreasing, prenatal toxoplasmosis will continue to pose a substantial threat to pregnant women and their children.

The main source of human *Toxoplasma* infections is seen to be consumption of undercooked meat and meat products [17]. Modern technology in meat processing may have reduced the degree of contamination, and hygiene measures such as strict rodent control and cats in livestock barns help to reduce infection pressure. Deep-freezing meat for a number of days also helps to kill *T. gondii* [7]. For some years, however, there has been a trend towards organic and free-range farming, and in such operations, there is a higher seroprevalence of infection [18].

House cats are another important link in the infection chain. A shift towards sterilised pet food products from supermarkets and the increasing tendency to keep these pets indoors may mean that there are fewer cats excreting oocysts.

The difference in the total incidences during and between pregnancies can be traced to the effect of primary prevention. Primary prevention includes no consumption of raw meat, avoiding contact with cats and strict hygiene when coming into contact with vegetables and garden soil. In many health systems, such as those in England and Wales and the United States [7,19], primary prevention is the only measure in place to prevent congenital toxoplasmosis. For methodological reasons, there is at present no clear evidence of general effectiveness and relevance of primary prevention [20].

The degree of effectiveness of primary prevention reflected by our study is disappointing. It mainly results from the different incidences (intragravid to intergravid) in the last 6 years of the study. The data indicate that only ca two out of seven infections were avoided by primary prevention. There is no general systematic primary prevention approach implemented

TABLE 2

Toxoplasma gondii infections: primarily seronegative pregnant women who presented for at least three examinations, Styria, Austria, 1996-2011

Year	Number	% of total
1996	2,235	31.0
1997	2,159	32.2
1998	2,114	32.6
1999	2,107	33.9
2000	2,229	35.5
2001	2,379	37.3
2002	2,525	41.2
2003	2,507	39.9
2004	2,649	41.5
2005	2,870	43.5
2006	3,220	54.4
2007	3,469	62.4
2008	4,046	60.0
2009	4,177	62.7
2010	4,332	64.8
2011	4,515	67.8

in Austria. Gynaecologists usually give advice about risk factors of *Toxoplasma* infection at the time of blood sampling for *Toxoplasma* tests. A desirable side effect of the extensive screening plan with repeated blood sampling should have been to make women of childbearing age aware of the dangers of primary infection. Although raw or undercooked meat (pork, beef, mutton, chicken, etc.) is seen as the main source of *Toxoplasma* infection, there are many other possible sources. Drinking water or ingestion of oocysts while swimming in natural waters could also lead to infection [21]. More recently, undercooked shellfish such as mussels and oysters have been considered infection sources [7,22]. Considering this multiplicity of possible sources of infection, primary prevention may have its limits [1].

Adherence to the MCB guidelines with respect to follow-up examinations for *Toxoplasma*-negative women has improved noticeably over the years. In 2011, some 67.8% of seronegative women had at least three tests in the MCB service. In another state (Upper Austria) in 2007, 35.5% of seronegative pregnant women had three or more serological toxoplasmosis tests [10]. In a Viennese study that ran from May 2001 to December 2002, the authors reported that after giving birth, only 22.5% of women at risk for infection showed evidence of three or more tests for toxoplasmosis in their MCBs [23].

Limitations

The problems with retrospective studies are well known. For this study, an extensive data collection covering a very long time period was available. Since

different assays have different sensitivities and specificities [24], comparison of results over a long time period is complex. Therefore we made a separate analysis of the time periods when the IIFT and ELISA assays (Vidia and Architect) were used (Figure 2). There was a decline in seroprevalence in each of these periods. The decrease in seroprevalence was also confirmed by the decreasing incidences of intragravid and intergravid seroconversions. This in turn indicates that our results are highly plausible.

