RAPID RISK ASSESSMENT

Hospital-acquired malaria infections in the European Union

30 April 2018

Main conclusions and options for response

Between January 2016 and April 2018, six sporadic hospital transmissions of malaria were identified in the European Union (EU). Although uncommon, hospital transmission of malaria has been described previously. While the countries reporting these six cases (i.e. Germany, Greece, Italy and Spain) have not observed an increase in the number of sporadic hospital-acquired cases of malaria since January 2016, the concomitant occurrence of these cases in four countries makes the overall event unusual. The mode(s) of transmission have not been determined for any of the cases. This rapid risk assessment presents the context, details investigations into the cases and offers options for prevention and control.

According to the scientific literature, the following modes of transmission should be taken into account in the investigation of hospital-acquired malaria:

- Parenteral introduction of blood that contains parasite-infected erythrocytes from one infectious individual to another patient during healthcare procedures;
- Blood transfusion, or bone marrow or organ transplant from a malaria-infected patient;
- Accidental contact of blood containing parasite-infected erythrocytes with an open wound.

Malaria transmission in a hospital can also be vector-borne, when a malaria-infected mosquito bites a hospitalised patient.

According to the literature, investigations are not always conclusive and entomological investigations may fail to identify rare events of vector-borne transmission such as transmission in hospital settings, airport malaria or luggage malaria.

Clinicians must be aware of the possibility of hospital-acquired malaria in hospitalised or recently discharged patients who develop an unexplained fever or a malaria-like clinical syndrome, especially if their hospital admission coincided with that of another patient admitted with malaria.

Healthcare providers should be aware that hospital transmission of malaria is rare but possible, irrespective of the *Plasmodium* species involved. However, hospital transmission has almost always been associated with *P. falciparum*. Patients with malaria should always be considered infectious by the parenteral route.

Prevention of transmission in hospitals requires that standard precautions are strictly implemented, including safe injection practices that prevent the sharing of patient care devices or equipment which may be contaminated by patient blood. Transmission of blood-borne pathogens is linked to the sharing of glucose monitoring, capillary blood sampling or insulin administration devices, multi-dose vials, or single-use ampoules among patients and the failure to change gloves after handling intravascular catheters or performing capillary blood testing. Reusable patient care equipment should be cleaned and disinfected between patients in accordance with manufacturer's instructions. The sharing of multi-dose vials among patients should be avoided. If it is necessary to share, a sterile syringe and sterile needle must be used each time the multi-dose vial is accessed.

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An investigation and assessment of infection prevention and control procedures and practices related to blood-borne transmission is indicated in the event of any suspected hospital transmission of malaria (e.g. handling of intravascular catheters, capillary blood sampling, use of glucometers, use of multi-dose drug vials, saline and heparin flushes, and use of gloves.) Investigations should also include an assessment of possible transmission through blood transfusion, bone marrow or organ transplantation, concurrent with a malaria case in the hospital, an assessment of possible vector-borne transmission by a local vector or associated with travel in a malaria-endemic country, airport/harbour or luggage malaria, and molecular typing of *Plasmodium* spp. isolates (when possible).

If a high local vector capacity supports increased risk of vector-borne transmission, prevention measures should be considered to avoid patients or personnel being bitten by mosquitoes (e.g. bed-nets, insect repellents) when a patient with malaria is hospitalised.

The risk of further spread of malaria in the EU associated with these recent events is considered negligible.

Competent authorities are encouraged to report new human cases of hospital transmission of malaria and the findings of public health investigations to the Epidemic Intelligence Information System for Antimicrobial Resistance and Healthcare-associated Infections (EPIS-AMR-HAI). Where appropriate, competent public health authorities are encouraged to issue notifications on EWRS, as per Article 9 of Decision 1082/2013/EU on serious cross-border threats to health.

Source and date of request

ECDC Internal Decision, 20 March 2018.

