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Miscarriage following dengue virus 3 infection in the first six weeks of pregnancy of a dengue virus-naive traveller returning from Bali to Italy, April 2016

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We report miscarriage following dengue virus (DENV)-3 infection in a pregnant woman returning from Bali to Italy in April 2016. On her arrival, the woman had fever, rash, asthenia and headache. DENV RNA was detected in plasma and urine samples collected the following day. Six days after symptom onset, she had a miscarriage. DENV RNA was detected in fetal material, but in utero fetal infection cannot be demonstrated due to possible contamination by maternal blood.

Case description

A woman in her 30s returned from Bali to Italy in early April 2016. The day she left Bali and arrived in Italy, she became ill with fever (> 38.5 °C), rash, asthenia and headache, which lasted for a further five days. She had had her last menstrual period mid-February and discovered she was seven-weeks pregnant on her return from Bali, having performed an off-the-shelf pregnancy test as soon as she landed in Italy. The day of her arrival (during the first 24 hours of fever), she presented at a hospital emergency department in Brescia (Lombardy region), where ultrasonographic examination confirmed she was pregnant. The size and cardiac activity of the embryo was normal. All haematochemical tests of the woman were normal (including white blood cell and platelet count), a rapid diagnostic test (BinaxNOW Malaria, Alere, Scarborough, United States) for malaria was negative, TORCH assays were negative for cytomegalovirus and toxoplasmosis, while she was immune to herpes simplex-1 virus and rubella virus, and she was discharged. Because of persistence of her symptoms, including a high temperature (> 38.5 °C), she returned the following day: she was mildly neutropenic but not platelet depleted. Ultrasound confirmed a live embryo. Blood and urine samples were collected and referred to the regional reference laboratory (Fondazione IRCCS Policlinico San Matteo in Pavia) to investigate potential arbovirus infections. Three days later, her platelet level started to fall, with the lowest count (30,000/µL; norm: 130,000–400,000/µL) recorded three days after that.

Three days after arriving in Italy, the woman's spouse, who had also been travelling in Bali, reported similar symptoms.

Laboratory findings

The diagnostic assessment included the following: (i) detection of dengue virus (DENV) 1–4 IgM and IgG antibodies in serum samples (using dengue virus IgM Capture DxSelect and dengue virus IgG DxSelect, Focus Diagnostics, United States), as well as detection of Zika virus (ZIKV) IgM and IgG antibodies (Anti-Zika virus ELISA (IgM) and Anti-Zika virus ELISA (IgG), Euroimmun, Germany); (ii) serology results were confirmed by neutralisation assay [1]; (iii) detection of DENV NS1 antigen in serum samples (dengue NS1 Ag STRIP, BIO RAD, France); (iv) detection of DENV RNA and ZIKV RNA in plasma and urine samples using a pan-flavivirus hemi-nested reverse transcription(RT)-polymerase chain reaction (PCR) targeting a conserved region of the NS5 gene [2] as well as virus-specific real-time RT-PCRs, targeting a conserved region in the 3' untranslated region of DENV 1–4 [3] and a portion of the envelope protein gene of ZIKV [4]; and (v) sequencing of positive pan-flavivirus amplicons.

DENV infection was diagnosed in the woman and her spouse, while ZIKV infection was ruled out.

Table

Virological results in two dengue virus-infected patients returning from Bali to Italy, April 2016

Patient	Days after symptom onset samples taken	IgG	IgM	DENV-3 NT Ab	NS1 antigen	Dengue virus-specific real-time RT-PCR Number of copies/mL			Pan-flavivirus RT-PCR		
						Plasma	Urine	Fetal material[b]	Plasma	Urine	Fetal material[b]
Woman[a]	+2 (7 WOP)	Neg	Neg	<1:10	Pos	7.0×10^8	1.0×10^3	NA	Pos	Pos	NA
	+9 (8 WOP)	ND	ND	ND	ND	ND	ND	3.9×10^3	ND	ND	Pos
	+12	Neg	Pos	1:80	Neg	20	1.6×10^3	NA	Pos	Pos	NA
	+24	Pos	Pos	1:160	Neg	Neg	Neg	NA	Pos	Pos	NA
Woman's spouse	+5	Neg	Pos	1:20	Pos	Neg	6.7×10^2	NA	Pos	Pos	NA
	+21	Pos	Pos	1:160	Neg	Neg	Neg	NA	Pos	Pos	NA

DENV: dengue virus; NA: not applicable; ND: not done; Neg: negative; NT Ab: neutralising antibody titre; Pos: positive; RT-PCR: reverse transcription-polymerase chain reaction; WOP; weeks of pregnancy.

[a] The woman had a miscarriage six days after symptom onset.

[b] Tested after miscarriage.

Two days after symptom onset, the woman's serum tested negative for DENV IgG and IgM, while NS1 antigenaemia and high levels of DENV RNA (7.0 × 108 copies/mL) were detected in her plasma (Table). DENV RNA was detected in her urine (1.0 × 103 copies/mL).

Sequencing of amplicons from the woman's plasma and urine confirmed infection by a DENV-3 serotype (GenBank accession number KX583642-KX583643). Interestingly, the DENV RNA load in her plasma was the highest observed at our institution for several years (the median value of 10 sequential recent imported DENV cases is reported for comparison: 3.1 × 104 DENV RNA copies/mL; range: 8.8 × 102 to 5.4 × 106 copies/mL). In addition, DENV was isolated from the plasma sample.

Six days after symptom onset, the woman had a miscarriage: there was no fetal cardiac activity. Three days later, she underwent surgical uterine evacuation and DENV RNA (3.9 × 103 copies/mL) was detected in the fetal material (GenBank accession number KX583644). A dramatic reduction of DENV RNA load in the woman's plasma was observed six days after the miscarriage (20 copies/mL), while NS1 Ag was negative. At this time (12 days after symptom onset), a plasma sample was positive for DENV IgM, while IgG tests were still negative. DENV real-time RT-PCR to detect DENV RNA in plasma and urine was negative 24 days after symptom onset, whereas it was still positive by pan-flavivirus hemi-nested RT-PCR. DENV IgG seroconversion was observed at that point (Table).