When dealing with serological results, there are always constellations that are difficult to interpret. That was also true with the assignment of intergravid seroconversions. 662 (90%) of the 732 ultimately defined intergravid seroconversions were unequivocal, but 70 uncertain cases were considered as intergravid seroconversion on the basis of all available information. The authors believe that the number of intergravid seroconversions represents the upper limit.

Due to missing data the correct assignment of examinations to a pregnancy was in rare cases uncertain. Repeating the analysis using only cases with secure classification, the results remained stable (data not shown). Despite these limitations, we have, for the first time, been able to directly measure the seroconversions and incidences of *Toxoplasma* infections and their dynamics over time.

Conflict of interest

None declared.

Authors' contributions

Designed the study: CB, SAH. Prepared and analysed data: CB, SAH, HJ, AB. Interpreted the results: CB, HJ, Wrote the first draft: CB, SAH, AB. Revised the article: CB, SAH, HJ, AB. All authors read and approved the final manuscript.

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Estimating true hospital morbidity of complications associated with mumps outbreak, England, 2004/05

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Mumps outbreaks in highly vaccinated populations continue to be reported globally. Therefore, quantifying the burden of mumps morbidity accurately will be necessary to better assess the impact of mumps vaccination programmes. We aim to estimate the true morbidity resulting from mumps complications in terms of hospitalised orchitis, meningitis, oophoritis and pancreatitis in England during the outbreak in 2004/05. This outbreak in England led to a clear increase in hospitalisations coded to mumps for complications of orchitis in those born in the 1970s and 1980s and possibly for meningitis in those born in the 1980s. A simple statistical model, based on analysing time trends for diagnosed complications in hospital databases with routine laboratory surveillance data, found that the actual morbidity was much higher. There were 2.5 times (166 cases) more mumps orchitis cases in the 1970s cohort and 2.0 times (708 cases) more mumps orchitis cases in the 1980s cohort than complications coded to mumps in hospital databases. Our study demonstrated that the mumps outbreak in England 2004/05 resulted in a substantial increase in hospitalised mumps complications, and the model we used can improve the ascertainment of morbidity from a mumps outbreak.

Introduction

Mumps is an acute viral infection which can present with fever, headache and swelling of the parotid glands (unilateral or bilateral). It can be asymptomatic in around 30% of children [1]. Reported complications of mumps infection include orchitis, aseptic meningitis, oophoritis, pancreatitis, encephalitis and permanent unilateral deafness [1-3].

