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First cases of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) infections in France, investigations and implications for the prevention of human-to-human transmission, France, May 2013

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In May 2013, Middle East Respiratory Syndrome Coronavirus (MERS-CoV) infection was diagnosed in an adult male in France with severe respiratory illness, who had travelled to the United Arab Emirates before symptom onset. Contact tracing identified a secondary case in a patient hospitalised in the same hospital room. No other cases of MERS-CoV infection were identified among the index case's 123 contacts, nor among 39 contacts of the secondary case, during the 10-day follow-up period.

On 7 May 2013, Middle East Respiratory syndrome-Coronavirus (MERS-CoV) infection was confirmed in France in a traveller who became ill after returning from the United Arab Emirates (index case). An investigation was immediately carried out among his contacts since onset of illness, as well as among individuals who had co-travelled with him to the United Arab Emirates. The aim of the investigation was to detect possible other cases and prevent human-to-human transmission. The secondary objective was to try to identify any likely circumstances of exposure to the virus during his travel.

MERS-CoV is a novel virus among the genus *Betacoronavirus*, which was initially identified in Saudi Arabia in September 2012, in two patients with severe pneumonia [1]. As of 7 May 2013, when the case in France was identified, 30 cases had been confirmed as infected with the virus worldwide, including four diagnosed in the United Kingdom (UK) and two in Germany [2,3].

Surveillance, contact tracing and case finding in France

French surveillance system

In France, suspected cases of MERS-CoV infection have to be reported by attending physicians to regional health agencies and hospital infection control teams. After validation of the classification as a possible case by a French Institute for Public Health Surveillance (InVS) regional office (CIRE), located in a regional health agency, a standardised notification form including socio-demographical information, clinical details, and history of travel in at-risk countries is completed for each possible case.

Up to 17 May, a possible case was defined as follows:

(i) any patient with a history of travel in an at-risk country, who presented with clinical signs and/or imaging consistent with acute respiratory distress syndrome (ARDS) or pulmonary infection, encompassing fever ≥38°C and cough within 10 days after return;
(ii) any contact of a symptomatic possible or confirmed case, presenting with acute respiratory infection, whatever the severity, with an onset of symptoms within 10

days of the last contact with a possible/confirmed case while symptomatic.

The list of at-risk countries, as defined in European Centre for Disease Prevention and Control (ECDC) rapid risk assessment dated 7 December 2012, included, Bahrain, Iran Iraq, Israel, Jordan, Kuwait, Lebanon, Palestine, Oman, Qatar, Saudi Arabia, Syria, United Arab Emirates, and Yemen [4].

For each possible case, respiratory samples (nasopharyngeal aspiration/swab, bronchoalveolar lavage (BAL) fluid when indicated, or induced sputum) are collected and sent to the National Reference Centres for influenza (Institut Pasteur, Paris (coordinating centre) or Hospices civils, Lyon) to be tested for the presence of MERS-CoV genome by real-time reverse transcriptase polymerase chain reaction (RT-PCR) [5,6].

A confirmed case is defined as a possible case with a positive MERS-CoV RT-PCR on respiratory samples [5,6].

Moreover, as part of the usual surveillance of both emerging or nosocomial infections, any cluster of hospitalised patients or healthcare workers (HCW) presenting with severe respiratory infections, regardless of any history of travel in at-risk countries, has to be notified to Public Health Authorities.

Contact tracing and case finding

The contact tracing of all identified cases is implemented as soon as the diagnosis is confirmed. Contacts are defined as all people who provided healthcare to a confirmed case without individual protection, shared the same hospital room, lived in the same household or shared any leisure or professional activity with a confirmed case since this case's onset of clinical symptoms of MERS-CoV infection (respiratory, digestive or even isolated fever ≥38°C). All contacts are followed-up during a 10-day period (equal to the maximum incubation period according to the knowledge of the disease at the time of the investigation described in this report) after their last contact with the confirmed case to check for clinical symptoms, and asked to measure their body temperature twice a day. The follow-up consists of daily calls from the InVS or CIRE for contacts who are not HCW or from the hospital infection control teams for HCW, to check for the occurrence of clinical symptoms and fever (≥38°C). Contacts are also provided with a hotline number to call anytime in case of any symptom.

For confirmed cases with a history of travel in an at-risk country, a contact tracing of all members of the travel group (co-travellers) is implemented. If the confirmed case had onset of symptoms during the travel, co-travellers are investigated as contacts. Because they potentially have been exposed to the same source of infection (co-exposed), co-travellers are followed-up during a 10-day period after their return from an at-risk country. They are interviewed about the nature and date of their activities, exposure to people presenting with respiratory symptoms, food consumption and exposures to animals, and to aerosols during the travel, in order to investigate the source of infection.

The investigations are carried out with respect to French regulations (authorisation of the Commission Nationale Informatique et Libertés n°341194v42).

Detected confirmed cases

The index case was a 64 year-old male patient with a history of renal transplant, who had returned from the United Arab Emirates on 17 April. He had onset of symptoms on 22 April consisting of fever (38.9°C) and diarrhoea but no respiratory signs. He was admitted in hospital A on 23 April where he was hospitalised until 29 April. On 26 April, the patient presented with dyspnoea and cough; he was transferred to hospital B for a single calendar day to undergo a BAL in a specialised respiratory unit and was re-admitted in hospital A. On 29 April, he was transferred to hospital C in an intensive care unit (ICU). All hospitals were in the same department, whereby hospitals A and B were in the same town, while C and D were in two other towns. Possible MERS-CoV infection was suspected on 1 May and the index case was isolated and individual precautions implemented for HCW and visitors. MERS-CoV infection was confirmed on 7 May. On 8 May, the index case was transferred to hospital D where he was admitted in ICU in a specialised unit with maximal precautions, including a negative pressure room. He died on 28 May 2013, 36 days after onset of symptoms.

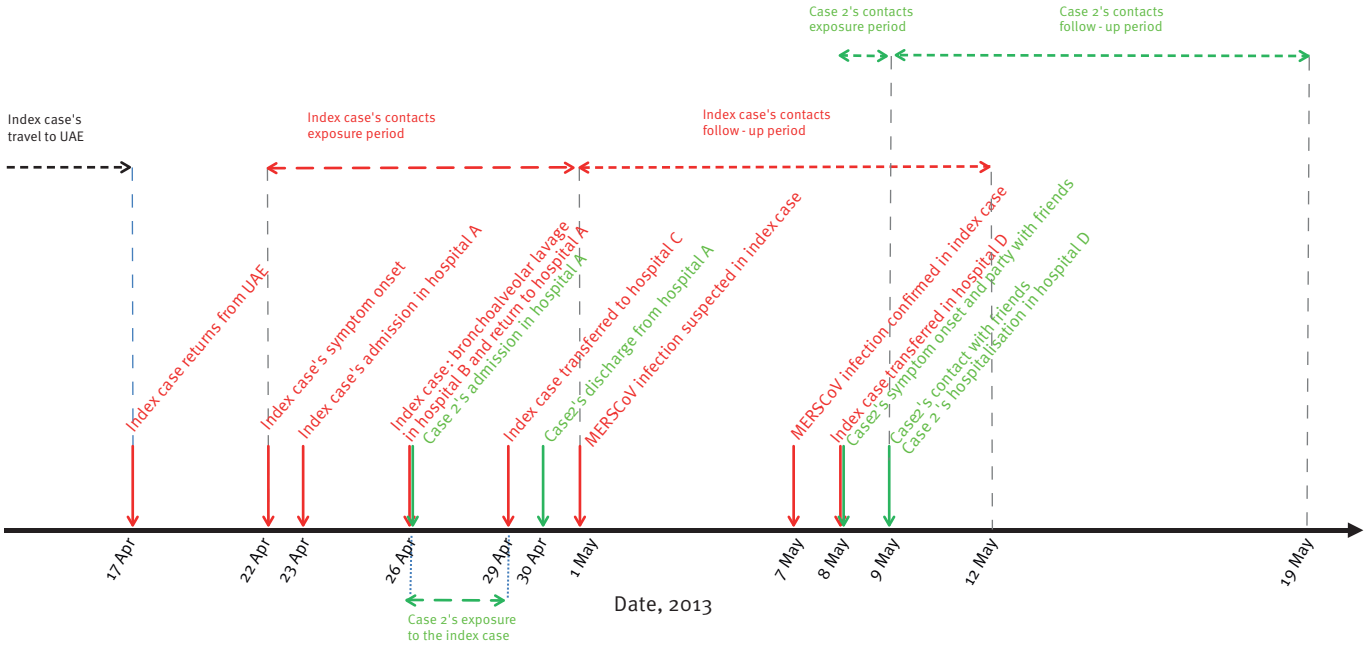
Case 2 was identified during the contact tracing of the index case. He was a 51-year-old male patient treated with steroids for several months prior to hospitalisation. He had no history of travel during the weeks before his hospitalisation. He shared with the index case a 20m² room with a single bathroom in hospital A from 26 to 29 April, while the index case presented with respiratory symptoms (Figure). The beds in the room were 1.5 m apart [7]. He was discharged on 30 April. Onset of symptoms suggestive of MERS-CoV infection occurred on 8 May, 12 days after first exposure. He first presented with malaise, muscle pain and fever (38.5°C) in the afternoon, and cough later that day. As case 2 was known as a contact of the index case, he was admitted in the infectious diseases ward in hospital D and isolated on 9 May. MERS-CoV infection was confirmed during the night of 11 to 12 May. Case 2 was admitted in ICU on 12 May where he is still isolated with the same precautions as the index case.

Contact tracing

The index case had travelled in the United Arab Emirates from 9 to 17 April 2013 with 37 co-travellers and his spouse. All co-travellers were interviewed from 10 to 13 May, and none had had any respiratory or digestive symptoms or fever, neither during the journey nor since their return. Except for the spouse,

Figure

Timeline of epidemiological features of two cases of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) infection and exposure and follow-up period of their contacts (n=162), France, April–May 2013



MERS-CoV: Middle East respiratory syndrome-Corona-Virus; UAE: United Arab Emirates.

as their interview took place 23 to 26 days after their last contact with the at the time asymptomatic index case, they were not followed-up. All had done the same itinerary and shared common activities with the index case. Their interview did not allow suggesting any hypothesis about the source of infection.

In total, 123 contacts exposed to the index case from his onset of symptoms (22 April) until his isolation (1 May) were identified and interviewed from 8 to 10 May. Six of them were family members who visited the index case in hospital A. Other contacts were 88 HCW and two patients (including case 2) in hospital A, four HCW in hospital B, 20 HCW and three patients in hospital C. Of the five contacts who were patients, only case 2 had shared a room with the index case. No contacts were identified in hospital D, as maximal infection control precautions had been immediately taken. Seven of the total 123 contacts matched the case definition for possible cases and were therefore tested for MERS-CoV infection (samples were taken between one and six days after contacts became symptomatic): only case 2 tested positive.

In total, 39 people were identified as contacts of case 2: 30 had attended a party with case 2 on 8 May, two had visited him at home on 9 May before admission to hospital D, and seven had visited him at home on 9 May and attended the party. Among those 39, 16 had a face-to-face conversation longer than 15 minutes with case 2 and were considered close contacts as described elsewhere [3]. All 39 contacts were interviewed on 12 May, and followed-up until 19 May for those with last contact on 9 May (n=9), and until 18 May for others (n=30). As of 19 May, all were asymptomatic.

Control measures

As soon a MERS-CoV infection was confirmed, the index case and case 2 were isolated, using airborne and contact precautions, in a negative pressure room with dedicated staff [8]. Case 2 had to wear a surgical mask until his medical condition required mechanical ventilation, and HCW who took care of the patients had to wear a filtering face piece (FFP)2 mask [8].

Close contacts of case 2 were asked not to return to work or school until the end of the follow-up, and were provided with surgical masks to wear when not alone and alcohol based hand rub. Other contacts could go on with their usual activities but had to carry a mask, and in case of symptoms, wear it and immediately go back home and call the dedicated hotline [8]. Particular measures for close contacts were implemented after case 2 was diagnosed, and were therefore not applied to contacts of the index case.

Both confirmed cases were notified to the ECDC and the World Health Organization (WHO), respectively on 8 May and 12 May.

Information about the disease and the outbreak was released to the public through the media, and to travellers via flyers and posters disseminated in airports. Specific information about the patients' management was disseminated to healthcare professionals through mailing lists and institutions' websites.

Discussion and conclusion

We report the investigation of the first two cases of MERS-CoV diagnosed in France since the emergence of the virus was first described in Saudi Arabia in 2012 [1]. The index case diagnosed in France was imported from the United Arab Emirates, and the second case resulted from a nosocomial infection. Considering that both cases spent four days (26 to 29 April) in the same hospital room, the incubation period of case 2 ranged from nine to 12 days. This emphasises the need for gathering more clinical information from future and past cases to be able to determine precisely the incubation period.