Results from virological and serological tests of her spouse's samples were somewhat different. Five days after symptom onset, DENV NS1 Ag was positive but DENV RNA in plasma was detected only by pan-flavivirus hemi-nested RT-PCR. In contrast, DENV RNA was detected in urine by both molecular assays. At that time, the sample was DENV IgM positive. Tests 21 days after symptom onset showed that NS1 Ag was negative in plasma and urine, as was DENV RNA using specific real-time RT-PCR, while both samples were positive using the pan-flavivirus hemi-nested RT-PCR. IgG seroconversion was seen at that point (Table).

Background

The recently reported clusters of microcephaly and other birth defects caused by ZIKV infection in South America [5] have prompted European countries to be on alert for arthropod-born infectious disease risks, especially regarding pregnant travellers and their sexual partners. While chikungunya and West Nile virus infections have not proved to be an increased risk of preterm delivery, miscarriage or low birth weight [6-8], maternal and fetal consequences of DENV infection during pregnancy can be severe [9-12]. In tropical and subtropical regions, four serotypes (DENV 1–4) may be endemic in the same human population and clinical manifestations may range from mild fever in primary infections to haemorrhagic syndromes in reinfections by a different serotype [13]. Other factors, such as high viraemia titre and increased dengue virus-specific serotype replication have been postulated in the pathogenesis of severe disease [14]. In pregnant women living in endemic regions, dengue fever and severe dengue can develop; low platelet counts have been seen in both primary and secondary infections [12]. Fetal death, premature birth and low birth weight, as well as vertical transmission at term causing neonatal thrombocytopenia, have been recorded [10]. In contrast, however, the impact of DENV infection on pregnancy outcome in dengue immunologically naive travellers and the relationship between peak viraemia and pregnancy outcome remain unexplored.

Discussion

DENV infection during the first trimester of pregnancy does not reveal a significant risk of vertical transmission of the virus [10]. However, since women in early pregnancy may not be hospitalised, the frequency of vertical transmission remains difficult to estimate [15-18].

The case described in our report chronologically links an acute DENV infection in the first weeks of pregnancy to an unfavourable outcome. It should be borne in mind, however, that one in five of all recognised pregnancies end in miscarriage [19] and maternal hyperthermia is recognised as an independent risk factor for miscarriage [20].

Although DENV RNA was detected in the fetal material tested after the miscarriage, in utero fetal infection cannot be demonstrated due to possible contamination with maternal blood. As fetal death followed the onset of DENV infection, adverse fetal outcome due to possible effects of high maternal viraemia on placental endothelial function cannot be excluded [14,21].

In the global alertness for ZIKV-induced microcephaly [5], DENV infection might represent an additional and underestimated risk factor for pregnancy.

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Conflict of interest

None declared.

Authors' contributions

Wrote the manuscript: MZ, FR, FB, LRT; managed the patient: LRT, GC; performed laboratory investigations: GC, EP, AS, FR; revised the manuscript: MZ, FR, FB, FC; coordinated the study: FB, MZ, FR, FC.

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Brucellosis in a refugee who migrated from Syria to Germany and lessons learnt, 2016

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A teenage woman migrating from Syria arrived in May 2015 in Germany. She gave birth to a healthy child in early 2016, but became febrile shortly after delivery. Blood cultures revealed *Brucella melitensis*. In retrospect, she reported contact with sheep in Syria and recurrent pain in the hip joints over about five months before diagnosis of brucellosis. We discuss consequences for adequate treatment of mother and child as well as for clinical and laboratory management.

As brucellosis is rare in many European countries and because there is even less experience with brucellosis in association with pregnancy, we here publish a case of a young pregnant woman who had migrated from Syria to Germany and was diagnosed with brucellosis directly after delivery of her child, and illustrate some lessons learnt.

Case report

A teenage female refugee from Syria delivered a full-term baby (birthweight 3,185 g) with vacuum extraction in Germany. Because of intrapartum asphyxia, mother and child were hospitalised in a special care unit. The child recovered without further complications. The mother developed a fever of 39 °C on day 1 after delivery. Investigation of the mother's blood revealed normal ranges of leukocytes, a slightly decreased haemoglobin level and an elevated concentration of C-reactive protein (CRP) at 38.0 mg/mL (normal < 5.0 mg/L). She was treated for three days with intravenous ampicillin sulbactam 3 g three times daily and metronidazole 500 mg twice daily. The patient was in good clinical condition and discharged from the hospital on day 4 with oral sultamicillin 375 mg and metronidazole 400 mg twice daily.

Two sets of blood cultures (BD Bactec Fx blood culture system) were taken during pyrexia. One anaerobic culture revealed a positive signal after 47 h incubation that was identified as *Fusobacterium nucleatum*. The aerobic blood culture revealed growth after 117 h and Gram staining showed faintly stained small Gram-negative coccobacilli (Figure 1). Small non-haemolytic colonies appeared on Columbia blood agar after 48 hours incubation at 36 °C in air with 5% CO2 enrichment (Figure 2) and were oxidase-positive. Using matrix-assisted laser desorption ionisation time of flight (MALDI-TOF) mass spectrometry (MS) (MALDI-TOF MS, Vitek MS Plus System bioMérieux, research use only (RUO) SARAMIS database), *Brucella* spp. was identified and confirmed as *Brucella melitensis* one day later when tested by real-time PCR in a reference laboratory using an accredited in-house assay.