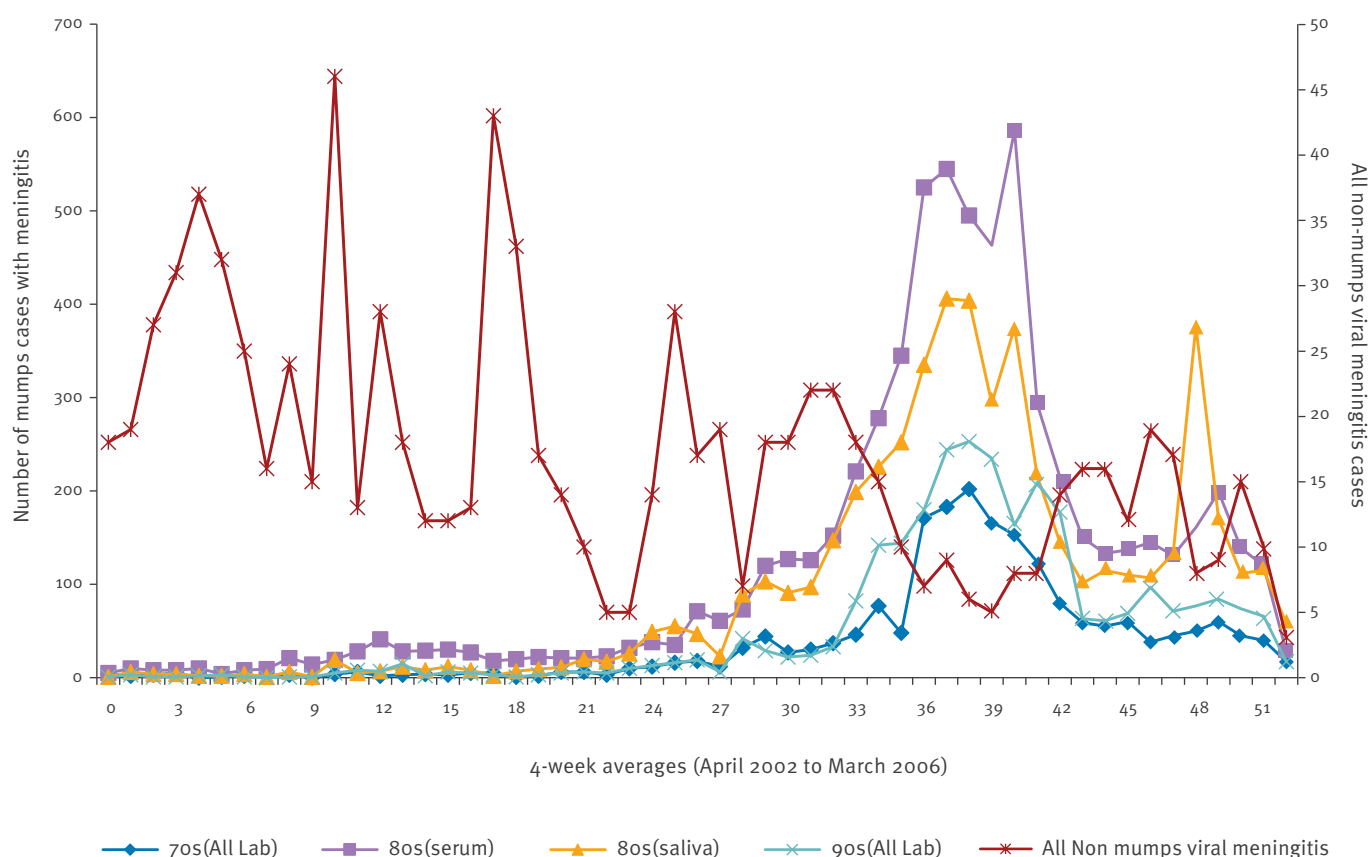
The measles, mumps, rubella (MMR) vaccine was introduced into the immunisation programme in the United Kingdom (UK) in October 1988 as a single dose for children aged 12 to 15 months. In 1996, to provide additional protection against all three infections, a second

dose was added to the schedule. In the first decade after MMR vaccine was introduced, rates of reported and confirmed mumps infection fell to extremely low levels in the UK (42 confirmed mumps cases in 1998). Since 1998, however, there have been a number of mumps outbreaks in adolescents culminating in a national epidemic in 2004 and 2005, affecting many universities and colleges across England and Wales. The age group mainly affected were those born before 1988, who had not been offered routine childhood MMR vaccination and who had avoided mumps exposure because of high coverage in younger children [4]. Although MMR coverage in two-year-old children was only ca 80%, the outbreak dynamics did not result in significant transmission in young children [5,6]. The introduction of a second MMR dose for pre-schoolers in 1996 could be a factor which may have contributed to this [4]. The number of laboratory-confirmed mumps cases rose from 500 in 2002 to 1,541 in 2003 to 8,129 in 2004 and 43,378 in 2005 [5].

Routine data on hospitalisations can provide information on complications of mumps if the diagnosis of mumps is obvious and coded correctly on discharge. Delayed or missed diagnoses have been shown to occur, particularly in the absence of a history of parotid swelling [7]. This may lead to an underestimation of complications attributable to mumps. As mumps outbreaks in highly vaccinated populations continue to be reported globally, quantifying the burden of mumps morbidity accurately will be necessary to better assess the impact of mumps vaccination programmes [7-16]. In this paper, we used regression analysis to assess the contribution of laboratory-confirmed mumps cases to the hospitalisations for orchitis, meningitis, oophoritis and pancreatitis in each birth cohort. Other common infections associated with meningitis were used as an additional parameter in the regression model for meningitis only. This enabled us to estimate the number of hospitalised orchitis, meningitis, oophoritis and

FIGURE 1

Four-weekly number of laboratory-confirmed mumps^a (n = 16,549) and non-mumps (n = 903) viral meningitis infections, England, April 2002–March 2006



^a Mumps infections stratified by birth cohort (and laboratory sample type for 80s cohort only).

pancreatitis attributable to the increase in mumps during the outbreak.