As of 7 June 2013, 55 cases were identified worldwide since the beginning of the worldwide outbreak [9], suggesting a limited human-to-human transmission, even if we assume that some cases may have not been diagnosed.

The index case was initially admitted with an atypical presentation consisting of digestive symptoms but no respiratory signs. Therefore, MERS-CoV infection was not suspected until the patient was in ICU with severe pneumonia. This finding raised the importance of disseminating information about emerging diseases in all hospital settings, including those wards that are not specialised in infectious diseases or critical care.

In-hospital transmission has previously been described in England, in a family member who visited a confirmed case in hospital [10]. A hospital cluster suggestive of nosocomial transmission has also been reported in Saudi Arabia, although the details of the transmission are still under investigation [11]. In France, a secondary infection was diagnosed in another hospitalised patient with underlying condition and long-term steroid treatment. The respiratory presentation of the index case strongly suggests an airborne transmission in the hospital room shared by both patients. However, some questions remain about the possible infectiousness of other body fluids or clinical samples, including stools as the index case presented with diarrhoea at an early stage of his disease, and a cross transmission through contaminated surfaces, medical devices or hands of HCW cannot be ruled out. During the severe acute respiratory syndrome (SARS) outbreak in 2003, a cluster of infections was detected in inhabitants of the same building. Virus aerosols originating from a flat where the index case of the cluster had had digestive symptoms, spread by drainage pipes, were assumed to be the origin of the infection of other cases in the cluster [12].

The large majority of reported MERS-CoV cases worldwide had underlying conditions and presented with severe respiratory infection requiring hospitalisation in ICU. Atypical presentations in immunocompromised patients may be really challenging for clinicians, especially as digestive symptoms are very common in travellers. Based on the index case's clinical presentation and on knowledge acquired from the SARS outbreak [13], the French case definition for possible cases was extended on 17 May to improve the sensitivity of the surveillance system. It now includes severe febrile clinical signs or febrile diarrhoea in immunocompromised persons or in those with chronic underlying conditions, returning from an at-risk country [14].

Despite the identification of few infections since 2012, MERS-CoV has demonstrated a real potential for nosocomial transmission, and stringent recommendations have to be implemented around possible cases as soon as MERS-CoV infection is suspected. The challenge presented by possible atypical presentations highlights the need for a better knowledge about both the virus and the disease.

Useful knowledge about the infection by MERS-CoV might be obtained from serological investigation in people who shared exposures of confirmed cases, or in contacts of confirmed cases. Such studies might help raising hypothesis about the extent of transmission and risk factors for infection and fatal outcome and must be encouraged.

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Conflict of interest

None declared.

Authors' contributions

Alexandra Mailles: wrote the manuscript. Alexandra Mailles, Christine Campese, coordinated the investigation of co-travellers of the index case. Pascal Chaud, Marie-Claire Paty, Caroline Semaille: coordinated the investigations of confirmed cases. Karine Blanckaert, Sylvie Hendricx: contact tracing and follow-up of healthcare workers. Pascal Chaud, Sylvie Haeghebaert: coordinated the contact tracing for index case and follow-up of healthcare workers and family contacts of the index case. Sylvie van der Werf, Bruno Lina, Valérie Caro, Sylvie Behillil, Jean-Claude Manuguerra, Vincent Enouf: implemented the biological diagnosis in France and carried out the diagnosis of all possible cases, including both confirmed case. Benoit Guéry, Xavier Lemaire, Nicolas Ettahar: clinical management of confirmed cases and symptomatic contacts. Delphine Antoine, Harold Noel, Guy La Ruche, Pascal Chaud, Hélène Prouvost coordinated the contact tracing and follow-up of contacts of case. Didier Che, Bruno Coignard, Daniel Levy-Bruh: expertise in risk assessment. Bruno Coignard, Daniel Levy-Bruhl, Françoise Weber: supervised the investigations and coordinated the relationship with health authorities in France and Europe. Christine Saura, Didier Che: implemented the surveillance system in France and coordinated all involved partners since September 2012. All: revised the manuscript and contributed with specific comments.

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Transmission scenarios for Middle East Respiratory Syndrome Coronavirus (MERS-CoV) and how to tell them apart

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Detection of human cases of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) infection internationally is a global public health concern. Rigorous risk assessment is particularly challenging in a context where surveillance may be subject to under-ascertainment and a selection bias towards more severe cases. We would like to assess whether the virus is capable of causing widespread human epidemics, and whether self-sustaining transmission is already under way. Here we review possible transmission scenarios for MERS-CoV and their implications for risk assessment and control. We discuss how existing data, future investigations and analyses may help in reducing uncertainty and refining the public health risk assessment and present analytical approaches that allow robust assessment of epidemiological characteristics, even from partial and biased surveillance data. Finally, we urge that adequate data be collected on future cases to permit rigorous assessment of the transmission characteristics and severity of MERS-CoV, and the public health threat it may pose. Going beyond minimal case reporting, open international collaboration, under the guidance of the World Health Organization and the International Health Regulations, will impact on how this potential epidemic unfolds and prospects for control.

As of 30 May 2013, 50 laboratory-confirmed cases of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) infection have occurred worldwide [1]. An apparently high case-fatality ratio (60%; 30 deaths as of 30 May 2013 [1]) and growing evidence that human-to-human transmission is occurring [2] make MERS-CoV a threat to global health. The current situation has already been compared to the early stages of the severe acute respiratory syndrome (SARS) epidemic in 2003 [3,4].

No animal reservoir has yet been identified for MERS-CoV, and yet human cases, mostly severe, have been detected over a wide geographical area in the Middle East and Europe. If most human cases to date have arisen from animal exposure, this implies a large but as yet uncharacterised zoonotic epidemic is under way in animal species to which humans have frequent exposure (Figure 1A). In this scenario, we might expect relatively small numbers of human cases overall, though with the limited surveillance data available to date, we cannot rule out the possibility that substantial numbers of human cases, with milder disease, have gone undetected.

Even if most human cases to date have been infected through zoonotic exposure, is it possible that MERS-CoV already has the potential to support sustained human-to-human transmission but has by chance so far failed to do so?

Alternatively, how feasible is it that most of the severe MERS-CoV cases detected to date were in fact infected via human-to-human transmission and that the epidemic is already self-sustaining in human populations (Figure 1B)? Under this transmission scenario, substantial numbers of human infections may have already occurred, with only a small proportion of them being detected. But is it feasible that such an epidemic would not have been recognised?

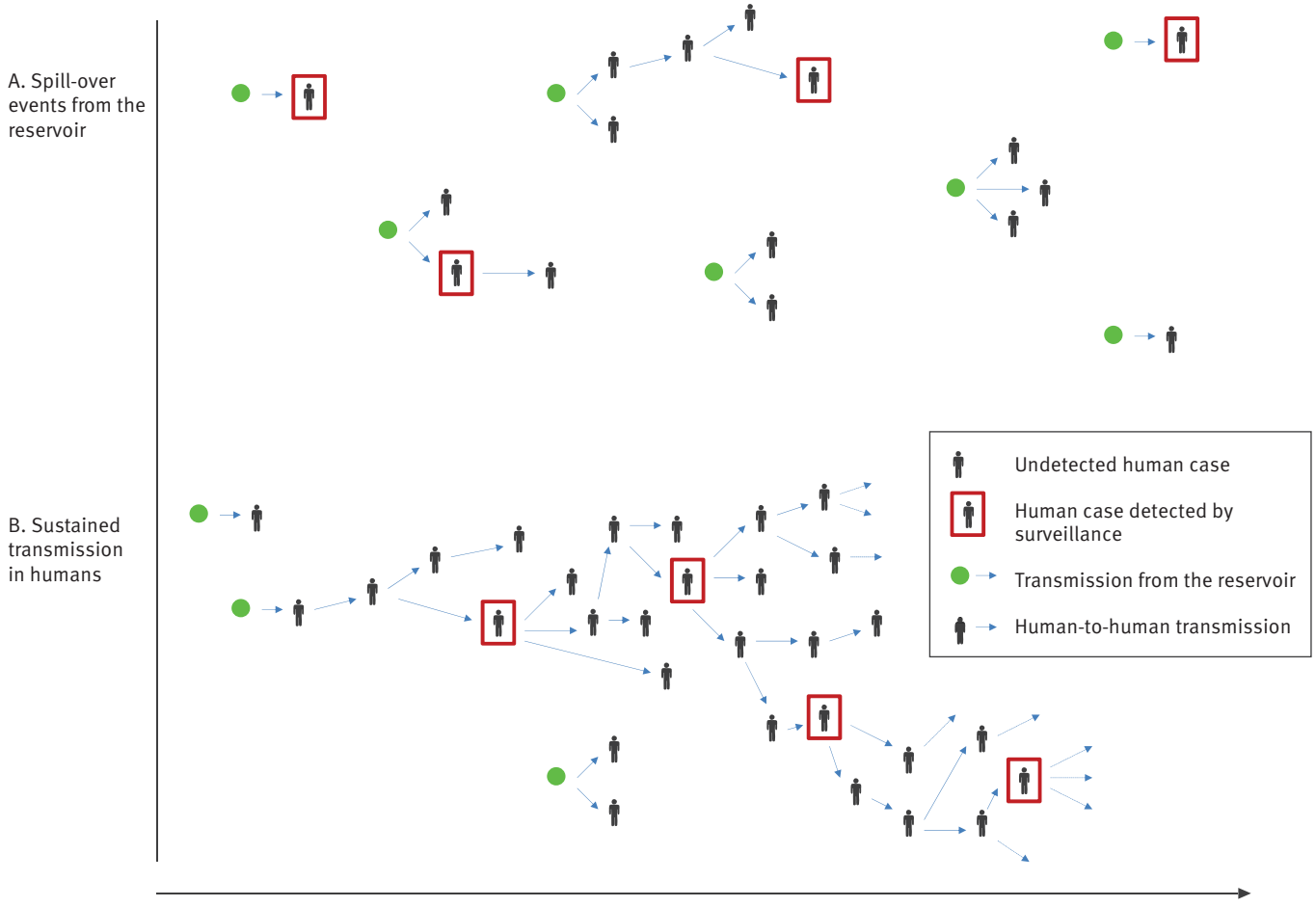
Each of these scenarios has very different implications for the assessment of severity, relevance of reservoir-targeted strategies and potential impact of MERS-CoV globally. Although it may not be possible to completely rule out any of the scenarios with the data currently available, it is timely to consider the priorities for data collection and analysis as cases accrue, so as to best be able to reduce uncertainty and refine the public health risk assessment.

Transmission scenarios for an emerging infection

The human-to-human transmissibility (and thus epidemic potential) of an emerging pathogen is quantified by the (effective) reproduction number, *R*, the average number of secondary infections caused by an index

Figure 1

Two illustrative scenarios for transmission of Middle East Respiratory Syndrome Coronavirus (MERS-CoV)



A. Few human-to-human infection events have occurred and observed clusters have arisen from separate spill-over events (i.e. introductions from the animal reservoir into human populations).

B. Many undetected human-to-human transmission events have occurred and the epidemic is already self-sustaining.

human infection. Depending on the value of *R*, different transmission scenarios are possible, as described below.

Scenario 1: subcritical outbreaks ($R<1$)

If $R<1$, a single spill-over event from a reservoir into human populations may generate a cluster of cases via human-to-human transmission, but cannot generate a disseminated, self-sustaining epidemic in humans. The number of human infections expected under this scenario is roughly proportional to the number of zoonotic introductions of the virus into the human population, with a multiplier, $1/(1-R)$, that increases with *R* (twofold if $R=0.5$, but 10-fold if $R=0.9$).

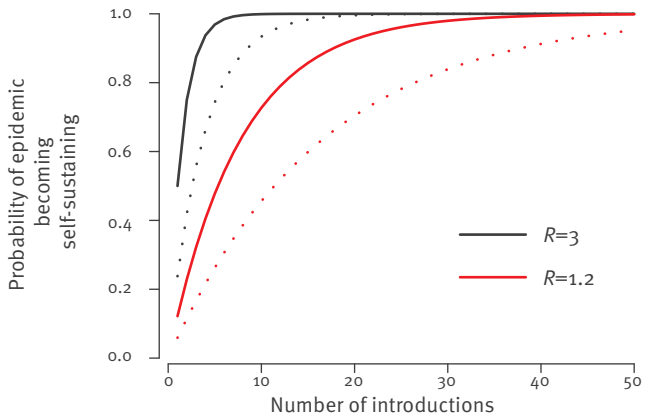
In this scenario, human infections can be mitigated by controlling the epidemic in the reservoir and/or preventing human exposure to the reservoir. Examples of this scenario are A(H5N1) and A(H7N9) avian influenzas.