Because of the results of the aerobic blood culture, the mother and child were immediately contacted and examined on day 11 after delivery. The mother reported that she was a sheepherder in Syria before arriving in Germany nine months previously and that she had suffered from recurrent hip pain for about five months before diagnosis of brucellosis. The serum sample was tested in parallel with two ELISAs revealing a high titre of 1:16,000 (normal: < 1:500; Virion-Serion GmbH, Würzburg, Germany) of *Brucella*-specific antibodies, with IgG of 29.1 U and IgM of 24.3 U (cut-off: 10 U; Novagnost Brucella IgG and IgM ELISA, Siemens Healthcare Diagnostics, Germany). Samples of mother's milk were not available for laboratory investigation. The patient was advised to start antibiotic therapy with doxycycline 100 mg twice daily and rifampicin 900 mg once daily for 12 weeks and to stop breastfeeding.

The examination of the baby by the paediatrician revealed no relevant clinical or laboratory abnormalities. The blood culture set up day 11 after birth

Figure 1

Gram staining of bacterial culture showing faintly stained small Gram-negative coccobacilli corresponding to *Brucella* spp., Germany, 2016

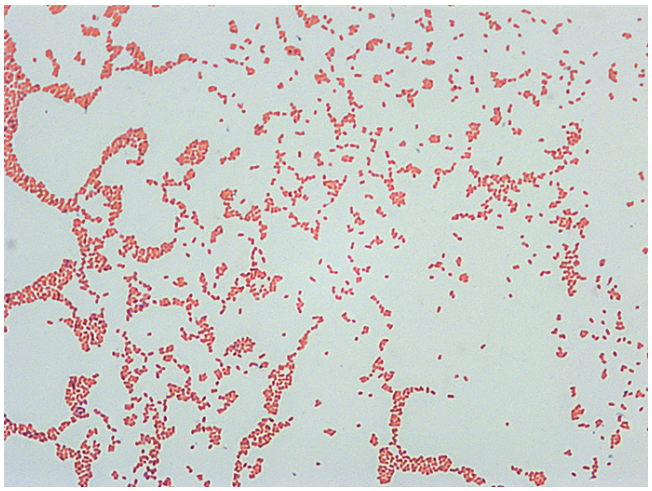

Figure 2

Bacteria isolated from blood culture forming small non-haemolytic colonies on Columbia blood agar after 48 hours incubation at 36 °C air with 5% CO2 enrichment, Germany, 2016

remained negative, the serum sample was positive only for Brucella-specific IgG antibodies (24.5 U).

In subsequent visits (four and seven weeks after delivery), mother and child had no relevant clinical or laboratory abnormalities. The antibiotics were well tolerated by the mother. Blood cultures from the mother drawn in week 7 after delivery remained negative; the IgM titre was slightly decreased to 18 U.

After the identification of *Brucella* spp., two persons from the delivery room were monitored and had serological follow-up. All serological tests at their first visit and three months later were negative.

Background

Brucellosis is a zoonosis that occurs worldwide, causing great economic losses and a high number of human infections in endemic regions, such as the Mediterranean, the Middle East including Syria, Iraq, Iran and Saudi Arabia, as well as Africa, Asia and Middle and South America. It is estimated that more than 500,000 people are suffering from brucellosis annually [1]. In contrast, only 22 to 47 cases annually were reported in Germany between 2010 and 2015. Most of these cases were associated with travel to endemic countries surrounding the Mediterranean Sea (Italy, Spain, Turkey). Between January 2015 and June 2016, 60 cases of brucellosis in Germany were reported to the Robert Koch Institute, 14 from the Middle East and North African countries, including four cases from Turkey. This seems to point towards a tendency of increased importations from these regions. Imported brucellosis plays an emergent role in Europe and globally [2,3].

B. melitensis (goats and sheep) is the most important species for human brucellosis, but other species like *B. abortus* (cattle), *B. suis* biovar 1 (swine) and *B. canis* (dogs) can also be associated with human cases. The small Gram-negative coccobacilli are most commonly transmitted to humans through contaminated food, e.g. unpasteurised milk, or by direct contact with infected animals [4,5]. They grow intracellularly, e.g. in mononuclear phagocytic cells, cause unspecific clinical manifestations especially in the acute stage, including typically undulant fever, which may last for years if not properly treated. The bacteria can be disseminated to various organs such as liver, spleen, bones and joints, genitourinary tract, skin, lung, heart and central nervous system. Therefore, human brucellosis has a broad variability of clinical signs and can mimic other infectious and non-infectious diseases. The infection is often diagnosed only when focal complications occur. Symptoms in children and teenagers with brucellosis are mostly fever, joint pain and hepatomegaly [6,7]. Intrauterine infections and transmission of the pathogen through breastfeeding has been reported, transmission of antibodies from mother to newborn is possible [7].

The laboratory diagnosis is mainly based on classical microbiological methods (but isolation could be cumbersome and is not always successful), on MALDI-TOF MS identification of colonies and on PCR that allows the discrimination of *Brucella* species [8-13]. In addition, multilocus variable-number tandem repeat analysis and other methods like whole genome sequencing, next generation sequencing and single nucleotide polymorphism analysis allow the generation of molecular epidemiology data that can be helpful in the identification of outbreak-related strains and the geographical relation between isolates [14,15]. The detection of IgG and IgM antibodies against *Brucella* antigens in serum is a reliable tool for diagnosing human brucellosis and classifying the stage of disease [16,17]. IgM

anti-*Brucella* antibodies indicate acute infection, and a titre increase in paired serum samples indicates an acute or reactivated infection.