Therefore, the aim of the study was to estimate the true morbidity resulting from mumps complications in terms of hospitalised orchitis, meningitis, oophoritis and pancreatitis in England during the mumps outbreak in 2004/05.

Methods

Data source

Selection of birth cohort cases and study period

For the purpose of this study, data were retrieved from 1 April 2002 to 31 March 2006 from all data sources. As the mumps outbreak mainly involved young adults, those born between 1970 and 1999 were included and cases were divided into three birth cohort decades for analysis: 70s (1970–79), 80s (1980–89) and 90s (1990–99). These birth cohorts were affected largely because they were not eligible for vaccination and had avoided exposure during childhood. As the outbreak occurred over a short time frame, the cohorts provide a suitable

proxy for age while at the same time keeping the modelling analysis less complex.

Laboratory-confirmed mumps cases

Confirmed mumps infections are reported by laboratories in England to Public Health England (PHE) (formerly the Health Protection Agency). Clinicians who diagnose mumps are also required by statute to notify the responsible public health officer for the local authority, usually a consultant in Communicable Disease Control. Since 1995, all notified cases of mumps have been followed by an offer of oral fluid testing for IgM at PHE. The oral fluid test had been shown to have a sensitivity of 90.3% and specificity of 97.6% [17]. A high proportion of notified cases provided samples and were tested using this method (50–80%) [18]. Some cases were confirmed on serum only using commercial mumps IgM assays. Cases confirmed by testing for IgM in oral fluid or in serum were used to provide data on trends in the 70s and 90s cohort. In 2005, during the peak of the outbreak, mumps oral fluid testing was temporarily suspended in those born between 1981 and 1986. Therefore, to avoid any bias resulting from change in testing, only mumps confirmed by serum IgM

FIGURE 2

Regression model of hospitalised orchitis (A and B) and hospitalised meningitis cases (C), England, April 2002–March 2006