Public health issue

Following the occurrence of several hospital-acquired malaria cases in the European Union (EU), ECDC has assessed the risk related to transmission of the parasite in hospital settings.

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All experts have submitted declarations of interest and a review of these declarations did not reveal any conflict of interest.

Experts from WHO have reviewed this risk assessment; however, the views expressed in this document do not necessarily represent the views of WHO.

Disease background information

Malaria is caused by infection with parasites of various *Plasmodium* species (i.e *P. falciparum, P. malariae, P. ovale, P. vivax* and *P. knowlesi*) transmitted by the bites of infected females of various *Anopheles* mosquito species [1]. Malaria caused by *P. falciparum* and *P. vivax* represents the majority of the malaria health burden worldwide, with estimated incidences of 207 million and 8.5 million cases respectively in 2016. [2].

P. falciparum can lead to life-threatening infection and is the most prevalent malaria parasite in Africa, but exists in numerous tropical and subtropical countries in the Americas and Asia. *P. vivax* infection usually leads to milder disease and relapses. *P. vivax* commonly infects humans in many countries outside of sub-Saharan Africa, notably in tropical countries in the Americas, Central and Southeast Asia, and Oceania [3].

P. malariae is widespread throughout sub-Saharan Africa and in the Amazon Basin of South America. It is also largely present in south-east Asia and on many of the islands in the western Pacific [4]. *P. ovale* is present in sub-Saharan Africa, south-east Asia and the islands of the western Pacific [5]. *P. knowlesi* is an emerging zoonotic malaria parasite responsible for malaria infection in south-east Asia [6].

The incubation period of malaria usually varies between seven and 15 days, but long incubation periods of several months have been observed, depending on the patients and the *Plasmodium* species. Parasitaemias associated with *P. ovale* and *P. malariae* are usually lower than those observed for *P. falciparum* or *P. vivax*. Infections with *P. vivax* and *P. ovale* in humans can lead to relapses due to quiescent liver stage forms (hypnozoites) of the parasite. Late relapses of *P. malariae* have been described, most probably in relation to the long-term persistence of blood stage parasite infection [4].

Several *Anopheles* mosquito species competent for malaria transmission are present in Europe. The World Health Organization's European Region, which encompasses the EU Member States, was declared malaria-free following the interruption of indigenous malaria transmission in 2015. Current efforts in the WHO European Region are focused on reducing the risk of malaria being reintroduced [7]. More information on malaria is available in the ECDC malaria factsheet.

Malaria parasites may be transmitted through substances of human origin (SoHO) [8-10]. In this context, the highest risk of malaria transmission is through contaminated erythrocyte-containing blood components and peripheral blood stem cells or bone marrow [11,12]. Transmission has also been reported as a result of kidney, liver and heart transplantation [13,14].

In non-malaria-endemic countries such as the EU Member States, the application of blood safety measures [15] has been successful in keeping the incidence of transfusion-transmitted malaria low, and the rejection rate of donors due to risk of malaria has been estimated, in various countries, to be 0.003–0.43% of all donations [16]. The EU Directive on human tissues and cells also recommends laboratory screening for the presence of malaria in donors, depending on their travel and exposure history and the characteristics of the donated cells and tissues [17]. The Council of Europe guide to the quality and safety of organs for transplantation [18] recommends checking travellers and immigrants from malaria-endemic countries (within the past five years) for malaria infection [18]. Parasitaemic donors of organs are usually rejected. However, grafts donated by successfully treated and recovered donors may be used, with some exceptions – e.g. liver donation where some species *(P. vivax* and *P. ovale*) may survive the treatment. In these cases, the prophylactic treatment of recipients for malaria should be considered.

In non-endemic countries, malaria may be transmitted by imported infectious mosquitoes or imported human cases. Airport/seaport malaria or luggage malaria results from the bite of an infected mosquito imported in an aircraft [19]. However, airport malaria remains a rare event with only a limited number of cases having been reported in recent years [19].