Scenario 2: supercritical outbreaks ($R>1$ but epidemic has not yet become self-sustaining in human populations)

If $R>1$, a self-sustaining epidemic in humans is possible but emergence following introduction is a chance event: many chains of transmission may extinguish themselves by chance, especially if *R* is close to 1. In the case of SARS, for example, where 'super-spreading' events played an important role in transmission (i.e. a small proportion of cases were responsible for a large proportion of onward transmission), it has been estimated that there was only a 24% probability that a single introduction would generate a self-sustaining epidemic [5] (following [5], we technically define 'super-spreading' events by an over-dispersion parameter $k=0.16$; the absence of super-spreading events is defined by $k=0.5$). This is because if the first cases were not part of a super-spreading event, they would be unlikely to generate further cases. However,

Figure 2

Probability that the epidemic has become self-sustaining in humans after n introductions from the reservoir if $R>1$



R: reproduction number.

This probability depends not only on *R* but also on the presence of super- spreading events (SSE) (without SSE: plain line; with SSE: dotted line). Values *R*=3 and *R*=1.2 were selected for illustrative purposes.

in this scenario, a self-sustaining epidemic is eventually inevitable if zoonotic introductions into the human population continue (Figure 2). As with the subcritical scenario (R<1), reducing infections from the reservoir is critical to reducing the public health risk.

Scenario 3: self-sustaining epidemic ($R>1$)

If $R>1$ and the epidemic has become self-sustaining in humans, the number of human cases is expected to grow exponentially over time. The rate of growth increases with R, but decreases with the mean generation time (GT), the time lag from infection of an index case to infection of those they infect. For example, for an eight-day GT – similar to that of SARS – once self-sustaining, the number of human cases is expected to double about every week if $R=2$, but only about every month if $R=1.2$. Although chance effects may mask exponential growth early in the epidemic, a clear signal of increasing incidence would be expected once the number of prevalent infections increases sufficiently [6]. If case ascertainment remains constant over time, the incidence of detected cases would be expected to track that of underlying infections, even if only a small proportion of cases are detected. Once the epidemic is self-sustaining, control of the epidemic in the reservoir would have limited impact on the epidemic in humans.

Publicly available data

As of 30 May 2013, 50 confirmed cases of MERS-CoV have been reported with symptom onset since April 2012 from Saudi Arabia, Jordan, Qatar, United Arab Emirates, the United Kingdom (UK), France and Tunisia [1,2,7-24]. There are additional probable cases from Jordan, Saudi Arabia and Tunisia [1,12,14]. Information on animal exposures is limited and the animal reservoir has not yet been identified. However, we suspect that some of the cases may have arisen from zoonotic exposure in the Arabian Peninsula. Human-to-human transmission is suspected in several familial and healthcare facility clusters in Saudi Arabia, Jordan UK and France. We understand that follow-up investigations of contacts of the confirmed MERS-CoV cases have taken place by Ministry of Health officials in affected countries, finding no evidence of additional symptomatic infection [7-10,15-19]. At this stage, it is difficult to ascertain whether other primary zoonotic or secondary human-to-human cases have been missed. Most cases have been reported as severe disease (40 of 44 with documented severity) and 30 (as of 30 May 2013) have been fatal [25]. Table 1 summarises data for each cluster.

Urgent data needs

Existing and additional data will help characterise the MERS-CoV transmission scenario. Many appeals for data have been brought forward by several experts and institutions such as the World Health Organization (WHO). We support this and summarise data requirements and the studies required to collect such data are summarised in Table 2. We illustrate here how these data may be analysed and interpreted with adequate statistical techniques [26-28].

Line-list data on confirmed cases

The spatio-temporal dynamics of cases may be used to ascertain whether the epidemic is self-sustaining and if so, to characterise human-to-human transmission [27-29]. It is therefore important that detailed epidemiological information is recorded for all confirmed and probable cases.

Identification of the reservoir species and exposure data

The importance of identifying animal reservoir(s) and understanding human exposure to reservoir species (e.g. direct contact, contact via contaminated food) is well recognised. Once the reservoir has been identified, any exposure of MERS-CoV human cases to that reservoir should be documented in epidemiological investigations. Currently, the uncertainty regarding reservoirs and modes of transmission mean that only five of 50 cases can reliably be classified as 'human-to-human' transmission, with the source of infection unclear for the remainder.

If none of the MERS-CoV cases detected by routine surveillance had exposure to the reservoir(s), this would clearly indicate that an epidemic in humans is already self-sustaining [26]. By contrast, if a substantial proportion of cases have been exposed to the reservoir(s), it may be possible to rule out the hypothesis that R≥1.

Table 1

Summary information per cluster of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) infection, as of 30 May 2013

Cluster ID	Country identified	Date of reporting	Date first symptom onset	Number of confirmed cases	Number of cases infected by human-to-human transmission	Number of reported probable cases	References
1	Saudia Arabia	20 Sep 2012	13 Jun 2012	1	0	0	[1,19]
2	Saudia Arabia	1 Nov 2012	5 Oct 2012	3	0	1	[1,13]
3	Saudia Arabia	4 Nov 2012	9 Oct 2012	1	0	0	[7,21]
4	Jordan	30 Nov 2012	21 Mar 12	2	0	9	[1,12]
5	United Kingdom	22 Sep 2012	3 Sep 2012	1	0	0	[8]
6	Germany	1 Nov 2012	1 Oct 2012	1	0	0	[1,9]
7	United Kingdom	11 Feb 2013	24 Jan 2013	3	2	0	[1,2]
8	Saudia Arabia	21 Feb 2013	NR	1	0	0	[1]
9	Saudia Arabia	7 Mar 2013	NR	1	0	0	[1]
10	Saudia Arabia	12 Mar 2013	24 Feb 2013	2	0	0	[1]
11	Germany	26 Mar 2013	NR	1	0	0	[1]
12	Saudia Arabia	9 May 2013	6 Apr 2013	21	Unknown	0	[20,22-24]
13	France	9 May 2013	22 Apr 2013	2	0	0	[1,11]
14	Saudia Arabia	14 May 2013	25 Apr 2013	1	0	0	[1]
15	Saudia Arabia	18 May 2013	28 Apr 2013	1	0	0	[1]
16	Tunisia	22 May 2013	NR	2	2	1	[1]
17	Saudia Arabia	22 May 2013	NR	1	0	0	[1]
18	Saudia Arabia	28 May 2013	12 May 2013	5	Unknown	0	[1]

NR: not reported.

A similar analytical approach can be used to assess local levels of transmission in countries where MERS-CoV cases are imported from abroad. We can determine if there is self-sustaining transmission in a country by monitoring the proportion of cases detected by routine surveillance with a travel history to other affected countries [26].

If reservoir exposure cannot be found in spite of detailed epidemiological investigations, this may indicate that the epidemic is already self-sustaining in humans. It is therefore important that efforts to identify the reservoir are documented even if they are unsuccessful. To date, very few of the 50 cases have reported contact with animals [1].

Thorough epidemiological investigations of clusters of human cases

Thorough and systematic epidemiological investigations – including contact tracing of all household, familial, social and occupational contacts, with virological and immunological testing – permits assessment of the extent of human infection with MERS-CoV among contacts of confirmed cases [29]. In this context, virological and serological testing is important for ascertaining secondary infections.

As stated above, if $R>1$, human-to-human transmission will eventually become self-sustaining after a sufficiently large number of virus introductions. So, if thorough cluster investigations indicate that all introductions to date have failed to generate large outbreaks, we can derive an upper bound for R (Figure 3). The distribution of cluster sizes can also be used to estimate R [30,31].

Routine surveillance is likely to be biased towards severe cases. As a consequence, the case-fatality ratio estimated from cases detected by routine surveillance may be a substantial overestimate. Secondary cases detected during thorough epidemiological investigations of human clusters are expected to constitute a more representative sample of cases in general, meaning more reliable estimates of severity will be obtained by recording clinical outcomes in this subset of cases. Seroepidemiological studies allow for better characterisation of the spectrum of disease, and for the calculation of the proportion of asymptomatic or subclinical infections [29].

Population-level data

Once reliable serological assays are available to measure levels of antibodies to MERS-CoV, it will be

Table 2

Assessing the transmission scenario of a zoonotic virus: data requirements, suggested investigations, parameter estimation and policy implications

Improved knowledge	Data requirements	Recommended study investigations	Parameter estimation	Policy implications
Identification of reservoir species and exposure data	• Identification of the source of infection, of animal reservoir specie(s) and of amplifier specie(s) • Exposure history of confirmed and probable cases	• Animal studies • Detailed exposure history collected during initial investigations of suspected cases	• Test if $R>1$	• Mitigation measures can be implemented to reduce transmission from the source to humans • Determine if epidemic is self-sustaining in humans
Thorough epidemiological investigations of clusters of human cases[b]	• Data as above, plus • Detailed epidemiological investigations of all cases to determine cluster size	Epidemiological, virological and serological[a] investigations of: • close familial, social and occupational contacts of MERS-CoV confirmed and probable cases • healthcare workers caring for MERS-CoV patients	• Estimate R • Estimate the generation time • Estimate severity parameters	• Make an assessment of severity • Determine if epidemic is self-sustaining in humans • Guide efforts for prevention of (human-to-human) transmission
Population-level infection data[b]	• Estimates of population-level seroprevalence	• Community-based seroepidemiological[a] studies	• Estimate the extent of infection in humans	• Identify risk groups for targeted mitigation measures to reduce transmission

MERS-CoV: Middle East Respiratory Syndrome Coronavirus.

[a] The development of serological testing is currently limited, though actively being developed.

[b] Protocols for epidemiological investigations can be found at [34,35].

important to undertake serological surveys in communities affected early to assess the prevalence of MERS-CoV infection. Should MERS-CoV cases continue to arise in those communities, a rapid follow-up study to collect paired serum samples would be highly valuable. Even a relatively small number of paired sera (about 1,000) could be used to estimate underlying infection rates and refine estimates of severity [32].

Conclusions

We have described three possible transmission scenarios for the emergence of a novel human pathogen from a suspected zoonotic reservoir, with different implications for risk assessment and control.

The most optimistic scenario is that $R<1$, and thus there is no immediate threat of a large-scale human epidemic. In this scenario, identifying the reservoir will inform efforts to limit human exposure. Detailed genetic investigations and estimation of R are also important for determining the selection pressure and opportunity for the virus to evolve higher human transmissibility [33].

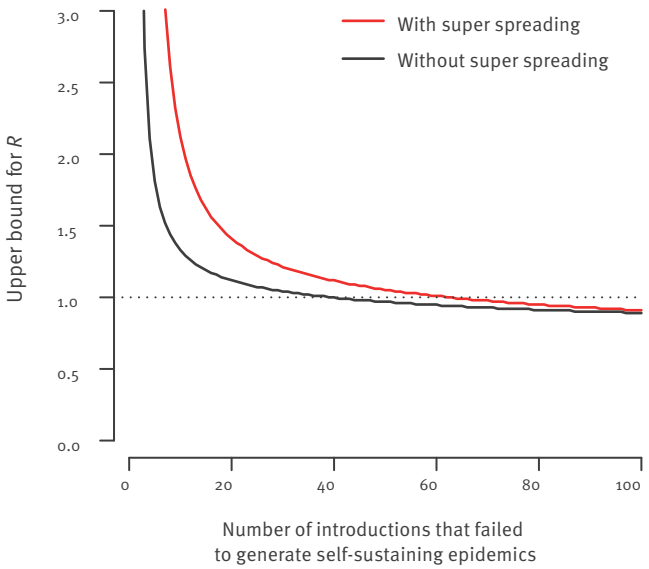
If $R>1$ but by chance MERS-CoV has not yet generated a self-sustaining epidemic, the total number of animal-to-human infections must have been relatively small. This would suggest that the severe cases that have been detected are not the tip of the iceberg and that disease severity is therefore high.

The final possibility is that $R>1$ and that human-to-human transmission is already self-sustaining. If this is the case, R must still be relatively low (i.e. <2) unless transmission only began to be self-sustaining in the recent past (e.g. early 2013). In this scenario, overall human case numbers might already be relatively large, suggesting that severity may be substantially lower than it appears from current case reports. Rapid implementation of infection control measures upon detection of MERS-CoV cases may be limiting onward spread beyond close contacts, and may explain the lack of clear-cut evidence from the epidemiological data available thus far that human-to-human transmission is self-sustaining.

Given the current level of uncertainty around MERS-CoV, it is important that adequate data are collected on future cases to underpin rigorous assessment of the transmission characteristics and severity of MERS-CoV, and the public health threat it may pose. This paper has reviewed the epidemiological investigations needed (Table 2); use of standard protocols – being developed by several groups; see available protocols

Figure 3

Upper bound for the reproduction number *R* as a function of the number of introductions from the reservoir that failed to generate self-sustaining epidemics



from WHO [34], the Consortium for the Standardization of Influenza Seroepidemiology (CONSISE) [35] and International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC) [36]) – where possible, would be beneficial. Going beyond minimal case reporting, open international collaboration, guided by the International Health Regulations, will impact how this potential epidemic unfolds and prospects for control.