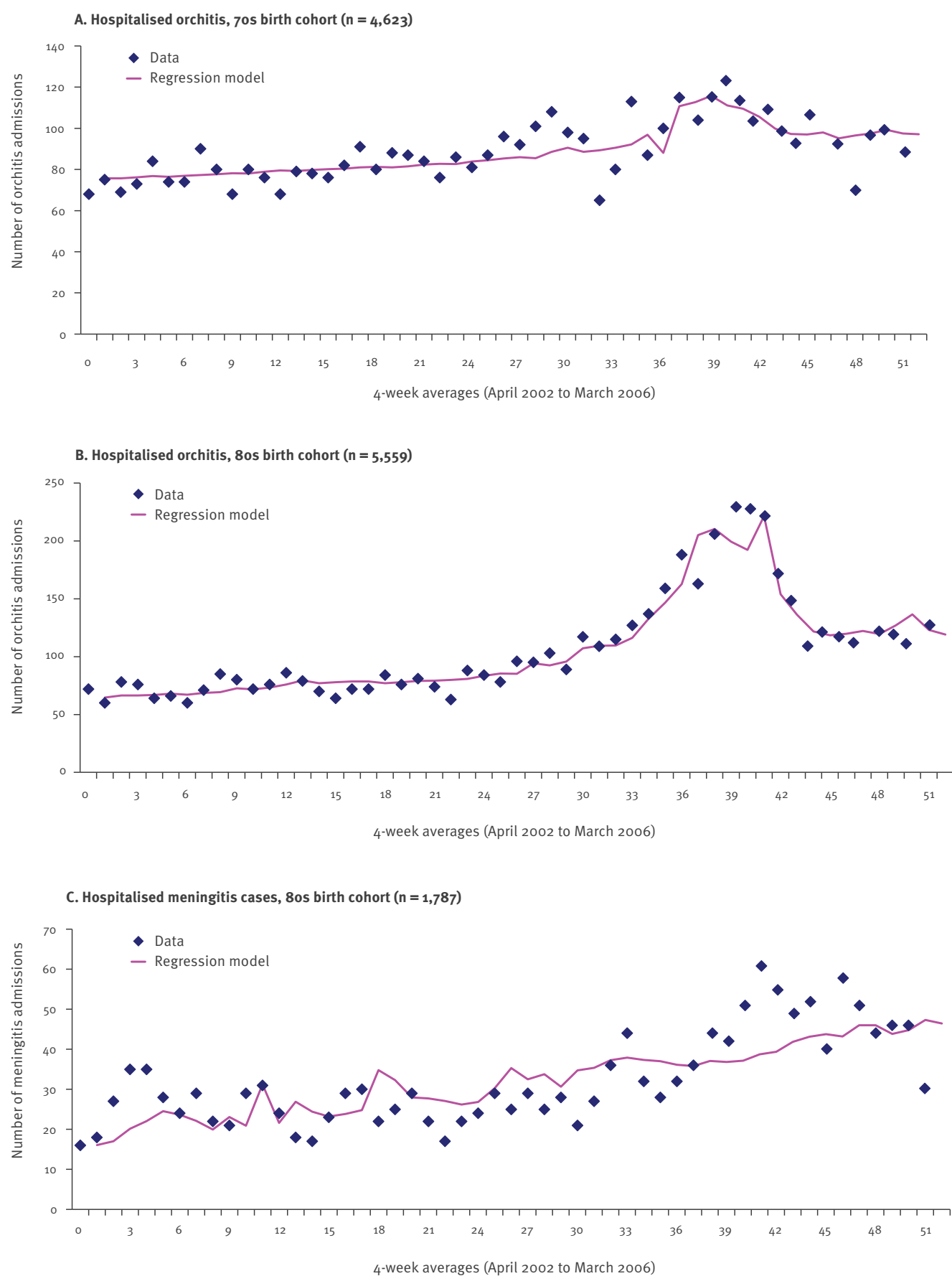


TABLE 1

Hospital admissions of orchitis, meningitis, oophoritis and pancreatitis cases (n=27,133) and cases coded as mumps (n=1,231), by birth cohort, England, April 2002–March 2006

Mumps complication	Birth cohort			
	70s	80s	90s	Total
Orchitis				
Hospital cases	4,623	5,559	2,265	12,447
Hospital cases coded as mumps	113	811	26	950
Meningitis				
Hospital cases	1,978	1,787	294	4,059
Hospital cases coded as mumps	16	118	9	143
Oophoritis				
Hospital cases	924	709	40	1,673
Hospital cases coded as mumps	0	0	0	0
Pancreatitis				
Hospital cases	6,025	2,585	344	8,954
Hospital cases coded as mumps	13	114	11	138

testing was used to derive trends for the 80s cohort in our model.

In summary, cases were defined as individuals born between 1970 and 1999 with a diagnosis of laboratory-confirmed mumps via either IgM in oral fluid or serum from 1 April 2002 to 31 March 2006.

Hospital admissions database

Hospital Episode Statistics (HES) capture all admissions (including day admissions) to National Health Service (NHS) hospitals in England. The diagnoses recorded at the time of discharge are coded using the International Classification of Diseases-10 (ICD-10). A minimum dataset for all admissions with any of the following codes was extracted: B26 (mumps), N45 (orchitis and epididymitis), A87 (viral meningitis), N70 (oophoritis) and K85 (acute pancreatitis) [19]. The anonymised HESID field, which is a unique ID generated from NHS Number, local patient identifier, postcode, sex and date of birth was used to link episodes from the same individual admitted over the period [20]. Length of hospital stay is calculated from days between the admission date and the discharge date.