Introduced malaria results from the bite of a local mosquito infected by an imported case. In the EU/EEA countries, several areas have experienced the re-introduction of malaria: Greece since 2009 [20-23], France (Corsica) in 2006 [24], and Spain in 2010 [25,26].

Hospital transmission of malaria

For the purpose of this document, hospital-acquired malaria is defined as non-vector-borne malaria acquired in a healthcare facility by a patient in whom the infection was not present or incubating at the time of admission.

Hospital-acquired malaria occurs through inoculation with blood or cells that contain parasite-infected erythrocytes during healthcare procedures. The transmission results from the introduction of the parasite into a person by artificial means, including blood transfusion, bone marrow or organ transplant, or the shared use of needles or syringes contaminated with blood.

Although uncommon, hospital transmission of *P. falciparum* malaria has been documented. Transmission has often been reported from one patient to another in the same ward by inoculation of blood that contained parasite-infected erythrocytes. In most of the reported cases, vector-borne transmission was considered unlikely by the investigators. Molecular analysis is able to demonstrate whether the *Plasmodium* isolates are identical. Diagnosis

and treatment are often delayed with potentially unfavourable outcome due to the low suspicion of malaria infection in non-endemic areas.

ECDC performed a literature review to identify transmission modes for malaria in hospitals, excluding transmission through blood transfusion, bone marrow and organ transplant and vector-borne transmission, as these are well-studied and not addressed in this rapid risk assessment (Annex 2).

Hospital transmission has been suspected or associated with several procedures or situations:

- saline and heparin flushes of intravenous devices [27-30];
- incorrect capillary blood sampling techniques for blood-glucose measurement [31];
- contamination of gloves during the manipulation of drip lines and venous cannulae [32];
- needle-stick injuries [33];
- minor and unnoticed bleeding from dry cracked hands during intravenous catheter insertion and manipulation [33];
- exposure during the manipulation of the catheter and/or parenteral infusion [34];
- contact transmission from an environment contaminated with occult blood into wounds or intravascular catheters [35];
- contaminated catheters and contrast medium for computed tomographic scanner [36];
- use of common components of the infusion apparatus, excluding the needle [3].

Due to the complexity of the investigations and the bias of recall, it should be noted that the exact mode of transmission remained unclear in a significant proportion of the events reported in the literature [26,27,35,37-42].

This evidence is a reminder that malaria is also a blood-borne infection and that the strict implementation of standard precautions (including safe injection practices and safe use of patient care equipment and devices that can be contaminated by patient blood) is crucial for preventing hospital transmission of malaria [43-45].

Event background information

Since January 2016, ECDC has identified six cases of hospital-acquired malaria in Italy (two cases), Spain (two cases), Greece (one case) and Germany (one case). Five of the cases were notified to ECDC and one was found in the literature. One of the cases died. None of the cases reported recent travel to a malaria-endemic country.

Table 1. Cases of hospital malaria transmission in the EU, by country, 2016–2018

Country of report	Age (years)	Date of onset	Place of infection	Suspected mode of transmission	Possible exposure	Outcome	*Plasmodium* species
Germany	33	2016	Nordrhein-Westfalen, Germany	Hospital transmission	Shared room with a malaria case	Alive	*P. falciparum*
Greece	39	18–20 Jul 2017	Ipeiros, Greece	Vector or hospital transmission	Shared ward with a malaria case	Alive	*P. falciparum*
Italy	4	29 Aug 2017	Trento, Italy	Hospital transmission	Shared ward with two malaria cases	Dead	*P. falciparum*
Italy	13	28 Oct 2017	Tuscany, Italy	Hospital transmission	Shared ward with a malaria case	Alive	*P. falciparum*
Spain	64	9 Mar 2016	Galicia, Spain	Hospital transmission	Stayed in emergency ward with a malaria case	Alive	*P. falciparum*
Spain	<1 (3 months)	19 Feb 2018	Madrid, Spain	Hospital transmission	Shared ward with a malaria case	Alive	*P. malariae* and *P. ovale*