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Conflict of interest

SC received consulting fees from Sanofi Pasteur MSD for a project on the modelling of varicella zoster virus transmission. The authors declare no other competing interests.

Authors' contributions

SC, MVK, SR, SAD, CF, NMF planned the analysis; MVK compiled the data; SC developed the methods and ran the analysis; SC wrote the first draft; SC, MVK, SR, SAD, CF, NMF edited the paper.

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Routine screening for *Coxiella burnetii* infection during pregnancy: a clustered randomised controlled trial during an outbreak, the Netherlands, 2010

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Between 2007 and 2010, the Netherlands experienced one of the largest outbreaks of Q fever. Since asymptomatic *Coxiella burnetii* infection has been associated with maternal and obstetric complications, evidence about the effectiveness of routine screening during pregnancy in outbreak areas is needed. We performed a clustered randomised controlled trial during the Dutch outbreak, in which 55 midwife centres were randomised to recruit pregnant women for an intervention or control strategy. In both groups a serum sample was taken between 20 and 32 weeks of gestation. In the intervention group (n=536), the samples were analysed immediately by indirect immunofluorescence assay for the presence of IgM and IgG (phase I/II) and treatment was given during pregnancy in case of an acute or chronic infection. In the control group (n=693), sera were frozen for analysis after delivery. In both groups 15% were seropositive. In the intervention group 2.2% of the women were seropositive and had an obstetric complication, compared with 1.4% in the control group (Odds ratio: 1.54 (95% confidence interval 0.60-3.96)). During a large Q fever outbreak, routine *C. burnetii* screening starting at 20 weeks of gestation was not associated with a relevant reduction in obstetric complications and should therefore not be recommended.

Introduction

Viral, bacterial and parasitic infections during pregnancy, such as human immunodeficiency virus, syphilis and toxoplasmosis, are a threat to both maternal and foetal health, even if the infection is asymptomatic. Routine screening for some of these infectious diseases is therefore recommended for all pregnant women [1]. Due to several outbreaks, the incidence of Q fever, a zoonosis caused by the bacterium *Coxiella burnetii*, has been increasing in the Netherlands and some other European countries since 2007 [2,3]. Most of the infected individuals are either asymptomatic or present with a mild influenza-like illness. However, *C. burnetii* may pose a serious threat to pregnant women because of the increased risk of chronic Q fever, often complicated by endocarditis [4-6]. In addition, both symptomatic and asymptomatic *C. burnetii* infection during pregnancy have been associated with obstetric complications due to placentitis, including preterm delivery, intrauterine growth restriction and foetal death [7,8]. Because most infected pregnant women remain asymptomatic [9], routine serological screening during an outbreak could be of great value to prevent chronic maternal infections and obstetric complications, but evidence from randomised trials is lacking. Since the Dutch Q fever outbreak has been unique in size, with over 3,500 cases over three years [10], we had the opportunity to perform a clustered randomised controlled trial (RCT) to assess the effectiveness of large-scale routine serological screening for *C. burnetii* infection of pregnant women during a Q fever outbreak.

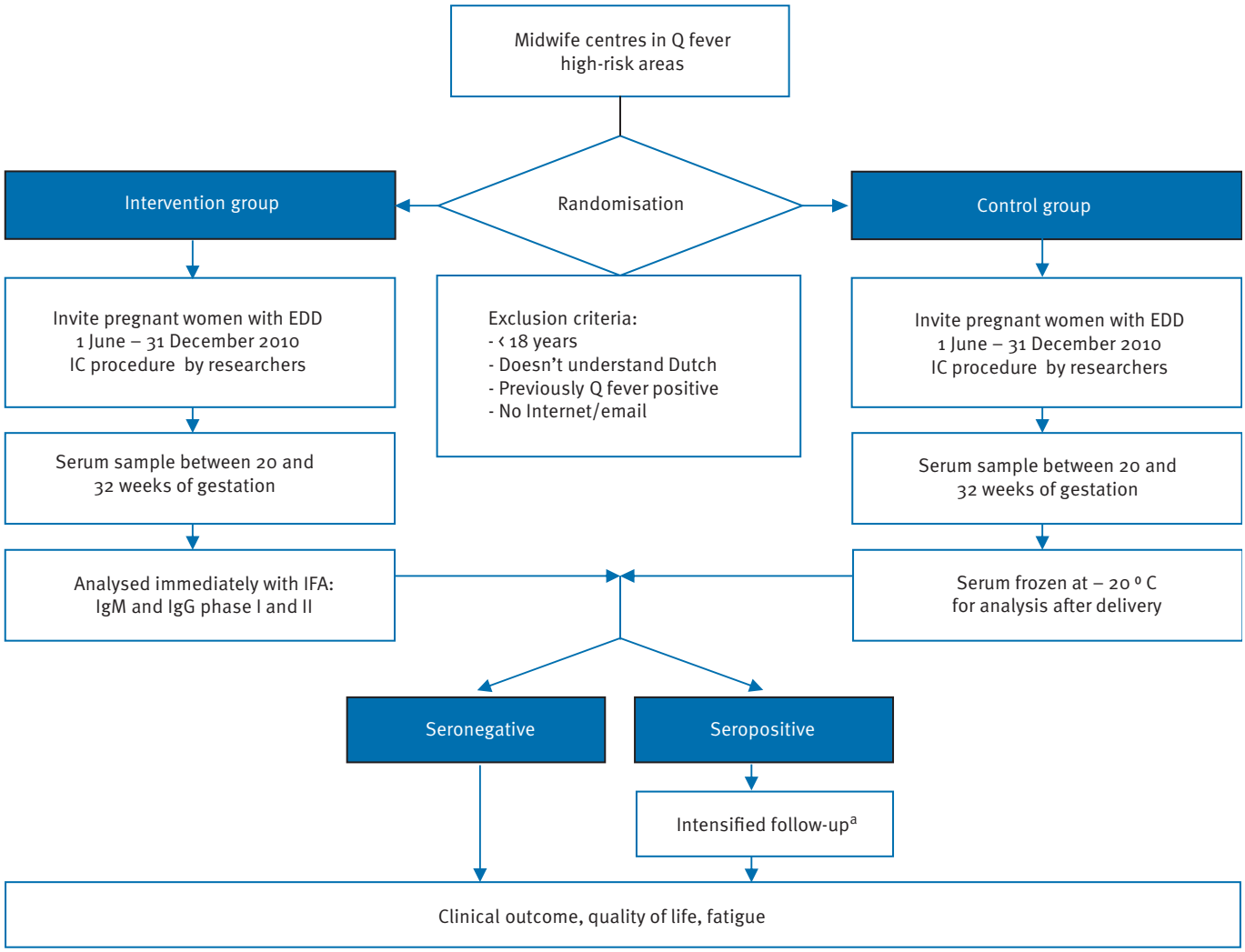
Methods

We conducted a clustered RCT in which primary care midwife centres were randomised to recruit pregnant women either for the intervention or for the control group (Figure 1).

The study was conducted according to the principles of the Declaration of Helsinki, and the study protocol was approved by the Medical Ethical Review Board of the

Figure 1

Flow chart of the study protocol, study on screening for *Coxiella burnetii* infection during pregnancy, the Netherlands, 2010 (n=1,229)



EDD: estimated date of delivery; IC: informed consent; IFA: indirect immunofluorescence assay.

[a] For the intervention group intensified serological follow-up and pregnancy monitoring with possible antibiotic treatment were performed during pregnancy under supervision of secondary healthcare. For the control group serological follow-up was performed after pregnancy in collaboration with the patients' general practitioner.

University Medical Center Groningen. All participants gave written informed consent.

The study was set in Q fever high-risk areas in the Netherlands. High-risk areas were defined as municipalities with a Q fever incidence of more than 50 cases per 100,000 inhabitants in 2009 or more than 20 cases per 100,000 inhabitants in the first half of 2010, according to the official Dutch surveillance data [11].

Randomisation

Randomisation was stratified by the number of goat farms in the municipality (up to seven or more than seven), a measure of the risk associated with contracting a *C. burnetii* infection according to a study by van der Hoek et al. [12], and by the size of the midwife centre (up to 300 or more than 300 pregnant women under care per year). Since this was an open-label study, midwives, other healthcare workers, participants and the researchers were aware of the outcome of the randomisation.

Inclusion and exclusion criteria for participants

Pregnant women, 18 years of age or older, with an estimated date of delivery between 1 June and 31 December 2010, supervised by a midwife in primary healthcare

were eligible for inclusion. In the Netherlands, midwives working in primary healthcare are only allowed to supervise low-risk, singleton pregnancies. Using this criterion, women with a known increased risk for complicated pregnancy outcome beforehand e.g. twin pregnancies or pregnant women with chronic illnesses, were excluded. Moreover, women who did not have access to Internet or an email address were also excluded because data collection was web-based. In the Netherlands, 91% of the households have Internet access [13]. The remaining 9% consist of elderly or single occupants, so very little exclusion from this restriction was expected. In addition, women who were unable to understand Dutch, unable to give informed consent, or were already diagnosed with Q fever, were ineligible for participation in the study. Since (diagnostic) testing was not performed on a regular basis before the study, very little exclusion from this restriction was expected also.

Intervention group

Participants in the intervention group were asked for a serum sample between 20 and 32 weeks of gestation to be screened for infection with *C. burnetii*. The samples were analysed immediately by indirect immunofluorescence assay (IFA) in the laboratory of the Jeroen Bosch Hospital, 's-Hertogenbosch, the Netherlands. Both immunoglobulin (Ig)M and IgG against *C. burnetii* phase I and phase II antigens (Nine Mile strain) were measured according to the manufacturer's instructions (Focus Diagnostics, Cypress, CA, USA). Each run included a positive and a negative control. For every positive sample the titre was determined to reduce the chance of false positivity. In line with the cut-off values used in the clinical setting for the diagnosis of Q fever in symptomatic patients, titres ≥1:32 were considered positive [14]. Whenever there was IgM seropositivity, follow-up was performed two to four weeks after the screening sample had been taken. A probable acute infection was defined as the presence of positive titres of IgM (phase I and/or II) in the first screening sample. A proven acute infection was defined as positive titres for IgM accompanied with (rising) titres of IgG phase I and/or II during follow-up. A previous infection was defined as the presence of only IgG (phase I and/or II) in the screening sample. A probable chronic *C. burnetii* infection was defined as an antibody titre of IgG phase I ≥ 1:1,024 [15].

In seronegative women, standard care was provided. In case of a (probable) acute or chronic *C. burnetii* infection, women were referred to an obstetrician and intensified serological and obstetric follow-up according to the local hospital protocol took place. Antibiotic treatment (cotrimoxazole (960 mg twice daily) or erythromycin (500 mg twice daily to four times a day, depending on the term of pregnancy) for at least five weeks) was started in collaboration with the local medical microbiologist in any case of a proven acute or chronic infection. In case of a previous infection, no treatment was started, but serological analysis was repeated in the third trimester of pregnancy to exclude reactivation or chronic infection.

Control group

Women in the control group were also asked for a serum sample between 20 and 32 weeks of gestation. These samples were centrally stored in the laboratory of the Jeroen Bosch Hospital at -20ºC and were analysed for antibodies against *C. burnetii* after delivery, as the intervention group. In this group, distinguishing a probable and proven acute infection was impossible since follow-up serology during pregnancy was not performed. In case of a positive test, the participant's general practitioner was advised to perform an extra serological analysis after delivery to exclude a probable chronic infection.

Both groups

If symptoms compatible with Q fever occurred during pregnancy, these participants were advised to visit a physician for regular diagnostics.

Outcome measures

The primary endpoint of the study pertained to the individual level and was a composite measure of a maternal or obstetric complication in seropositive women. A maternal complication was defined as a serological profile suggesting a probable chronic infection. Obstetric complications included preterm delivery (defined as delivery <37 weeks of gestation), a child small for gestational age (defined as birth weight <10th percentile [16]), and perinatal mortality (defined as foetal or neonatal death between 22 weeks of gestation and one week post partum).

Secondary endpoints were the separate components of the composite measure and maternal fatigue and quality of life one month post partum. Fatigue was assessed using the 'Shortened fatigue questionnaire' [17]. Quality of life was assessed using the validated 'EQ5D questionnaire' [18].