Non-mumps meningitis trend data

For the viral meningitis model only, to adjust for the possible effect from non-mumps-related causes of meningitis, we included as an additional independent variable trends of laboratory-confirmed infections with other viruses known to be commonly associated with meningitis. The trend information used for non-mumps-related causes of meningitis was derived from temporal data on confirmed meningitis cases due to coxsackievirus A and B, echovirus or untyped enterovirus during the study period. Because of the small

numbers involved, this was included in aggregate form and not broken down by birth cohort.

Statistical analysis

We developed least square regression models which associated hospital records of orchitis, meningitis, oophoritis and pancreatitis with parameters of laboratory-confirmed mumps cases and time. The formula for hospital records of complication Y in a four-week period j was:

Formula 1

$$Y_j = C + \alpha L_j + \gamma_j$$

where Y is the total number of recorded complications in the HES database, j is unit time in intervals of four weeks, C is a constant representing background cases of complication Y attributable to non-mumps causes, α is the coefficient for laboratory-confirmed mumps infection, L is the number of laboratory-confirmed mumps and γ is the coefficient for unit of time (four weeks). We included γ to factor in age trends of mumps morbidity. Specifically for meningitis, laboratory-confirmed meningitis cases not due to mumps were included as an extra parameter in the equation.

The values of α were estimated by least square regression in Microsoft Excel version 11. The final model only included statistically significant explanatory parameters while non-significant parameters were dropped. The least significant parameter was removed first and one at a time. A parameter was only retained in the model if α remained significantly ($p < 0.05$) associated with hospital record of complication. The model was also eyeballed to ensure that the fit to actual data was reasonable, taking into consideration the goodness-of-fit of the model as denoted by R².

The statistical models for each birth cohort and complication generated a value for the number of hospital records of complication associated with a single laboratory-confirmed case of mumps. This was used to estimate the morbidity attributable to the mumps outbreak in each birth cohort and complication by the sum of αL_j during the study period from the equation above, which is denoted by:

Formula 2

$$\sum \alpha L_j$$

Complications and birth cohorts for which the association with the parameter laboratory-confirmed mumps was not statistically significant (not $p < 0.05$) were dropped from the final model. In such a scenario, the model estimated no complications attributable to the mumps outbreak.

The method has been used in a similar way to investigate hospital admissions due to rotavirus, the

TABLE 2

Orchitis and meningitis morbidity in hospitalised mumps cases attributable to mumps outbreak, by birth cohort, England, 2004/05 (n =1,798)

Birth cohort	Orchitis			Meningitis		
	70s	80s	90s	70s	80s	90s
Intercept (C)	75.13	63.44	28.49	29.4	10.47	5.65
Coefficient for mumps case (α)	0.14 (0.08–0.21)	0.23 (0.20–0.27)	a	a	a	a
Coefficient for unit time (γ)	0.32 (0.10–0.54)	0.5(0.18–0.92)	0.58 (0.44–0.73)	a	0.64 (0.47–0.81)	a
Coefficient for non-mumps meningitis	NA	NA	NA	0.50 (0.25–0.75)	0.31 (0.04–0.59)	a
R ²	0.59	0.88	0.57	0.25	0.56	0
Estimated cases attributable to mumps ($\Sigma \alpha L_i$)	279 (193–511)	1,519 (1,366–1,870)	0	0	0	0

NA: not applicable.

^a Parameter dropped from model as not significant.

contribution of respiratory syncytial virus to bronchiolitis and pneumonia-associated hospitalisation and to evaluate the contribution of serogroup C meningococcal infections to clinically diagnosed cases of meningitis and septicaemia [21–23].