Discussion

The accidental cultivation of *Brucella melitensis* in blood cultures drawn during post-partum fever demonstrates the complexity of this diagnosis. The anamnestic, clinical and laboratory data of our patient were consistent with a protracted or chronic course of brucellosis activated during pregnancy and/or delivery. However, the exact point in time and place of infection of our patient cannot be determined. As she was working as sheepherder in her home country before coming to Germany, she may have been infected in Syria, but infection along the route of migration is also possible. Prolonged antimicrobial therapy is imperative for achieving cure; monotherapy is associated with a high rate of relapse. In this case, the 12-week regimen based on oral doxycycline 100 mg twice a day in combination with rifampicin 900 mg/day in a single dose was preferred because of its oral application and fewer adverse effects compared to the combination of doxycycline with aminoglycosides (such as streptomycin or gentamicin).

Because of possible transmission either transplacentally or through breastfeeding (during the 11 days until diagnosis), the baby was examined. Antibacterial treatment was not started for the baby because there were no relevant clinical or laboratory abnormalities. Regular examinations, at least every three months over a period of one year, were advised.

Although person-to-person transmission of brucellosis is rare, transmission may occur in contact with contaminated blood and by aerosol-producing diagnostic procedures [18]. For patients suffering from human brucellosis during hospitalisation, standard precautions and contact precautions for those with draining wounds are required. In the case described here, mother and newborn were in a rooming-in unit and under contact precautions because of hygiene regulations for refugees.

Handling of cultures with human pathogenic *Brucella* spp. requires biosafety level 3 (BSL3) conditions, i.e. consequent work in a class II biologic safety cabinet. However, for primary diagnostic samples, accidental cultivation of *Brucella* spp. in a routine (BSL2) laboratory cannot be avoided. This entails working without safety cabinets for reading agar plates, performing phenotypical tests, etc. It was estimated that brucellosis represents the most common laboratory-acquired infection in clinical routine laboratories [19-21], and an enhanced safety policy are necessary to prevent laboratory acquisition of *Brucella* as well as regular training of the laboratory staff to be aware of how to handle and cultivate highly pathogenic bacteria such as *Brucella* spp. In our case, technicians had regularly been trained to recognise the combination of small faintly stained Gram-negative coccobacilli ('fine sand'), slow-growing small colonies on Columbia blood agar and the requirement of aerobic conditions with CO_2 enrichment, and were aware of the necessary biosafety measures. When *Brucella* spp. was suspected during reading of the agar plates, handling outside a safety cabinet was immediately stopped. All following steps for identification were performed under a class II biosafety cabinet with adequate precautions; as the risk of infection of laboratory personnel was high, we consider that these measures were effective in preventing an infection.

Lessons learnt

The clinical microbiological laboratory plays a key role in the diagnosis and management of human brucellosis. It should provide a rapid and exact identification of every Gram-negative rod that is cultivated from blood culture or surgical material to exclude *Brucella* spp. or other highly pathogenic bacteria. Currently, the most suitable tool for identification of bacteria is MALDI-TOF MS because it provides rapid, accurate, sensitive and cost-effective identification of human pathogenic bacteria. Attention should be paid using commercially available MALDI-TOF MS systems including standard diagnostic databases to identify *Brucella* spp. and other highly pathogenic bacteria. The related MALDI-TOF MS databases do not usually support validated laboratory diagnoses of highly pathogenic bacteria, which should be improved in the future. In addition, when highly pathogenic bacteria such as *Brucella* spp. are suspected or the results are ambiguous, material should be sent to a reference laboratory for further confirmation and identification at species level. When sending live material, the international dangerous goods regulations for air transportation (International Air Transportation Association (IATA)) [22] and ground transportation (Accord européen relatif au transport international des marchandises Dangereuses par Route (ADR)) [23] need to be considered. Alternatively, for PCR and genome analyses, sending bacterial DNA can be sufficient. Genetic material prepared by an evaluated protocol including safe inactivation of the pathogen can be sent without considering specific shipment regulations.

We can assume that physicians in non-endemic European areas have a poor awareness of imported brucellosis in patients arriving from regions with endemic brucellosis, exemplified in our case by people who have migrated from regions in the Middle East. Although our patient had been in Germany for nine months and in ambulatory care during pregnancy, questions on travel history, animal contacts and consumption of raw animal products or duration of symptoms were not asked [4,24]. A connection between hip pain and brucellosis was not made.

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Conflict of interest

None declared.

Authors' contributions

Roland Grunow and Sonja Swidsinski wrote the manuscript. Dietmar Schlembach, Sabine Jackowski-Dohrmann treated the patient. Sonja Swidsinski, Bettina Eberspächer and Silke Klee, Daniela Jacob performed the initial and confirmatory microbiological tests. Roland Grunow, Sonja Swidsinski, Vera Loenning-Baucke, Daniela Jacob, read and revised the manuscript.

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Research article

Epidemiology of *Strongyloides stercoralis* in northern Italy: results of a multicentre case–control study, February 2013 to July 2014

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Strongyloides stercoralis **is a soil-transmitted helminth widely diffused in tropical and subtropical regions of the world. Autochthonous cases have been also diagnosed sporadically in areas of temperate climate. We aimed at defining the epidemiology of strongyloidiasis in immigrants and Italians living in three northern Italian Regions. Screening for *S. stercoralis* infection was done with serology, confirmation tests were a second serological method or stool agar culture. A case–control approach was adopted and patients with a peripheral eosinophil count ≥ 500/mcL were classified as cases. Of 2,701 individuals enrolled here 1,351 were cases and 1,350 controls; 86% were Italians, 48% women. Italians testing positive were in 8% (97/1,137) cases and 1% (13/1,178) controls (adjusted odds ratio (aOR) 8.2; 95% confidence interval (CI): 4.5–14.8), while positive immigrants were in 17% (36/214) cases and in 2% (3/172) controls (aOR 9.6; 95% CI: 2.9–32.4). Factors associated with a higher risk of infection for all study participants were eosinophilia ($p < 0.001$) and immigration ($p = 0.001$). Overall, strongyloidiasis was nine-times more frequent in individuals with eosinophilia than in those with normal eosinophil count.**

Introduction

Strongyloides stercoralis is a soil-transmitted helminth affecting millions of people worldwide [1,2]. Its transmission occurs in areas where poor hygienic conditions and humid, warm climate permit the free-living cycle of the parasite. The larvae present in the soil can penetrate human skin, therefore barefoot walking and agricultural activities pose people at risk of acquiring the infection. *S. stercoralis* produces larvae that can reinfect the host by a so called auto-infective cycle, a peculiarity shared only by *Capillaria* spp [3], so that an infected person remains infected life-long, if not properly treated [4]. This is the reason why strongyloidiasis can be diagnosed in people who have left endemic countries already several years before.