Germany

In February 2016, the index case (case first noticed by the health authorities) [46], who had shared a room with an imported malaria case in a hospital ward in Germany for under 24 hours subsequently developed malaria [34]. The *Plasmodium* isolates from both patients showed an identical DNA pattern by PCR–based fingerprinting comprising microsatellite PfRRM and the gene encoding merozoite-specific protein 1 (*P. falciparum* MSP1). Vector-borne transmission by local *Anopheles* mosquitoes was ruled out as the event took place in winter. No blood transfusion or intravenous drug use was reported.

The mode of hospital transmission was not determined. No concurrent blood draws or finger-stick blood tests were documented. Only one of the patients received subcutaneous injections. Different parenteral antibiotics were given to the patients via peripheral intravenous catheters, which were routinely sealed using a plastic stylet without prior saline flushes. No invasive procedure was performed on the index case.

Greece

On 17 August 2017, Greece reported one case of *P. falciparum* with the most likely place of exposure being a healthcare facility in the region of Ipeiros, in north-western Greece. The index case presented symptoms compatible with malaria with an onset between 18 and 20 July 2017, was hospitalised on 21 July and discharged on 12 August 2018.

The case, with no recent travel history to a malaria-endemic area, had previously been hospitalised in another facility between 17 June and 10 July 2018, where he shared the same ward between 22 and 25 June with a patient treated for imported *P. falciparum* malaria. The date of symptom onset was compatible with transmission occurring in the ward.

Epidemiological investigations ruled out transfusion- and transplant-transmitted malaria. The case had received a blood transfusion on 25 July, after the onset of symptoms. Blood samples of the case taken before the blood transfusion on 24 July were retrospectively retrieved and tested positive for malaria.

During their stay, both patients received parenteral treatment through peripheral vein catheters and were tested daily for glucose through capillary blood sampling techniques using the same available glucometer but different lancets. Saline flushes were conducted using single syringes for each patient. No needle injury was reported during this period.

The blood samples from the index imported case were not sent to the malaria reference centre for further molecular typing, therefore the comparison of the isolates was not possible.

Entomological investigations in the area around the hospital and the patient's residence did not reveal the presence of *Anopheles* mosquitoes. However, this is a peri-urban area, suitable for *Anopheles* mosquitoes, and this targeted investigation could not rule out the previous or current presence of *Anopheles* mosquitoes in the area. No further locally-acquired malaria case has been reported in the area despite increased awareness among local clinicians. The closest local airport has no international flights to endemic areas and is located about 10 km from the hospital and residence of the patient. Luggage malaria was also considered unlikely, as the imported index case arrived in Greece from Cameroon on 9 June 2017 and stayed in another village (35 km away from the place of exposure) where he had already opened his luggage after returning from Cameroon.

The investigation concluded that the case could be the result of a hospital transmission or vector-borne transmission in the hospital.

Italy

Trento

On 5 September 2017, Italy reported a fatal case of malaria in a four-year-old girl with no travel history to a malaria-endemic country [47].

She had been admitted to a hospital in the Veneto region on 13 August 2017 and diagnosed with diabetes mellitus. After returning from the Veneto region, she had been admitted to a hospital in the Trento region for diabetes from 16 to 21 August 2017, and diagnosed with pharyngitis on 31 August 2017.

On 2 September 2017, she was admitted and diagnosed with *P. falciparum* malaria and subsequently transferred to the tropical diseases reference centre in Lombardy where she died on 4 September 2017. Epidemiological investigations identified two patients infected with *P. falciparum* who were hospitalised in the same ward during her stay in the Trento hospital between 16 and 21 August 2017; both cases were imported. The investigation at the Trento hospital did not identify breaches in medical procedures that could have resulted in transmission.