Results

Laboratory-confirmed infections

The timing of the mumps outbreak in 2004/05 was apparent in laboratory-confirmed mumps cases in all three birth cohorts, particularly those born in the 80s cohort (Figure 1). Cases confirmed by oral fluid (saliva) in the latter cohort declined slightly earlier than in the other birth cohorts because of the restriction on oral fluid testing in this age group. Trends over time in the cases confirmed by serum testing in the 80s cohort, however, were similar to overall trends in the other cohorts. There were 900 laboratory-confirmed infections due to coxsackievirus A and B, echovirus and untyped enterovirus between April 2002 and March 2006. The trend in non-mumps causes of meningitis fluctuated over time, decreasing during the mumps outbreak period.

Mumps-coded hospital admissions for orchitis, meningitis, oophoritis or pancreatitis (HES database)

Between April 2002 and March 2006, there were a total of 2,284 hospitalisations with a diagnostic code for mumps among those born between 1970 and 1999. Of these, 950 (42%), 143 (6%), 138 (6%) and 0 (0%) also had a second diagnosis code for orchitis, meningitis, pancreatitis and oophoritis, respectively. For hospitalised mumps orchitis, the 80s cohort contributed the largest numbers with 811 cases, while the 90s cohort was least represented with only 26 cases. The average length of stay for hospitalised mumps was shortest in the 90s cohort with 1.3 days, compared with 2.2 days and 1.9 days in the 70s and 80s cohorts, respectively.

The pattern of highest burden in the 80s cohort and lowest burden in the 90s cohort was similar for mumps meningitis and pancreatitis but at a smaller scale due to the smaller number of such complications (Table 1).

Non-mumps-coded hospital admissions for orchitis, meningitis, oophoritis and pancreatitis (HES database)

Between April 2002 and March 2006, there were a total of 12,447 hospital admissions for orchitis, 4,059 hospital admissions for meningitis, 1,673 hospital admissions for oophoritis and 8,954 hospital admissions for pancreatitis in patients born between 1970 and 1999 (Table 1).

The number of hospital admissions for orchitis in the 70s and 80s birth cohorts peaked in 2004/05 coinciding with the peak of the mumps outbreak (Figure 2). Looking at the raw data, there also appeared to be a slight increase in hospitalisations for meningitis cases at this time in people born in the 80s (Figure 2C). However, the model did not demonstrate any statistically significant increase over the period. None of the other hospital records of orchitis, meningitis, oophoritis and pancreatitis by birth cohort showed any obvious spikes coinciding with the increase in laboratory confirmed mumps cases.

Modelling to estimate true morbidity associated with mumps outbreak

The regression model was produced for hospitalisation with orchitis, meningitis, oophoritis and pancreatitis for the three birth cohorts (70s, 80s and 90s). Only the models for orchitis in the 70s and 80s cohort were statistically significant for all parameters. The models found 2.5 times more mumps orchitis in the 70s cohort (166 cases) and 1.9 times more mumps orchitis in the 80s cohort (708 cases) when compared with the number of cases in each cohort that were coded as mumps in hospital databases (HES). Apart from the

two outcomes above, all other outcomes had no excess morbidity attributable to the mumps outbreak according to the model generated (Table 2).

Discussion

The 2004/05 mumps outbreak in England led to an increase in hospitalisations coded as mumps orchitis in those born in the 1970s and 1980s and possibly those coded as mumps meningitis in those born in the 1980s. Our regression models echo these findings but found that the true burden of hospitalised mumps orchitis was 2–2.5 times greater than the number of cases actually coded as mumps in hospital databases. There was no obvious increase in other complications or age cohorts coinciding with the outbreak but there was a suggestion of higher numbers of mumps-coded pancreatitis (114 cases) in hospital records in those born in the 1980s.