The few studies conducted in the United States (US) and in Europe to evaluate the prevalence of strongyloidiasis in immigrants and refugees from endemic countries, either through population or hospital-based studies, probably underestimated the real burden of the infection as long as microscopic stool examination was the only test used for screening [5]. In fact, the methods commonly employed for stool microscopy such as formalin-ether concentration, have a low sensitivity. Preferred faecal-based methods for the detection of *S. stercoralis* are Baermann funnel concentration and agar plate culture (APC), but the method that has so far demonstrated the highest sensitivity is serology [6]. Studies conducted in the field, classically underestimate the burden of strongyloidiasis if there is no special focus on this infection i.e. through using an appropriate diagnostic method. This is why the 'old' estimates of prevalence from the late 1980s and 1990s [7,8] were recently questioned [1,2].

The transmission of strongyloidiasis occurs especially in tropical and subtropical areas. However, in some temperate countries, autochthonous transmission occurred in the past [9,10], or might be still ongoing

Figure 1

Map of northern Italy showing where participating sites are situated, study of *Strongyloides stercoralis* epidemiology, northern Italy, February 2013–July 2014

The names of the study sites are in red.

[11,12]. Therefore, cases of *S. stercoralis* infection can be diagnosed in people who have never moved from the Mediterranean coast.

Strongyloidiasis can be fatal in immunocompromised patients so prompt diagnosis and effective treatment are crucial for all those infected, in order to prevent later complications, such as disseminated strongyloidiasis [4]. Chronic infection is characterised by mild, unspecific symptoms such as pruritus, abdominal pain or discomfort, respiratory impairment which are not easily attributable to *S. stercoralis* and there is no full agreement among experts on considering eosinophilia as a predictor of the infection [13]. However, in non-endemic countries a high eosinophil count might be a sufficient index of suspicion in travellers or in patients over 65 years with history of barefoot walking in a formerly endemic area [14,15].

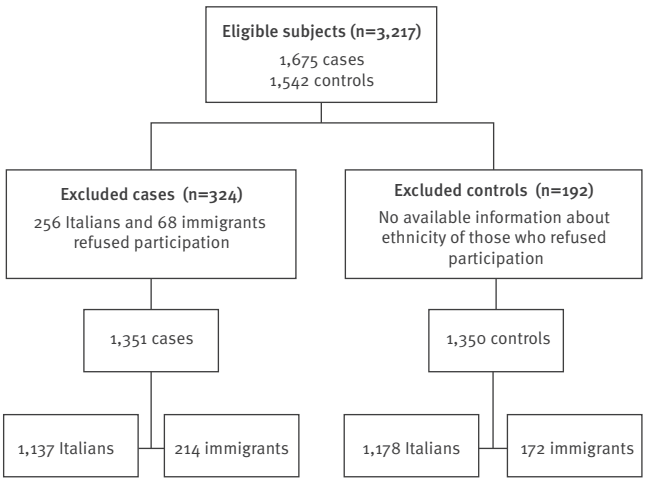
The treatment of choice for strongyloidiasis is ivermectin that has demonstrated a higher efficacy than albendazole [16]. Although the drug is included in the World Health Organization (WHO) list of essential medicines [17], it is not accessible for the vast majority of infected people in the world [18,19]. In fact, this essential drug is still donated to endemic countries, but with the strict limitation of use for *Wuchereria bancrofti* and *Onchocerca volvulus* control programmes [20]. In Italy, ivermectin has never been registered for human use.

In a previous pilot study, we screened 132 Italian individuals born in 1940 or earlier, with eosinophilia and no significant travel history, presenting to the clinical laboratories of two health districts. The serology test, an in-house immunofluorescence antibody test (IFAT), was positive in 28% of cases, suggesting that strongyloidiasis can be a relevant cause of eosinophilia in this group of individuals [9].

In the present study, we extended the previous screening in order to estimate the prevalence of strongyloidiasis in six provinces of three Italian Regions. The population analysed included both adult

Figure 2

Flowchart for inclusion of participants in study of *Strongyloides stercoralis* epidemiology, northern Italy, February 2013–July 2014



immigrants and Italians born before 1952, with or without eosinophilia.

Methods

Study design and setting

We conducted a multicentre case–control study.

Participants were enrolled between 2 February 2013 and 27 July 2014. The enrolling sites were the outpatient blood sampling sectors of seven hospitals located in three Italian Regions: Veneto (Negrar, San Bonifacio, and Treviso sites), Lombardia (Brescia, Mantova sites), and Friuli Venezia Giulia (Trieste, Udine sites) (Figure 1). The Centre for Tropical Diseases of Negrar (CTD) and the Health Prevention Department, Verona, were the coordinating centres.

On 1 January 2013, according to the Italian National Institute of Statistics [21], the total resident population in the six provinces of the three Regions involved in the study was 4,215,423 people (3,742,724 Italian and 472,699 foreign residents). With regard to Italian residents, 1,074,367 (28.7%) were >60 years (born before 1952), which was the age cut off for inclusion of Italians in the present study. As for immigrants, 351,347 (74.3%) were >17 years old, which was the age criterion for their inclusion in the present study.