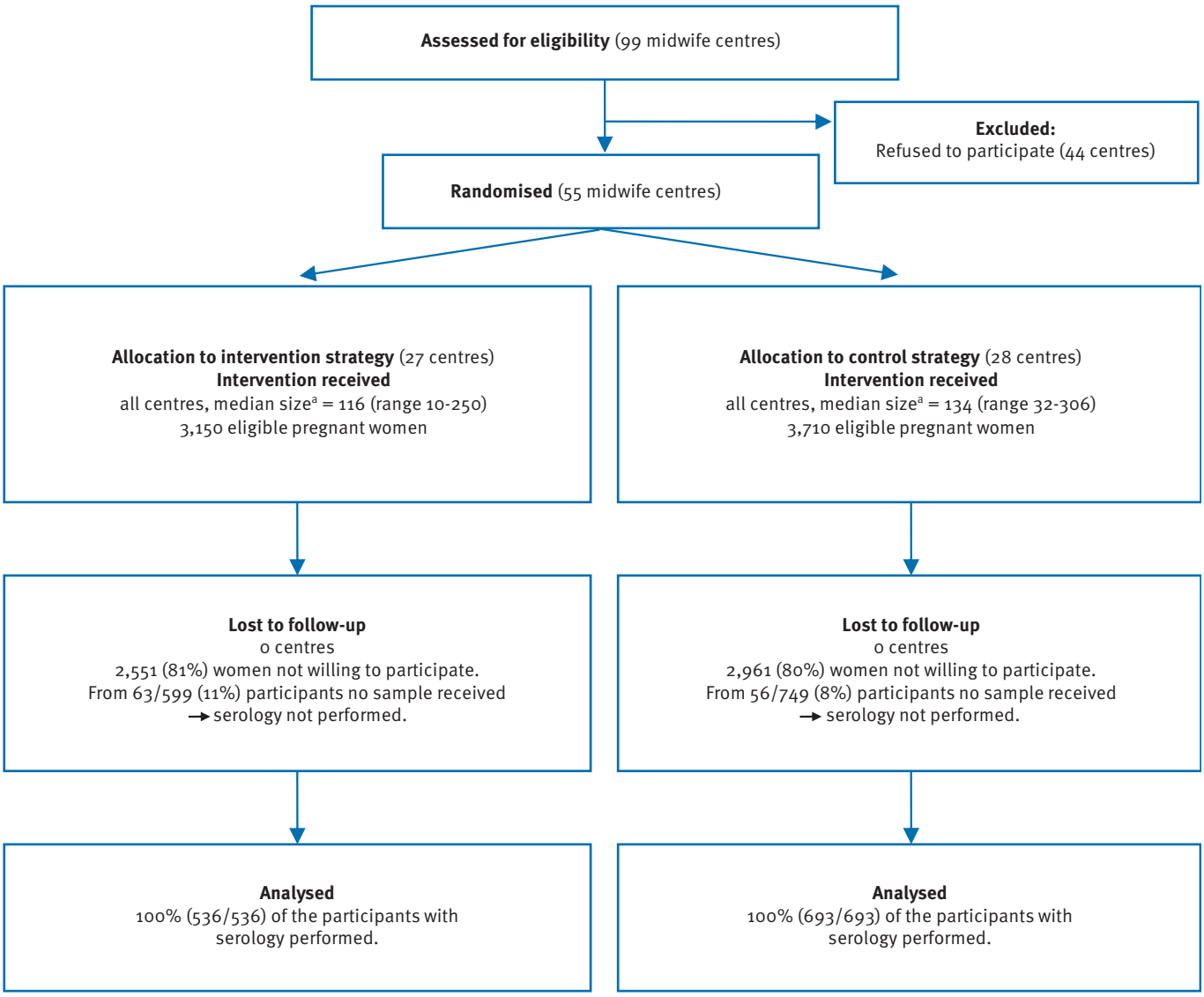
Sample size calculation

Since midwifery in primary healthcare follows strict protocols and serology was performed in one laboratory for all participants the presence of clustering in the infrequent primary outcome of the study was expected to be minimal. Therefore the sample size calculation was performed at the individual level.

Based on the literature and pilot data from the Netherlands, we expected that 12% of pregnant women in the Q fever high-risk areas would be seropositive [19,20]. Of these, we estimated at least 25% would have one of the previously defined complications. Thus, 3% of all pregnant women in Q fever high-risk areas would meet the primary outcome. A reduction of the complication rate by at least 50% as a consequence of early detection through screening during pregnancy was defined as clinically relevant. We considered reductions smaller than 50% unlikely to trigger a

Figure 2

Flow chart of the progress of clusters and participants, study on screening for *Coxiella burnetii* infection during pregnancy, the Netherlands, 2010 (n=1,229)



[a] Size of the midwife centre according to the number of eligible pregnant women under care.

change in practice given the implications on healthcare resources. Based on these expectations, we estimated needing at least 3,400 participants with complete follow-up to achieve a statistical power of 80% (two-sided α=0.05).

Statistical methods

Data were analysed according to intention-to-screen principle. Baseline demographic information was summarised by group using frequencies with percentages for categorical variables and means with standard deviations for continuous variables. Odds ratios (OR) and corresponding 95% confidence intervals (CI) were calculated using generalised linear mixed models (GLMM) to adjust for possible clustering effects. For continuous variables the mean difference with 95% CI was calculated. For the primary endpoint also the crude OR with 95% CI was calculated using binary logistic regression analysis, to provide an indication of the extent of clustering. A two-sided p value of 0.05 or less was defined as being statistically significant. Statistical analyses were performed using R version 12.1 and PASW Statistics version 18.0 (SPSS inc. Chicago, Illinois, USA).

Table 1

Baseline characteristics of the clusters (n=55) and participants, study on screening for *Coxiella burnetii* infection during pregnancy, the Netherlands, 2010 (n=1,229)

	Intervention group (%)	Control group (%)
Midwife centre characteristics		
Number	27	28
Size		
≤300 women per year	14 (52)	13 (46)
>300 women per year	13 (48)	15 (54)
Goat farms in municipality		
≤7	13 (48)	14 (50)
>7	14 (52)	14 (50)
Participant characteristics		
Number	536	693
Age (in years) mean± SD	31.9 ± 3.8	31.7 ± 3.7
Nulliparous	252 (47)	295 (43)
Ethnic origin non-western[a]	14 (2.6)	12 (1.7)
Level of education[b]		
Low	29 (5.4)	49 (7.1)
Medium	177 (33)	228 (33)
High	319 (60)	411 (59)
Other/Unknown	11 (2.1)	5 (0.7)
Maternal smoking during pregnancy	54 (10)	54 (7.8)
Body mass index (kg/m^2) mean± SD[c]	23.8 ± 3.7	24.1 ± 4.0
Primary hypertension	5 (0.9)	3 (0.4)
Hypothyroidism	6 (1.1)	11 (1.6)
History of preterm delivery	20 (3.7)	24 (3.5)
History of miscarriage[d]		
None	411 (77)	550 (79)
One	97 (18)	115 (17)
Repeated	27 (5.0)	27 (3.9)
Gestational age (weeks) moment of sampling mean± SD	28.7 ± 4.7	29.9 ± 4.8
Coxiella burnetii seropositive	82 (15)	101 (15)

[a] Non-western is defined as any ethnic background other than Western-Europe, North-American or Australian.

[b] Low: no formal education, primary school, lower-middle secondary school and lower professional school; medium: medium professional school and higher secondary school; high: higher professional school and university.

[c] Prior to pregnancy.

[d] n=535 for intervention group and n=692 for control group.

Results

Between March 16 and July 17, 2010, 55 of the 99 eligible midwife centres were willing to participate and were randomised: 27 to the intervention and 28 to the control strategy (Figure 2). In total, these centres supervised 6,860 eligible pregnant women of whom 1,348 (20%) signed informed consent. Among these women a blood sample was collected for 1,229 participants: 536 participants (44%) in the intervention group and 693 (56%) in the control group. At the moment of screening, none of the participants suffered from clinical signs of symptomatic Q fever [4], such as pneumonia or hepatitis.

Of 119 participants no blood sample was received, either because they forgot to give a sample or because the sample was lost. These women were excluded from the analysis since the primary outcome measure could not be determined. Of 104 participants in the intervention group and 196 participants in the control group, the sample was taken outside the protocol period, i.e. before 20 weeks of gestation (n=7 and n=5,

Table 2

Complications in seropositive participants, study on screening for *Coxiella burnetii* infection during pregnancy, the Netherlands, 2010 (n=1,229)

	Intervention group		Control group		Unadjusted OR[a] (95% CI)	P value[a]	Adjusted OR[b] (95% CI)	P value[b]
	Total n=536 (%)	Seropositives n=82 (%)	Total n=693 (%)	Seropositives n=101 (%)				
Overall complication[c]	12 (2.2)	12 (14.6)	10 (1.4)	10 (9.9)	1.56 (0.67-3.65)	0.30	1.54 (0.60-3.96)	0.37
Preterm delivery	8 (1.5)	8 (9.8)	5 (0.7)	5 (5.0)	2.09 (0.68-6.41)	0.20	1.80 (0.37-8.72)	0.47
Small for gestational age	4 (0.7)	4 (4.9)	5 (0.7)	5 (5.0)	1.04 (0.28-3.87)	0.96	1.04 (0.28-3.87)	0.96
Perinatal mortality	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	Not applicable			

CI: confidence interval; OR: odds ratio.

[a] Crude odds ratio and p value calculated with binary logistic regression analysis.
[b] Odds ratio and p value calculated with generalised linear mixed models, taking into account a clustering effect.
[c] Primary outcome measure.

Table 3

Fatigue and quality of life one month post partum for all participants, study on screening for *Coxiella burnetii* infection during pregnancy, the Netherlands, 2010 (n=1,229)

	Intervention group n=536 (%)	Control group n=693 (%)	OR (95% CI)[a]	Mean difference[a] (95% CI)	P value[a]
Fatigue score mean±SD[b]	14.6 ± 5.7	13.5 ± 5.5	NA	1.08 [0.43-1.72]	<0.001
Quality of Life[c]					
Mobility ≥ 2	58 (12)	86 (14)	0.86 (0.60-1.23)	NA	0.42
Self-care ≥ 2	3 (0.6)	3 (0.5)	1.31 (0.26-6.50)	NA	0.75
Usual activities ≥ 2	74 (15)	99 (16)	0.97 (0.70-1.35)	NA	0.85
Pain/discomfort ≥ 2	132 (27)	179 (28)	0.94 (0.72-1.24)	NA	0.68
Anxiety/depression ≥ 2	27 (5.5)	38 (6.0)	0.92 (0.56-1.53)	NA	0.75
EQ VAS±[d]	80.1 ± 11.6	81.4 ± 12.1	NA	1.18 (-0.39-2.75)	0.14

CI: confidence interval; NA: not applicable; OR: odds ratio.

[a] Odds ratio, mean difference and p value calculated with generalised linear mixed models, taking into account a clustering effect.
[b] n=506 and 662 for the intervention and control group, respectively. Range of the score from 4 (not fatigue) to 28 (extreme fatigue).
[c] First part of the 'EQ5D' questionnaire, n=488 and 636 for the intervention and control group, respectively. Score of 1=no problems, 2= with any problems, 3=with major problems.
[d] Second part of the 'EQ5D' questionnaire, self-reported health score on a scale from 0 to 100, score of 100=best imaginable health state, score 0= worst imaginable health state. Participants with a score lower than 11 were excluded (n=30), since a mistake while filling out was assumed.

Table 4

Pregnancy outcome for seropositive versus seronegative participants[a], study on screening for *Coxiella burnetii* infection during pregnancy, the Netherlands, 2010 (n=1,229)

	Seropositive n=183 (%)	Seronegative n=1,046 (%)	OR (95% CI)[b]	Mean difference[b] (95% CI)	P value[b]
Gestational age at delivery (in weeks) mean±SD	39.6 ± 1.8	39.7 ± 1.7	NA	0.12 (-0.15-0.38)	0.38
Preterm delivery <37 weeks	13 (7.1)	58 (5.5)	1.30 (0.70-2.43)	NA	0.41
Preterm delivery <34 weeks	3 (1.6)	13 (1.2)	1.32 (0.37-4.69)	NA	0.66
Birth weight (in grams) mean±SD	3,512 ± 527	3,507 ± 546	NA	4.8 (-81-90)	0.91
Small for gestational age	9 (4.9)	78 (7.5)	0.64 (0.32-1.30)	NA	0.22
Perinatal mortality	0 (0.0)	6 (0.6)	NA		0.60[c]
Overall complication[d]	22 (12)	133 (13)	0.94 (0.58-1.52)	NA	0.79

CI: confidence interval; NA: not applicable; OR: odds ratio.

[a] Using a cut-off titre of ≥1:32

[b] Odds ratio, mean difference and p value calculated with generalised linear mixed models, taking into account a clustering effect.

[c] Calculated with Fisher's exact test, since generalised linear mixed models could not provide a p value.

[d] Composite measure of any preterm delivery, small for gestational age, or perinatal mortality.

respectively) or after 32 weeks of gestation (n=97 and n=191). However, there was no difference in the baseline and outcome variables between the participants with and without this protocol deviation (data not presented, available from authors on request), hence they were included in the analysis.