During the years of low mumps incidence following introduction of the MMR vaccine, mumps morbidity due to orchitis, meningitis, pancreatitis and oophoritis was rare. The resurgence of mumps despite high coverage with two-dose MMR in many countries may require improved monitoring of mumps complications as well as may require consideration of new strategies. Mumps orchitis was the most common reason for hospitalisation, accounting for 42% of all hospitalised mumps cases, similar to large outbreaks reported from the Netherlands and from Jewish communities in the United States and Israel [7,15,24]. The high numbers of mumps orchitis in current mumps outbreaks may be attributed to the high attack rates in adolescents and young adults. Although mumps orchitis has been shown to cause acute azoospermia and oligospermia, the potential of mumps orchitis to lead to infertility in post-pubertal males remains unclear [25–29].

Our model demonstrated the limitation of using hospital surveillance records alone to study admissions attributable to mumps. To improve the quality of the data, clinicians would need to actively seek a history of mumps in patients who present with orchitis, meningitis and pancreatitis, and to record the history of recent mumps in the discharge summary. Better recording, however, is unlikely to fully resolve the issue of underascertainment of mumps complications as many patients may not have been aware that they have had mumps. Based on our model, the true burden of mumps orchitis would be 2 or 2.5 fold higher compared with cases actually coded as mumps in hospital databases during a mumps epidemic. However, even this level is likely to be a minimum since we have only looked at hospitalised morbidity. Nevertheless, our findings should provide a quantification to better estimate the true burden of hospitalised mumps morbidity. This has important implications for improving vaccination uptake at the frontline as well as describing the overall impact of mumps vaccination as the programme evolves.

Analysis of the hospitalised mumps population showed that the number of mumps complications varied between birth cohorts. Overall, those born in the 1990s had fewer complications and a shorter mean hospital stay (by an average of 0.75 days) compared with the other birth cohorts. Regression modelling identified a similar pattern with higher morbidity from orchitis in those born in the 1970s and 1980s compared with the 1990s birth cohort. It is unlikely that this is simply due to a difference in the number of mumps cases during the outbreak. Laboratory surveillance data suggest that the number of confirmed cases was similar in the 1970s and 1990s cohorts. A possible explanation is the protective effect of MMR vaccination against complications. Previously, we found that MMR vaccination reduces the risk of hospitalisation, orchitis and meningitis despite vaccine failure [30]. A similar protective effect of MMR vaccine especially against mumps orchitis have also been found in United States, the Netherlands and Israel [15,24,31]. In England, the 90s cohort, unlike the other cohorts, were likely to have received at least one or more doses of MMR as MMR vaccine was only introduced into the national programme in October 1988.

The model was unable to detect an increase in hospital morbidity for meningitis, oophoritis or pancreatitis in the affected cohorts. This is consistent with the observed lack of oophoritis coded as mumps but at odds with the increase in pancreatitis with a mumps code (138 cases) in routine hospital databases. Moreover, the statistical model was not able to detect a spike in hospitalised meningitis in the 80s cohort coinciding with the mumps outbreak. This highlights the limitation of the model and suggests that the burden of morbidity from the mumps outbreak is likely to be a minimum estimate. Other limitations included the assumption that all the parameters accounting for the reported variation in four-weekly numbers of hospitalisations were included in the model. In addition, we could only investigate complications that resulted in acute hospital admissions and we could not investigate long-term sequelae such as deafness resulting from mumps.

Conclusion

Our study showed that the mumps outbreak in England 2004/05 resulted in a substantial increase in mumps complications of orchitis, pancreatitis and possibly meningitis with subsequent hospitalisations. We have shown that analysing time trends for all diagnoses of complications in hospital databases with routine laboratory surveillance data in a simple statistical model can improve the ascertainment of morbidity from a mumps outbreak. This method increased the morbidity due to mumps-related orchitis hospitalisations by a factor of 2 or 2.5 when compared with those coded as mumps alone.

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Conflict of interest

None declared.

Authors' contributions

CFY and MR were involved in the study design, data analysis, interpretation of result and drafting the manuscript.

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