Participants

Investigators proposed the screening to individuals meeting the inclusion criteria and consecutively presenting as outpatients to perform a full blood count to one of the collaborating laboratories, during 20 randomly-selected weeks. For the study purpose, we adopted the following definitions:

- Cases: individuals with peripheral eosinophil count ≥ 500/mcL;
- Controls: individuals with eosinophil count < 500/mcL;
- Italians: individuals born and resident in Italy;
- Immigrants: individuals who were born in an endemic area and resided there for at least the first two years of life, and without Italian citizenship.

Each selected week, every centre had to recruit 10 cases and 10 controls. Inclusion criteria were: Italians born before 1952 (as in CTD experience with hundreds of patients the infection was extremely rare in younger Italian individuals with no travel history), immigrants aged ≥ 18 years. Each participant gave informed written consent. Individuals included in the study received a copy of the result of the test(s) performed and, in case of positive or uncertain result, a treatment with ivermectin (200 µg/kg, stat dose) was offered. A case report form (CRF) with essential clinical data was filled for those with positive test results.

Laboratory methods

Screening for *S. stercoralis* infection was performed with a commercial ELISA test, IVD Research, CA, USA ELISA (IVD ELISA) during 2013, then Bordier ELISA until the end of the study period, due to unavailability of the former test; positive samples were tested with an in-house IFAT [22]. Discordant samples were analysed with Bordier ELISA during 2013, until when IVD ELISA was used as the screening test. Subsequently, due to unavailability of the latter test, Bordier ELISA was used as the screening test, and a third testing for discordant samples was no longer possible. The three tests have been described in detail elsewhere [23]. Patients testing positive in the screening tests were invited to supply a faecal sample for Koga agar plate culture for *S. stercoralis* [24] and/or for copro-parasitological test (formalin-ether concentration).

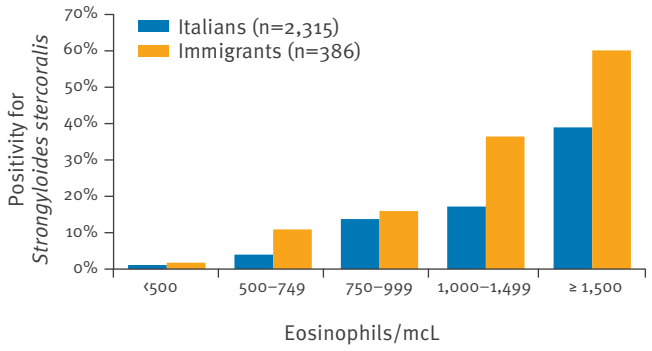
For the study purpose, patients were defined as 'positive' in case of two concordant positive serologic tests and/or a positive screening test AND a positive APC / copro-parasitological test. Individuals with only one positive screening test and negative stool test were classified as 'uncertain' in case a third serologic test was unavailable.

Study size

There is scarce data on the prevalence of strongyloidiasis in Italy. Previous, smaller studies, found a prevalence between 10 and 15% in Italians with eosinophilia aged >60 and >68 years, respectively, and around 4% in controls of the same age group with normal eosinophil count [9,14]. On the basis of these surveys, the study size was calculated considering an odds ratio (OR) for suspected/confirmed strongyloidiasis in cases vs controls of 3, a case/control ratio of 1:1, a prevalence

Figure 3

Percentage of positivity for *Strongyloides stercoralis* in relation to eosinophil count in Italians and in immigrants, study of S. stercoralis epidemiology, northern Italy, February 2013–July 2014 (n=2,701)



of strongyloidiasis in the control group of 3%, a confidence level at 95%, a study power of 80% and a design effect of 1.5. Eventually, a total of 950 Italians were to be tested, 475 cases and controls, respectively. Therefore, we initially established to enroll at least 500 individuals per group (1,000 Italian individuals in total).

The literature demonstrates a high variability in the prevalence of strongyloidiasis in immigrants, depending on their country of origin and on the screening method used [5]. Studies based on serology demonstrated a prevalence between 10 and 36%, irrespective of the eosinophil count. To calculate the study size we assumed an OR (for strongyloidiasis in cases vs controls) of 3 and a study power 80%. Based on these data, the minimum number of immigrants to be tested was 185 for each group, resulting in a total number of 370. Therefore, we attempted to enroll 200 individuals per group, 400 immigrants in total.

Overall, the minimal sample size required was of 1,400 individuals. As the sample size calculation was based on very weak estimates, particularly for Italian individuals for whom no formal, previous prevalence study was available, the proposed target sample was twice as large i.e. 2,800 individuals, 200 cases and 200 controls to be recruited by each study site.

Statistical methods

Data at each centre were entered in a pre-structured Excel file and analysed using Stata 10 software (Stata Corp., Texas, US). For quantitative variables, data distribution was checked for normality by Shapiro-Francia test.

Since data were not normally distributed, they were analysed using the non-parametric Mann–Whitney test and the variations among groups were calculated as medians with interquartile ranges (IQR). Associations among categorical variables were analysed by Pearson's chi-squared test or Fisher's exact test as appropriate, and presented as observed frequencies and proportions. Trend analysis was performed by chi-squared test for linear trend. The OR of finding the outcome of interest (i.e. *S. stercoralis* infection) in relationship to the eosinophil count (defining cases and controls) and to other variables of interest (sex, age, recruitment site, geographical area of origin) were calculated by logistic regression. For all tests, the level chosen to indicate statistical significance was $p < 0.05$ (two-tailed).

Ethical issues

The Ethics Committee of the coordinating centres (Comitato Etico della Provincia di Verona) approved the study protocol on 17 January 2012. The study protocol was then submitted to the Ethics Committees of each of the study sites, and formally approved.

All study participants received an information sheet and a letter for their general practitioner, explaining aim and methods of the study; signed informed consent form was required.