Molecular sequencing of the *Plasmodium* isolates from the girl and the other two children hospitalised concomitantly, performed by the National Institute of Health in Rome, indicated that the *Plasmodium* isolates of the girl and one of the two children were genetically identical, confirming with reasonable certainty that it was hospital transmission and excluding the involvement of a vector, either from local *Anopheles* mosquitoes or luggage malaria.

Entomological investigations in the area of Trento did not reveal the presence of *Anopheles* mosquitoes. Neither did entomological surveys in Bibione (Veneto region), where the girl spent her holidays during the first half of August 2017 (from 1 to 13 August).

Tuscany

On 6 December 2017, Italy reported a hospital-acquired case of malaria in Tuscany.

The index case was a 13-year-old boy who did not report recent travel history to a malaria-endemic country. In October 2017, he had, on three occasions, visited the same hospital emergency room: first on 13 October for respiratory difficulties, myoclonic movements of the limbs, headache, vomiting and apyrexia; the second time on 28 October, for suspected virosis and fever (the fever had started on 26 October); and the third time on 31 October, due to the persistence of the fever.

On 16 November 2017, he tested positive by PCR for *P. falciparum*. The diagnostic was confirmed by thick drop and thin smear from capillary blood. His condition improved immediately after the start of antimalarial therapy.

The imported malaria case was a 15-year-old male who had been hospitalised with suspected malaria on 13 October 2017 in the same hospital. He had reported recent travel to Senegal.

Both children had been registered in the same emergency room on 13 October 2017 and were both hospitalised in the same ward during their stay in the hospital (imported case from 13 to 27 of October and hospital-acquired case from 13 to 19 October). The overlapping times and interventions carried out on the two boys were carefully analysed.

Blood samples and peripheral blood smears of both patients were sent to the National Institute of Health in Rome. The microscopic and molecular analyses confirmed that the parasites present in the blood samples were *P. falciparum*. The molecular analysis indicated that the two *Plasmodium* isolates were genetically identical (full identity of the sequences of three polymorphic molecular markers).

The hospital-acquired case received a transfusion on 6 November 2017. Pre-transfusion blood and aspirated bone marrow samples retrieved retrospectively tested positive by PCR for *P. falciparum*, thus excluding the possibility of transmission by transfusion.

Entomological investigations in the area of the hospital did not reveal the presence of *Anopheles* mosquitoes so the possibility that the infection of the index case was due to *Anopheles* mosquitoes being present in the environment was considered highly unlikely.

The entomological investigation, together with the results of molecular analysis, confirmed with reasonable certainty that the case was hospital-acquired, excluding the involvement of a vector either from local *Anopheles* mosquitoes or luggage-malaria

Spain

Madrid

On 27 February 2018, Spain reported a case of malaria infection in a three-month-old girl who lived in Madrid and did not have any travel history to a malaria-endemic area. This index case had been hospitalised from 1 to 7 February in a hospital in Madrid because of a disease unrelated to malaria. The reported date of onset of symptoms was 24 February, the same day that she was readmitted into the same hospital with a diagnosis of malaria infection by *P. malariae* and *P. ovale*.

Epidemiological investigations ruled out maternal-fetal transmission and she had not received transfusion of blood or blood products, or a bone marrow or organ transplant. During her first hospitalisation, the patient was in the same ward during the same period (1–6 February) as another child from Equatorial Guinea with imported malaria.

Although the specific mechanism of transmission was not identified, both cases shared the same combination of *Plasmodium* species (*P. malariae + P. ovale*). Subspecies characterisation of *P. ovale* showed *P. o. curtisi* in both cases and the subtyping of *P. ovale* merozoite surface protein (*P. ovale* MSP1 fragment 3) showed an identical DNA sequence.