Baseline characteristics

Baseline characteristics are shown in Table 1. The mean gestational age at the time of sampling was 28.7 weeks for the intervention group and 29.9 weeks for the control group. Fifteen per cent of the women in both groups were seropositive for *C. burnetii* in the first sample taken. Fifty-two of the 1,229 participants had a probable acute infection: 30 (5.6%) in the intervention group and 22 (3.2%) in the control group; 131 participants had a previous infection: 52 (9.7%) in the intervention group and 79 (11.4%) in the control group. After follow-up, seven women in the intervention group (1.3%) were confirmed as having an acute *C. burnetii* infection and antibiotic treatment was started at a median stage of pregnancy of 28 weeks (range 22-36 weeks) for a duration of one to five weeks, depending on the serological follow-up and term of pregnancy. In the other 23 patients (77%) with a probable acute infection, follow-up serology ruled out this suspicion and was consistent with a previous infection. Follow-up showed no cases of probable maternal chronic infections in either of the two groups, so only obstetric complications in seropositive women were recorded as an endpoint. None of the women in the intervention or control group were treated with antibiotics during pregnancy for symptomatic Q fever.

Primary endpoint

For all the participants the primary outcome measure was available. There was no difference in the primary endpoint between the intervention and the control group (Table 2); the risk estimate obtained from the clustered analysis for an obstetric complication in seropositive women in the intervention group compared with the control group was 1.54 (95% CI 0.60-3.96). The non-clustered analysis showed a similar OR of 1.56 (95% CI 0.67-3.65). There were six cases of perinatal mortality: four foetal deaths and two early neonatal mortalities. For all of them the mothers were seronegative.

Secondary endpoints

Analyses of the separate components of the composite measure showed that the difference in the primary endpoint in favour of the control group, though non-significant, seemed to be the result of a small difference in the risk of preterm delivery (Table 2).

The fatigue score one month post partum was approximately 1 point higher in the intervention group compared with the control group (14.6 versus 13.5, p<0.001). Quality of life did not differ between the two groups (Table 3).

Explorative analysis showed that *C. burnetii* seropositivity during pregnancy, even when the cut-off titre for seropositivity was increased to ≥1:64 (data not shown),

was not associated with gestational age at delivery, birth weight or any of the defined obstetric complications (Table 4). Of the seven women in the intervention group with an acute infection, two delivered preterm and one delivered a child small for gestational age.

Discussion

We showed that, during a Q fever outbreak, large-scale routine serological screening for *C. burnetii* infection during pregnancy starting at 20 weeks of gestation seemed not to be associated with a relevant reduction in obstetric complications in seropositive women. Therefore, our data do not support such a preventive programme. This result was due to the low incidence of acute *C. burnetii* infection (1.3%), the absence of patients with a probable chronic infection and the fact that *C. burnetii* seropositivity was not associated with adverse pregnancy outcomes.

Surprisingly, we observed that participants of the intervention group had a somewhat higher fatigue score one month post partum than controls. Although the clinical relevance may be questionable, other screening strategies for infectious diseases during pregnancy have shown that screening for, and therefore awareness of, infectious diseases may induce negative psychological effects [21]. Importantly, despite the fact that our study was performed in a Q fever high-risk area and participation of midwife centres was satisfactory (56%), the participation rate of pregnant women was unexpectedly low (20%). Although it's likely that this low percentage reflects a reluctance to take part in a randomised controlled trial, this might also indicate that the acceptance of such a preventive programme among this group might not be straightforward. From an earlier study on this topic we learned that women's appraisal of programme efficacy and convenience, their knowledge about the disease and perceived Q fever risk is crucial for their intended programme uptake [22].

Since three out of seven women with an acute *C. burnetii* infection in the intervention group had a complication, monitoring of pregnant women diagnosed with Q fever is still advisable and counselling about treatment should be performed. Further studies on monitoring and treatment, especially of symptomatic infected pregnant women, are needed.

Strengths and limitations

A major strength of our study is that it is the first randomised, prospective study in a community based - non-selected - pregnant population focusing on the effectiveness of routine screening for *C. burnetii* infection. Since the Dutch Q fever outbreak between 2007 and 2010 was unique in its magnitude and duration, we had the opportunity to perform this study in a high-risk area. However, probably due to the drastic veterinary measures taken by the Dutch government, the incidence of acute *C. burnetii* infections steeply declined since 2010 [10]. Inclusion of participants after the second half of 2010 would not have been informative and was perceived as unethical. Therefore, we did not reach our projected number of participants, which increases the risk of a type II error. However, this risk seems to be minimal, because the lower estimate of the 95% CI of the primary outcome (OR 0.60) precludes the a priori defined 50% risk reduction in relevant outcomes.

There are also some further limitations to address. In this study screening started at 20 weeks of gestation. There are two main reasons why we chose this design. First of all, we aimed to avoid treatment with a drug (cotrimoxazole) that is not completely investigated during the most vulnerable phase of pregnancy [23]. Earlier screening and withholding treatment until 20 weeks of gestation was perceived as unethical and therefore not an option. Secondly, at 20 weeks of gestation pregnant women could combine the venepuncture for this study with a structural ultrasound, which is offered to all pregnant women in the Netherlands. With this we intended to increase the participation rate. Because of this design, screening in the first trimester of pregnancy is still untested, and effectiveness of such a strategy cannot be excluded. However, a recent Danish study showed no association between *C. burnetii* infection and spontaneous abortion up to 22 weeks of gestation [24], indicating that screening earlier in pregnancy would probably also be ineffective.

Given that 44% of the eligible midwife centres and 80% of the eligible pregnant women were not willing to participate, it may not be possible to generalise the results. However, since major patient characteristics such as maternal age and proportion of nulliparous women are comparable with other large population based cohort studies from the Netherlands [25,26], we believe the degree of selection bias is minimal and our results are applicable to other Q fever outbreaks similar to the one in the Netherlands. Nevertheless pregnant women with a non-western ethnicity were underrepresented in our study population so our results should be interpreted with caution for this group, especially because it is known that the seroprevalence in pregnant women with a non-Dutch ethnic background is higher [27].

In the 119 women who signed informed consent, but from whom no blood sample was available, Q fever cases could have been missed. However, participant characteristics and complication rates in this group were similar to the group with a blood sample analysed; therefore the risk of selection bias seems to be low.

Serological screening during pregnancy in general is challenging. A high rate of false-positive tests has been described, especially for IgM assays [28,29]. Furthermore, the specificity of tests may be low if the incidence of the disease is relatively low and the prevalence is relatively high. Of every positive sample the

titre was determined and we performed serological follow-up of all IgM positive women to prevent treatment of false-positive acute cases.

In contrast to our results, previous studies reported a strong association between undetected and untreated *C. burnetii* infection during pregnancy and complicated pregnancy outcome [7,8,30]. One explanation for this might be that in the previous non-randomised studies, selection bias could have led to an overestimation of the risks. Otherwise, differences in pathogenicity between different *C. burnetii* strains could exist. Genotyping of Dutch samples is ongoing [31]. Since in the Netherlands a relatively high number of chronic Q fever cases have been described in patients with aneurysms [32], it could be hypothesised that the strains involved in the Dutch outbreak are highly virulent for people with underlying vascular diseases, while pregnant women are relatively protected [33]. However, further discussion on this topic is beyond the scope of this paper.

There are also studies in line with our results. In three large studies conducted in Q fever high-risk areas in Denmark, the Netherlands and France no association between seropositivity and complicated pregnancy outcome was found [24,27,34].

Conclusions

This clustered randomised controlled trial showed that 15% of the pregnant women in a Q fever outbreak area were seropositive, but the incidence of acute *C. burnetii* infection was low. Although the broad confidence interval did not exclude a small beneficial effect of screening, routine screening during pregnancy starting at 20 weeks of gestation seems not to be associated with a relevant reduction of obstetric complications in seropositive women. Therefore, in the current setting, this study does not support such a preventive programme.

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Conflict of interest

None declared.

Authors' contributions

JMM participated in the design of the study, performed the study, analysed and interpreted the data and drafted the manuscript; ACAPL participated in the design of the study as an expert on laboratory testing and performed the diagnostic analyses; CJCMH participated in the design of the study as an expert on obstetric care; JCEM was responsible for the logistics surrounding laboratory testing; PMS participated in the design of the study as an expert on laboratory testing and performed the diagnostic analyses; WvdH participated in the design of the study as an expert on national public healthcare and performed the data on Q fever risk areas; AR participated in the design of the study as an expert on local health care in the most affected areas of the Netherlands; EdV participated in the design of the study as an expert on paediatrics; RPS participated in the design of the study as an expert on epidemiology; JGA participated in the design of the study as an expert on obstetric care and supervised the analysis and report; EH initiated and designed the study, wrote the grant application, and supervised the data collection, analysis and report. All authors revised the draft manuscript and approved the final manuscript.

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No increase in primary nosocomial candidemia in 682 German intensive care units during 2006 to 2011

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We evaluated the epidemiology of and trends in primary nosocomial candidemia within a network of 682 German intensive care units (ICUs) during 2006 to 2011. Nosocomial laboratory-confirmed bloodstream infection (NLCBI) was diagnosed using standard definitions from the United States Centers for Disease Control and Prevention. Incidences were calculated by NLCBI per 1,000 patients and incidence densities per 1,000 patient-days and per 1,000 central-line days. In the 682 ICUs, there were 2,220,803 patients, 7,943,615 patient-days and 5,363,026 central-line days. A total of 381 of the 6,666 NLCBIs were associated with *Candida albicans*, 142 with non-*albicans Candida*. Non-*albicans Candida* made up 26% of all the *Candida* isolates. The mean incidence density of *Candida* central line-associated NLCBIs was 0.09 per 1,000 central-line days and remained unchanged between 2006 and 2011. Crude ICU mortality was 21.9% for *C. albicans* and 29.7% for non-*albicans Candida*. *Candida* was the fourth leading cause of primary NLCBIs, accounting for 6.5% of all bloodstream infections acquired in ICUs. Based on an incidence density of 0.07 per 1,000 patient-days, extrapolation of our data resulted in 465 primary nosocomial *Candida* NLCBIs in German ICUs per year. Our data show that there was no increase in primary *Candida* NLCBIs during 2006 to 2011.

Introduction

Candida species are frequently isolated in nosocomial bloodstream infections [1]. Depending on the geographical region, *Candida* is the third to the tenth most commonly isolated pathogen in blood cultures [2-4].

Candida spp. are common inhabitants of the mucosal surfaces in the tracheal, gastrointestinal and genitourinary tracts. In most cases, candidemia is deemed to arise endogenously, preceded by colonisation with the infecting strain [5]. Patients in an intensive care unit (ICU) are at particular risk for candidemia because of their debilitated condition, presence of central lines and the fact they are often subject to renal dialysis or receipt of broad-spectrum antibiotics or parenteral nutrition [6].

Bloodstream infections can be either primary or secondary [7]. Most primary infections are due to colonised intravascular catheters. Colonisation occurs from bacteria embedded in a biofilm matrix, originating mostly from a patient's skin microflora.

Secondary infections are disseminated from infections acquired at other sites, such as the peritoneum, urinary tract, lung, postoperative wounds and skin.

The mortality rate of infected persons due to candidemia varies considerably, from 20% to 60% [8-11]. The prognosis is better for primary than for secondary candidemia [7]. Expanded use of antifungals has probably influenced the temporal trends of primary and secondary candidemia, as has the species causing candidemia [3,12].

Antifungals are widely advertised and prescribed for therapy and prophylaxis. The pharmaceutical industry valued the global market for human antifungal therapeutics at USD 9.8 billion (EUR 7.6 billion) in 2009 and expected it to increase at a compound annual growth rate of approximately 3.8% to reach USD 11.3 billion (EUR 8.8 billion) in 2014 [13].

In Germany, hospitals are obliged to collect and analyse data on nosocomial infections and drug-resistant pathogens [14]. These routine data are reported to the National Reference Centre for the Surveillance of Nosocomial Infections.

The aim of our study was to evaluate the epidemiology of and analyse the trends in primary nosocomial candidemia within a network of 682 German ICUs between 2006 and 2011.

Methods

Study population

The Krankenhaus Infektions Surveillance System is a voluntary, web-based national surveillance system for nosocomial infections in Germany, to which about a quarter of all German ICUs report data [15]. From

January 2006 to December 2011, all ICU patients who developed nosocomial laboratory-confirmed bloodstream infections (NLCBIs) were included in our study. A total of 682 ICUs reported the type of ICU, and size and type of hospital. Of these, 365 ICUs were interdisciplinary, 119 surgical, 109 medical, 17 neurosurgical, 17 paediatric, 16 cardiac surgical, 10 neurological and 29 other ICUs.

For every patient with an NLCBI, the time from admission to the ICU to time of onset of infection is reported, as is sex, age, central-line use within 48 hours before the infection, type of pathogen (up to four pathogens) and mortality. The onset of infection is defined as either the onset of the first clinical symptom or the day the samples were taken for microbiology cultures that led to diagnosis of the infection. The earlier date is defined as the onset of infection. All ICUs also report the number of central central-line days and patient days.
ICUs report only primary NLCBIs [16].