Results

Participants

A total of 3,217 individuals fulfilled the inclusion criteria; 516 were not included in the study because they were unable to give informed consent or refused to participate. The total number of individuals included in the study and analysed was 2,701 (Figure 2).

The study population comprised 1,392 men (52%) and 1,309 (48%) women. Each participating centre recruited ca 400 individuals.

Among 2,315 Italians, the proportion of women was 41% (n=464) for the 1,137 cases and 53% (n=625) for the 1,178 total controls. Median age was 73 years (range: 61–99; IQR: 67–78) and 72 years (range: 61–94; IQR: 67–77) for cases and controls, respectively. Median value of eosinophil count was 630/mcL (range: 500–24,890; IQR: 550–790) and 150/mcL (range: 0–490; IQR: 100–220) for cases and controls, respectively.

Among 386 immigrants, women represented 48% (n=103) of the 214 cases, and the proportion was higher for the 172 controls, 68% (n=117). Median age was 38 years (range: 18–87; IQR: 30–48) and 40 years (range: 18–83; IQR: 29–53) for cases and controls, respectively. Median value of eosinophil count was 655/mcL (range: 500–2,380; IQR: 570–830) and 145/mcL (range: 0–460; IQR: 70–240) for the cases and the controls, respectively.

Immigrants originated from Europe, especially eastern Europe and the Balkans (26%, n=101), Asia (22%, n=83), Sub-Saharan Africa (21%, n=82), North Africa and Middle East (18%, n=68), and Latin America (13%, n=52).

Table 1

Signs and symptoms compatible with strongyloidiasis in individuals testing positive who answered a questionnaire, study of *Strongyloides stercoralis* epidemiology, northern Italy, February 2013–July 2014 (n = 54)

Signs and symptoms	Number of Italians (%) n=43	Number of immigrants (%) n=11	Total number (%) n=54
Pruritus	23 (53.5)	4 (36.4)	27 (50)
Skin rash	13 (30.2)	2 (18.2)	15 (27.8)
Respiratory symptoms	16 (37.2)	3 (27.3)	19 (35.2)
Abdominal pain	9 (20.9)	1 (9.1)	10 (18.5)
Diarrhoea	1 (2.3)	2 (18.2)	3 (5.6)

Prevalence in cases and in controls

Overall, of 2,701 participating individuals, 149 (5%) were classified as positive (110 Italians and 39 immigrants) and 32 (1%) as uncertain (29 Italians and 3 immigrants).

Among Italians with eosinophilia (cases) 8% (97/1,137) were positive vs 1% (13/1,178) without eosinophilia. Considering a total population of the same age group of 1,074,367 in the six provinces, and an average of 4% of subjects of the same age with eosinophilia (data not shown), we obtain a rough estimate of 4,000 Italians over 60 years of age with *S. stercoralis* infection.

Among immigrants, positive cases were 17% (36/214) vs 2% positive controls (3/172), respectively. The proportion of positives was significantly higher among cases, both for Italians ($p < 0.001$) and immigrants ($p < 0.001$). Moreover, the higher the eosinophil count, the higher was the proportion of infected individuals, in both groups. Among Italians, the proportion of positive individuals ranged from 4% (31/780) for those with eosinophil counts between 500 and 749/mcL, to 39% (21/54) for those with eosinophil counts ≥ 1,500/mcL ($p < 0.001$) (Figure 3).

Among immigrants, this proportion ranged from 11% (15/138) for those with eosinophils between 500 and 749/mcL, to 60% (6/10) for those with eosinophils ≥ 1,500/mcL ($p < 0.001$) (Figure 3), albeit numbers were small in the latter group. Moreover, among the Italian cases, the proportion of positive individuals showed an upward trend with increasing age ($p < 0.001$) and varied depending on the study site ($p = 0.01$), with a peak in individuals born before 1936 (46/380; 12%) and in those recruited in the sites located in agricultural regions of the Po valley (e.g. San Bonifacio site: 19/146; 13%).

Immigrant cases had the following distribution, according to the region of origin of the patients: Latin America (6/31), Sub-Saharan Africa (14/48), Asia (11/55), Europe (2/44), and North Africa (3/36).

Some of the individuals who were positive in the screening test refused to provide a stool sample, therefore the results of stool tests were available only for 70% (104/149) of patients with positive serology of which 28% (n=29/104) had a positive stool result. Ninety-nine of 149 positive patients (66%), plus seven individuals with uncertain result, received ivermectin treatment, offered free of charge to all eligible patients. Information about possible risk factors for complicated strongyloidiasis was available for 83% (124/149) positive individuals: 16% (20/124) presented a current or past condition considered to constitute a risk for the development of severe strongyloidiasis. In the latter group, most (17/20) were treated, while two individuals refused and one died of metastatic breast cancer soon after being tested.

Analysis on the subgroup of 54 of 149 positive individuals who answered the questionnaire showed that the majority had signs and symptoms compatible with strongyloidiasis (Table 1) and had been exposed to a risk factor for infection (farm work 32/54; walking barefoot in earlier years 37/54). Only two of 43 responding Italians reported a stay longer than one month in endemic countries, where they might have had contact with contaminated soil. The remaining Italians did not present a relevant travel history, so we assume that the infection was probably acquired in Italy.

By logistic regression, eosinophilia ($p < 0.001$) and immigration ($p = 0.001$) were independent risk factors for infection for all participants. After adjusting for birth cohort, sex and site of recruitment for Italians, or age, sex and geographical area of origin for immigrants, presence of eosinophilia ≥ 500/mcL was significantly associated with infection both in Italians (adjusted OR: 8.18; 95% CI: 4.53–14.76; $p < 0.001$) and in immigrants (aOR: 9.62; 95% CI: 2.85–32.41; $p < 0.001$). Among Italians, year of birth and site of recruitment maintained a significant association with infection also at the multivariate analysis (Table 2); the same occurred among immigrants with regard to area of origin (Table 3).