It was not possible to establish the mode of transmission, but transmission probably occurred during the hospitalisation when both children were in the same ward and both were receiving intravenous fluids. So far infection control investigations and patient safety procedures have not identified any specific breach. The cleaning, disinfection, sterilisation and pest control procedures of the hospital met all the usual requirements. However, infection prevention and control measures at the hospital have been reinforced. Both cases were discharged and fully recovered from malaria.

The entomological investigation excluded vector transmission. No larvae or adult mosquitoes were found in the hospital. The winter season and ambient conditions were not compatible with the mosquito's life cycle. The two closest airports (10 and 14 km from the hospital) do not have international flights to malaria-endemic areas. The largest airport with international flights to malaria-endemic areas is located 40 km from the hospital.

Galicia

In 2016, a 64 year-old man who lived in Galicia and had no travel history was diagnosed with malaria. This index case was under treatment due to myeloma. However, blood or blood-product transmission were ruled out. The patient received an autologous bone marrow transplant in 2013 and 2014, and blood transfusions in 2014 and 2015.

The fever onset date was 9 March 2016. He was admitted to hospital on 13 March and was diagnosed with malaria due to *P. falciparum* on 16 March 2016. This index case had been previously admitted several times to the hospital in 2016 (discharge dates: 22 January and 19 February due to respiratory infections, and 4 March due to febrile syndrome). He also visited an emergency room where there was an imported case of malaria (*P. falciparum*) on 15 January 2016, and clinical specimens from both cases were analysed.

The molecular typing investigations were inconclusive. Both patients showed infection by *P. falciparum* (microscopy, RDT and PCR). Genotyping was done by typing *Pf*MSP1 (families K1, RO33 and Mad) and *Pf*MSP2 (families FC27 and IC) genes. Molecular typing was inconclusive because it was only possible to obtain PCR amplification in the *Pf*MSP1 K1 from the index case and the size of the fragment in the imported case was different from the ones obtained in the index case.

Although an entomological investigation was not conducted, vector-borne transmission from local *Anopheles* mosquitoes was deemed unlikely because the event took place in winter and the environmental conditions were not compatible with the mosquito's life cycle. The index case lived 25 km from the seaport and 10 km away from the airport. There are no flights to/from malaria-endemic areas at this airport but the seaport has international transit of merchandise and containers, some from malaria-endemic areas.

Despite the complexity of this investigation and due to the multiple admissions and procedures of the index cases, it was concluded that hospital transmission was the most likely mode of transmission.

ECDC threat assessment for the EU

In 2016 and 2017, six events involving the hospital transmission of *P. falciparum, P. malariae* and *P. ovale* took place in Germany, Greece, Italy and Spain. Although hospital transmission has been confirmed, the investigations were inconclusive as to the exact mechanism of transmission. Breaches in infection control that could have resulted in person-to-person transmission could not be identified. Transmission through blood transfusion, or bone marrow or organ transplant was ruled out.

Entomological investigations did not identify malaria-competent vectors in the hospital surrounding area for any of the events reported, however such investigations cannot always ascertain the absence of competent vectors in the area. The results of molecular analysis of the *Plasmodium* isolates from the cases in Germany, Italy and Spain indicated that the most likely route of transmission was hospital transmission through direct contact with blood that contained parasite-infected erythrocytes during provision of healthcare.

Although there is a potential risk that other patients or healthcare workers may have been exposed at the time of the events, it is unlikely that it would have been extensive and the risk of further spread of malaria in such contexts within the EU is negligible.

The investigation of these cases shows the clear added value of molecular analysis in building evidence of patient-to-patient transmission of malaria.

Hospital transmission is almost always associated with *P. falciparum.* This may be explained by the fact that the observed parasitaemia is higher for *P. falciparum* infection than for infection with other *Plasmodium* species [4,5,48]. However, the other *Plasmodium* species can also be transmitted within healthcare settings. This is exemplified by a concomitant transmission of *P. malariae* and *P. ovale* in Madrid, Spain in 2018.