NLCBIs were defined as central-line associated if a central line was in place at the time of, or within 48 hours before, the onset of the infection.

Fungal pathogens were reported as *C. albicans*, non-albicans spp., *Aspergillus* spp. or other fungi and could be isolated in a blood culture as the only pathogen (monomicrobial case) or as one of several pathogens (polymicrobial case).

As the frequency of NLCBIs might be biased because of different types of ICUs, the type of hospital and, most importantly, by the frequency of microbiological diagnostics (blood cultures) we analysed changes over time for all ICUs and also for a subgroup of ICUs that participated continuously over the six years.

Definition of nosocomial primary candidemia

The Krankenhaus Infektions Surveillance System uses the United States Centers for Disease Control and Prevention (CDC) standard definitions [16]. ICUs reported only primary NLCBIs [17]. Nosocomial primary candidemia was defined as occurring in an ICU patient without signs and symptoms of infection at the time of admission to the ICU, with one or more blood cultures positive for *Candida*, while candidemia was not related to *Candida* infection of another site.

Statistics

Incidence was calculated as the number of NLCBIs per 1,000 patients. Incidence density was calculated as the number of NLCBIs per 1000 patient days or per 1,000 central-line days. Time from ICU admission to onset of infection and crude ICU mortality was calculated for monomicrobial cases only.

All analyses were performed using SPSS (IBM SPSS statistics, Somer, NY, United States), SAS (SAS Institute, Cary, NC, United States) and Epi Info 6 (CDC, Atlanta, GA, United States).

Results

From January 2006 to December 2011, 682 German ICUs submitted data to the Krankenhaus Infektions Surveillance System. A total of 2,220,803 patients were included, accounting for 7,943,615 patient days, 5,363,026 central-line days and a median length of stay of 3.6 days (interquartile range (IQR): 2.8–5.0). ICUs submitted data for a median of 39 months (IQR: 18–64). The number of ICUs increased over time, from 347 in 2006 to 455 in 2009 and to 527 in 2011. A total of 205 ICUs submitted the data continuously from 2006 to 2011.

A total of 6,666 NLCBIs associated with 7,453 pathogens were reported. Among the 6,666 NLCBIs, 5,970 (90%) were monomicrobial cases while the rest were polymicrobial (618 cases with two pathogens, 65 with three and 13 with four). A total of 6,382 (96%) NLCBIs were central-line associated.

Fungi were isolated 575 times from 563 (8%) of the NLCBIs. Of these 575, a total 381 (66%) were associated with *C. albicans* and 142 (25%) with non-*albicans Candida*. Some 288 (76%) of the cases with *C. albicans* infection were monomicrobial.

The mean incidence density of the NLCBIs stratified by pathogen type in 2006 to 2011 is shown (Figure 1). In 4,591 (69%) of the NLCBIs, Gram-positive bacteria were reported, in 1,458 (22%) Gram-negative bacteria and in 563 (8%) fungi. If only monomicrobial NLCBIs (n=5,960) were analysed, Gram-positive bacteria were the causative agent in 4,021 (67%), Gram-negative bacteria in 1,109 (19%) and fungi in 428 (7%).

The mean incidence density of fungal NLCBIs per 1,000 patient days did not change significantly between 2006 and 2011 (0.09 (95% CI): 0.07–0.11) in 2006; 0.08 (95% CI: 0.07–0.10) in 2011 (Figure 1).

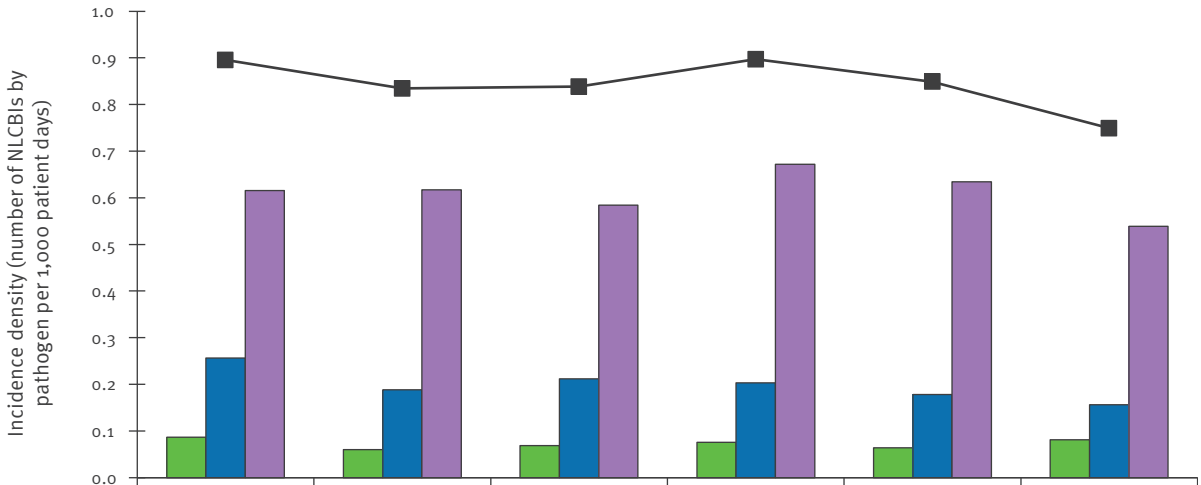
The incidence of fungal NLCBIs per 1,000 patients also did not change significantly between 2006 and 2011. It was 0.30 in 2006 (95% CI: 0.24–0.37) and 0.29 in 2011 (95% CI: 0.24–0.35).

With respect to candidemia, the mean incidence density of *Candida* spp. from 2006 to 2011 was 0.07 per 1,000 patient-days (Figure 2A) and of the central-line associated NCBLIs, it was 0.09 per 1,000 central-line days (Figure 2B).

The mean incidence density of *Candida* spp. revealed no significant difference over time. It was 0.08 per 1,000 patient-days in 2006 (95% CI: 0.06–0.10) and 0.07 per 1,000 patient-days in 2011 (95% CI: 0.06–0.09) (Figure 2A).

Figure 1

Mean incidence density of all nosocomial primary laboratory-confirmed bloodstream infections (n=6,666) and associated microorganisms (n=7,453) in 682 intensive care units, Germany, 2006–2011



	2006	2007	2008	2009	2010	2011
Fungi	0.09	0.06	0.07	0.08	0.06	0.08
Gram-negative bacteria	0.26	0.19	0.21	0.20	0.18	0.16
Gram-positive bacteria	0.62	0.62	0.58	0.67	0.63	0.54
All NLCBIs	0.90	0.83	0.84	0.90	0.85	0.75

NLCBIs: nosocomial laboratory-confirmed bloodstream infections.

Gram-positive bacteria: *Staphylococcus aureus*, coagulase-negative staphylococci, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Enterococcus* spp., *Corynebacterium* spp.

Gram-negative bacteria: *Haemophilus* spp., *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., *Proteus* spp., *Serratia* spp., other *Enterobacteriacea*, *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Stenotrophomonas maltophilia*, *Acinetobacter* spp., *Bacteroides* spp., *Legionella* spp.

Fungi: *Candida albicans*, non-*albicans* Candida spp., *Aspergillus* spp., other fungi.

The mean incidence density of NLCBIs with non-*albicans Candida* per 1,000 patient days was 0.02 in 2006 (95% CI: 0.01–0.03) and 0.02 in 2011 (95% CI: 0.01–0.03) and of *Candida albicans* 0.06 in 2006 (95% CI: 0.05–0.08) and 0.05 in 2011 (95% CI: 0.04–0.07).

If only ICUs with continuous participation over all six years were included in the analysis (n=205), there was also no significant change over time in the *Candida* incidence in this subgroup analysis (data not shown).

For monomicrobial NLCBIs, the length of stay in the ICU before onset of infection differed, depending on the pathogen. The median time was generally shorter for Gram-positive pathogens (13–16 days) than for the Gram-negative pathogens *Klebsiella* spp. and *Pseudomonas aeruginosa* (18 and 19 days, respectively) and was 15 days for *C. albicans* (Figure 3).

C. albicans ranked fourth among the most frequently isolated pathogens in NLCBIs, after coagulase-negative staphylococci, *Enterococcus* spp. and *Staphylococcus aureus*. *C. albicans* accounted for 4.8% of all NLCBIs and all *Candida* spp. for 6.5% (Table 1). Non-*albicans Candida* made up 26% of all *Candida* spp. With respect to crude ICU mortality of NLCBIs *Candida* spp. took second place, with a mortality of 23.9% after *P. aeruginosa* with 24.5%.

We extrapolated the data of our study of *Candida* NLCBIs: it resulted in 465 primary nosocomial *Candida* NLCBIs in German ICUs (based on an incidence density of 0.07 per 1,000 patient days and a total of 7,042,898 ICU-patient days in 2008) [18,19].

The reported number of cases and incidence per 100,000 population of candidemia in countries with nationwide, coded-discharge diagnosis or a laboratory-based notification system (Denmark, Finland, Germany, and United States) is shown in Table 2.

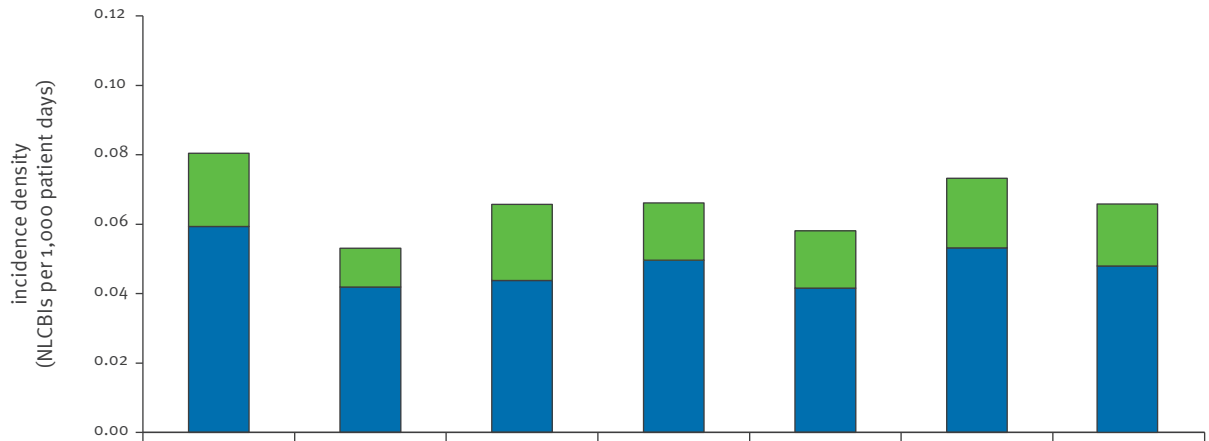
Discussion

The most important finding of our multicentre study of German ICUs was that the mean incidence density

Figure 2

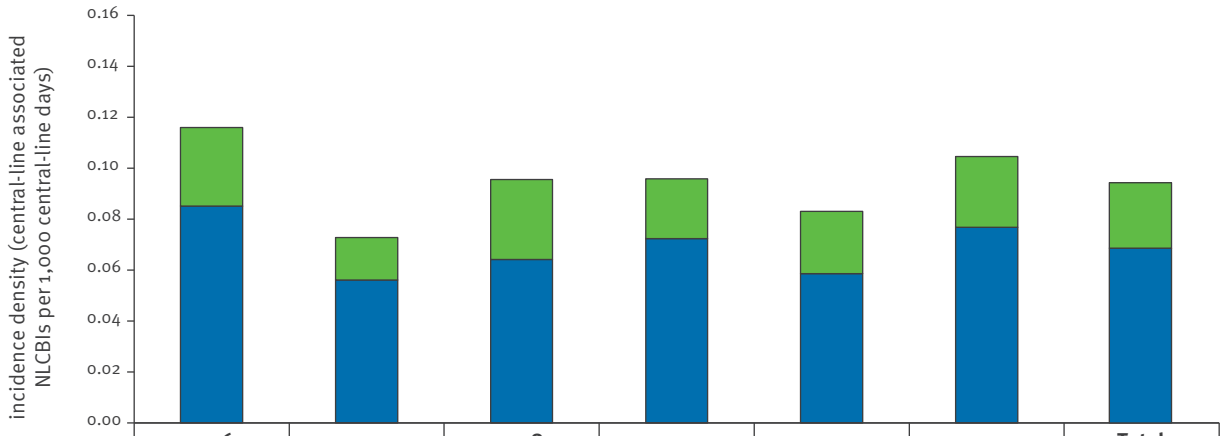
Mean incidence density of the number nosocomial primary laboratory-confirmed bloodstream (NLCBIs) infections per 1,000 patient days (panel A) or central-line associated NLCBIs per 1,000 central-line days (panel B) with *Candida albicans* and non-*albicans Candida*[a] species in 682 intensive care units, Germany, 2006–2011

A



	2006	2007	2008	2009	2010	2011	Total
Candida albicans	0.06	0.04	0.04	0.05	0.04	0.05	**0.05**
non-*albicans Candida*	0.02	0.01	0.02	0.02	0.02	0.02	**0.02**
All *Candida* spp.	**0.08**	**0.05**	**0.07**	**0.07**	**0.06**	**0.07**	0.07

B



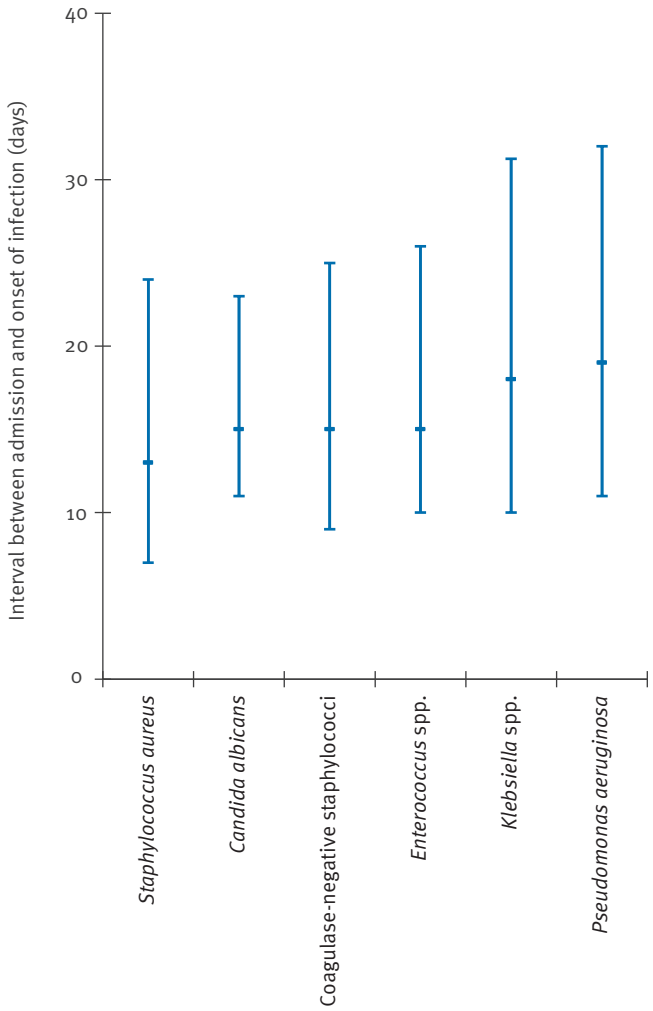
	2006	2007	2008	2009	2010	2011	Total
Candida albicans	009	006	006	007	006	008	**0.07**
non-albicans *Candida*	0.03	0.02	0.03	0.02	0.02	0.03	**0.03**
All *Candida* spp.	**0.12**	**0.07**	**0.10**	**0.10**	**0.08**	**0.10**	0.09

Note: numbers may not sum up because of rounding to the second decimal place.

[a] Mono- and polymicrobial cases.

Figure 3

Interval between date of admission to the intensive care unit (ICU) and onset of infection of the most frequently isolated pathogens in monomicrobial nosocomial laboratory-confirmed primary bloodstream infections in 682 ICUs, Germany, 2006–2011



The median and interquartile range are depicted.

of *Candida* spp. central line-associated bloodstream infections was 0.09 per 1,000 central-line days from 2006 to 2011 and remained unchanged during this time. Furthermore, *C. albicans* was the fourth leading cause of primary NLCBIs in 682 German ICUs, accounting for 4.8% of all bloodstream infections acquired in the ICUs and it remains an important infection.

Data from the European Centre for Disease Prevention and Control (ECDC) reported that of the most frequently isolated microorganisms in ICU-acquired bloodstream infections in 11 European countries, the proportion of *Candida* spp. was 6.3% in 2004, which increased to 7.5% in 2006 and decreased again to 6.3% in 2008 [20].

Data from 1,116 ICUs reporting to the United States National Nosocomial Infection Surveillance System showed that the incidence density of NLCBIs due to *Candida* decreased significantly from more than 0.9 NLCBI per 1,000 central-line days in 1989 to about 0.35 per 1,000 central-line days in 1999 [21]. Data from 2006 to 2007 reported 0.6 NLCBI per 1,000 central lines (2,223,650 central-line days and 1,342 *Candida* isolates in NLCBIs) [2]. This shows that the pooled mean incidence density of *Candida* central-line-associated NLCBIs of 0.09 of our 682 German ICUs was several folds lower than that in United States ICUs. We cannot fully explain why the incidence densities in the United States and German ICUs were very different. Differences in healthcare systems should be taken into consideration and differences in the job description of medical staff might also contribute. Unlike in the United States, taking blood cultures cannot be delegated to nurses in Germany but have to be performed by physicians themselves. This might lead to underdetection of isolates that cause infections. The frequency of blood cultures per 1,000 patient days in German ICUs was considerably below the mean of all European ICUs (55 blood cultures per 1,000 bed days in German ICUs compared with 73 per 1,000 bed days in all European ICUs in 2004) [22].

In the United States, *Candida* was in 2008 the third leading pathogen responsible for NLBSIs, after coagulase-negative staphylococci and enterococci – ahead even of *S. aureus* and outnumbering all Gram-negative bacilli [2]. *C. albicans* and other *Candida* species account for 11.8 % (each 5.9%) to all central-line-associated bloodstream infections, according to data of the National Healthcare Safety Network [2].

Candida ranked second in the Extended Prevalence of Infection in the ICU (EPIC) II study, which included culture-positive infections in 1,265 ICUs in 75 countries in 2007 [23]. In contrast to our study, EPIC II focused on all infections (not only nosocomial); on all bloodstream infections (not only on primary bloodstream infections) and EPIC II was a prevalence study. Furthermore, the majority of all ICU infections in Western Europe were respiratory tract infections and only 14.8% were bloodstream infections. Only 8.2% of all bloodstream infections were caused by *Candida* if only monomicrobial bloodstream infections were analysed [9]. In our study, 6.5% were caused by *Candida*. *Candida* lies far behind the Gram-positive pathogens, coagulase-negative staphylococci, *S. aureus* and *Enterococci*.

Unfortunately, non-*albicans Candida* are not differentiated at species level in the Krankenhaus Infektions Surveillance System. However, the National Reference Centre for Systemic Mycoses published 2004–2005 data on the incidence and antifungal susceptibilities of *Candida* spp. in Germany: the majority of non-*albicans Candida* were *C. glabrata* (accounting for 44.9%), followed by *C. parapsilosis* (22.5%), *C. tropicalis* (15.2%), *C. kefyr* (5.1%) and *C. krusei* (3.9%) [24].

In our study, non-albicans accounted for only a quarter of all *Candida* species. In other words, the frequency of *C. albicans* was 74%, which was comparable with the 72% *C. albicans* in EPIC II in western European ICUs, whereas *C. albicans* was isolated less frequently in other geographical regions (e.g. only in 57% in Latin American ICUs) [9]. In a Swiss nationwide study, *C. albicans* remained the predominant *Candida* species recovered in 66% of all candidemias over a period of 10 years (1991-2000), which is also in accordance with our results [25]. Nonetheless, the predominance of *Candida* species differs geographically.

There are numerous studies demonstrating the shift from *C. albicans* to non-albicans species and describing the temporal and geographical influences on *Candida* species distribution [3,4,26]. Several factors may have contributed to these differences in species distribution and in frequency of isolation. They include attention to infection control, catheter-care guidelines and probably most importantly lack of drug pressure. The rise of non-albicans species is generally correlated with the therapeutic and prophylactic use of fluconazole [4]. Similar to antibiotic use, antimycotic use can be hypothesised to be higher in the United States, for example, because of a more defensive type of medicine with more calculated or prophylactic anti-infective therapy because of the high risk of medical malpractice lawsuit [27]. This might influence endogenous colonisation. Although there are scarce comparative quantitative data on antifungal consumption, also within Europe, antifungal use, risk groups and healthcare budgets vary largely [28,29]. In Denmark, for example, over the last years from 2004 to 2009, consumption increased by 140% [30].

Many studies state that candidemia is recognised as a leading cause of morbidity and mortality in severely ill patients and that crude (all-cause) mortality rates range between 20% and 60% [10]. We advocate differentiating between primary and secondary candidemia, because this has an impact on mortality rates, i.e. primary *Candida* bloodstream infections have lower mortality rates. It is of interest that non-*albicans Candida* species had the highest crude mortality rates (of almost 30%), which underlines the importance of early and standardised detection of *Candida* species and drug-susceptibility testing.

Nationwide data for candidemia from the German Institute for the Hospital Remuneration System (all hospitals in the country, both primary and secondary bloodstream infections) revealed that the number of patients with candidemia was 3,712 cases

Table 1

Most frequently isolated pathogens in 5,970 monomicrobial primary nosocomial laboratory-confirmed bloodstream infections and related crude mortality in 682 intensive care units, Germany, 2006–2011

Pathogen	Number (%) of NLCBIs	Number (%) of related ICU deaths
Coagulase-negative staphylococci	2,128 (35.6)	339 (15.9)
Staphylococcus aureus	895 (15.0)	150 (16.8)
MSSA	568 (9.5)	80 (14.1)
MRSA	327 (5.5)	70 (21.4)
Enterococcus spp.	954 (16)	194 (20.3)
Candida spp.	389 (6.5)	93 (23.9)
C. albicans	288 (4.8)	63 (21.9)
non-*albicans Candida*	101 (1.7)	30 (29.7)
Klebsiella spp.	254 (4.3)	43 (16.9)
Pseudomonas aeruginosa	159 (2.7)	39 (24.5)
Escherichia coli	213 (3.6)	43 (20.2)
Enterobacter spp.	183 (3.1)	27 (14.8)
Serratia spp.	95 (1.6)	13 (13.7)
Acinetobacter spp.	60 (1)	14 (23.3)
Total	**5,970 (100)**	**1,077 (18.0)**

ICU: intensive care unit; NLCBIs: nosocomial laboratory-confirmed bloodstream infections; MRSA: meticillin-resistant *Staphylococcus aureus*; MSSA: meticillin-sensitive *Staphylococcus aureus*.

Table 2

Cases and incidence of candidemia in Denmark, Finland, Germany and United States

Data for the year specified	Candidemia			
	Denmark[a] 2009	Finland[b] 2007	Germany[c] 2008	United States[d] 2000
Number of cases	470	161	3,712	16,500
Incidence per 100,000 population	8.6	3.1	4.7	5.6

[a] Data from six departments of clinical microbiology, which serve a third of the Danish population [30].

[b] Data from the Finish National Infectious Disease Register, to which all clinical laboratories in Finland notify all fungal (and bacterial) isolates from blood [34].

[c] Data from the German Institute for the Hospital Remuneration System (InEK) identified by the presence of the *International classification of diseases, tenth revision* (ICD-10) diagnosis code B37.7. [31].

[d] Data from the United States Agency for Healthcare Research and Quality identified by the presence of the *International Classification of Diseases, Ninth Revision* (ICD-9) diagnosis code 112.5 [35].

– identified by presence of the International Statistical Classification of Diseases and Related Health Problems ICD-10 diagnosis code for candidemia – in 2008 in Germany (population of 82 million inhabitants) [31]. Use of these ICD-10 diagnosis codes seems unlikely to lead to the underestimation of the burden of candidemia, because they are required for reimbursement of hospital expenses. In the light of the results of our study, as well as remuneration data and increase in the consumption of antifungals, it seems reasonable to include antifungal use in antibiotic (or antimicrobial) stewardship programmes.

Our study has several strengths. The data result from a large network of 682 ICUs based on a comparatively long study period of six years. Surveillance data from the Krankenhaus Infektions Surveillance System are representative and validated [32,33]. Standard definitions were applied in all ICUs.

Several limitations of our study have to be taken into consideration: firstly, differences in the frequency of taking blood cultures across different ICUs. Furthermore, misclassification by the laboratories (e.g. non-*albicans Candida* for Saccharomyces) cannot be excluded. In addition, our data highlight only primary and not secondary NLCBIs. Secondary NLCBIs also play an important role in the ICU. The frequency of non-albicans bloodstream infections can also be influenced by the duration of incubation and subculture practices. A major limitation of the study is that non-*albicans Candida* species were not further classified. However, this is also the case in other surveillance systems on healthcare-associated infections, such as the United States National Healthcare Safety Network [2].

In conclusion, primary *Candida* NLCBIs showed no increase in the six-year study period in a network of 682 German ICUs. Primary *Candida* NLCBIs remain a rare event in spite of an upsurge in invasive procedures and therapies in an aging population and they should therefore not be overestimated.

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Conflict of interest

None declared.

Authors' contributions

EM conceived of the study, and wrote the manuscript. FS managed the data and performed the statistical analysis. CG and PG designed and coordinate the National Nosocomial Infection Surveillance System and helped to draft the manuscript. All authors read and approved the final manuscript.

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