These events are a reminder that hospital transmission of malaria is possible. The risk of hospital transmission of malaria increases if standard precautions are not strictly applied. In particular, precautions that prevent the transmission of blood-borne infections, such as sharing patient care devices or equipment that may be contaminated by patient blood (including syringes, multi-dose vials, glucose monitoring and insulin administration devices) or failing to change gloves between patients after procedures involving any contact with patient blood. In areas with a possible circulation of competent vectors that can transmit malaria, vector-borne transmission is also possible, if mosquito protection measures are not applied.

Annex 1. List of items to consider during investigation of a potential hospital transmission of malaria

Epidemiological investigation

- Date of symptom onset for the index case, malaria-compatible symptoms prior to laboratory diagnostics, use of substances of human origin (transfusion/transplant) and medical care (peripheral/central venous catheter, multiple intravenous drug administrations, laboratory sampling, etc.)
- Investigation of an alternative mode of contamination (intravenous drug use, shared needle, etc.)
- History of travel to malaria-endemic areas.
- Residence/work close to an airport or a harbour – to rule out airport malaria.
- Travel history (patient, family or relatives) in malaria endemic country – to rule out luggage malaria.
- Locally-acquired cases of malaria reported in the area, or cases of febrile illness among the patient's relatives, or report of mosquito bites.
- Dates of hospitalisation, treatment and outcome of the index case.
- Identification of a potential link with the suspected or confirmed potential malaria source case in a healthcare setting. The malaria source case can be a patient or a healthcare worker. The concurrence is broadly defined as time spent in the vicinity of a suspected or confirmed malaria case (such as same hospital room, same emergency ward or same ward) or sharing medical personnel.
- Investigation of infection prevention and control procedures and practices that may lead to exposure to blood containing parasite-infected erythrocytes, as described in the literature, such as:
  - capillary blood sampling by finger stick (e.g. blood glucose monitoring);
  - direct exposure to Substances of Human Origin (SoHO);
  - shared contaminated multiple-use infusion devices or successive infusion by same healthcare staff;
  - application of heparin to peripheral locks (multi-dose heparin container) or saline flush.
- Define the period between the first potential exposure in healthcare settings and both the onset of malaria-compatible symptoms and the final diagnostic.

Entomological investigation

- Entomological investigation about the presence of competent and active vector in/around the healthcare setting and in/around the household of the index case under investigation.
- Estimation of an extrinsic incubation period (EIP) defined as the interval between the acquisition of *Plasmodium* by a local vector and its ability to transmit *Plasmodium* to humans.
- Assess the possibility of vector-borne transmission in comparing the EIP and the human malaria incubation period for the identified *Plasmodium* species with the time between first potential exposure in the healthcare setting and the onset of malaria-compatible symptoms.

Molecular investigation

- *Plasmodium* species-level identification.
- Molecular typing of the *Plasmodium* isolate for the index case and the suspected source case.

Annex 2. Literature review

Original research articles were retrieved from PubMed, Embase.com, and Cochrane Library on 16 April 2018.

The search strategies submitted combined the concepts of malaria with cross infection. Controlled vocabulary (i.e. MeSH and Emtree terms) and natural vocabulary (i.e. keywords) were used to represent the concepts in the search strategies.

We found that the use of the title and abstract fields, and the controlled vocabulary for these concepts considerably increased the noise in the results. Hence, proximity operators in multiple fields were used with Embase.com to increase the adequacy of the results. Additional searches were submitted in PubMed using the title field and the MeSH terms to complement the retrieval.

The results were in all languages and limited to records published from the year 2000 onwards.

A total of 295 articles were retrieved. After de-duplication, 268 articles were included in the review from which 18 articles were selected. Of these articles, nine described suspected or associated modes of transmission. In addition, by reviewing the references of the articles we found six additional relevant articles. However, only two describing modes of transmission were different to the articles already retrieved by the search strategy.

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