Discussion

The high number of screened individuals, especially Italians, in our study, permitted to obtain a valuable estimate of the prevalence of strongyloidiasis in the

Table 2

Logistic regression analysis of factors associated with Italians testing positive for *Strongyloides stercoralis*, study of *S. stercoralis* epidemiology, northern Italy, February 2013–July 2014 (n = 2,315 of which 1,137 cases and 1,178 controls)

Factors	OR	95% CI	P value
Eosinophil count ≥ 500/mcL	8.18	4.53–14.76	< 0.001
Sex (male vs female)	0.93	0.63–1.39	0.730
Year of birth			
1947–1951	1.00	Reference	NA.
1937–1946	2.56	1.24–5.28	0.011
1936 or before	3.95	1.90–8.20	< 0.001
Recruitment site			
Trieste	1.00	Reference	NA
Udine	1.34	0.54–3.32	0.52
Negrar	1.67	0.69–4.00	0.25
Mantova	2.33	1.00–5.41	0.050
Brescia	2.47	1.07–5.70	0.033
Treviso	2.97	1.33–6.64	0.008
San Bonifacio	3.43	1.54–7.68	0.003

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

studied regions in the north of Italy: 8 and 17%, respectively, in Italians and immigrants with eosinophilia, 1 and 2% in those with a normal eosinophil count, irrespective of signs/symptoms of the infection. This finding is relevant for autochthonous Italians, for whom prevalence data were previously limited and patchy, and this study demonstrated a considerable proportion of infected individuals. In addition, 2% of Italian controls without eosinophilia with positive/uncertain test result is worth of note. The findings indicate that the infection is not an extinguished problem among elderly Italians living in the study areas.

The geographical pattern of infection prevalence is consistent with a higher transmission in agricultural areas of Po valley during the first decades of the past century, with a downward trend over time likely due to improvement of hygiene and sanitary conditions. Parts of the country, in the centre and in the south, presented in the past characteristics that make a location suitable for the free-living cycle of *S. stercoralis*. It is thus probable that a similar epidemiological picture might be prevalent in a large part, if not in the whole, of Italy. This could also be true for other countries in the Mediterranean basin, where sporadic autochthonous strongyloidiasis cases have been diagnosed [10,12].

Among immigrants, the proportion of positive individuals was high among cases with eosinophil counts ≥ 500/mcL, particularly if individuals originated from Sub-Saharan Africa, Asia, and Latin America.

Prevalence data are fundamental to implement screening and prevention programmes. We believe our results support the establishment of risk categories for screening individuals at risk of developing strongyloidiasis, such as elderly Italians (and, probably, Europeans from other Mediterranean countries) and immigrants with eosinophilia. In the latter group, it might be even cost-effective to treat all patients without testing [4]. This should, however, be demonstrated by a well-designed study, also considering that a pre-treatment diagnostic evaluation (obligatorily including serology) is crucial to monitor cure at follow-up [25].

One in three of the infected individuals refused the treatment that was offered free of charge after a thorough explanation of the risk associated with untreated, chronic infection. Even general practitioners were not always keen to collaborate. Our experience suggests that strongyloidiasis is not always perceived as a relevant health problem, not only by the general population, but also by the medical community. To overcome this problem, it would be advisable to create national guidelines for the screening and management of eosinophilia that should consider strongyloidiasis among the differential diagnoses. Moreover, considering that strongyloidiasis can be fatal in immunocompromised individuals, *S. stercoralis* should be included in guidelines/protocols for screening of candidate patients for immune-suppressant therapies, such as the oncological and rheumatological ones.

Limitations

We faced some difficulties in finding eligible immigrants for inclusion in the study, therefore the number of immigrants enrolled was slightly lower than planned. This was the reason, in addition to that provided in the Methods part, to recruit a higher number of Italians than the initially calculated sample size, as we did not deem it appropriate, to stop the recruitment in this group and continue only with immigrants. We did not include in the analysis the countries of origin as numbers for such analysis were too small and instead we analysed the continents/macro-areas. We still believe the results are useful, considering the paucity of similar data in the literature. Although the included individuals were not randomly extracted from the general population, the enrolment of out-patients, coming to the hospital laboratory to perform a very simple and common test (full blood count), results in a sample that can be comparable to the general population in that age range, in particular for the larger Italian group. The controls were unmatched, but consecutively recruited on a randomly selected day on a 1:1 basis, within the same main group (Italian or immigrant) and age range.

Finally, the accuracy of serology is high, but false-positive and false-negative results can occur [23]. The use of a second, confirmatory serological test in addition to the faecal-based tests, when available, was aimed to increase the specificity of the results. Sensitivity can be lower in immunocompromised individuals [26], however, we believe that this may have had a minimal influence on the overall results, given the high number

Table 3

Logistic regression analysis of factors associated with testing positive for *Strongyloides stercoralis* among immigrant individuals, study of *S. stercoralis* epidemiology, northern Italy, February 2013–July 2014 (n = 386 of which 214 cases and 172 controls)

Factors	OR	95% CI	P value
Eosinophil count ≥ 500/mcL	9.62	2.85–32.41	< 0.001
Sex (male vs female)	1.70	0.82–3.54	0.16
Age (+1 year)	1.00	0.97–1.03	0.83
Geographical area of origin			
Europe	1.00	Reference	NA
North Africa/Middle East	1.77	0.28–11.27	0.55
Asia	5.01	1.02–24.59	0.047
Latin America	6.33	1.20–33.40	0.030
Sub-Saharan Africa	9.54	2.01–45.19	0.004

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

of individuals screened [19]. The screening test had to be changed, however Bordier ELISA and IVD demonstrated similar accuracy in our previous study [23]. Therefore, we believe that the number of patients positive at screening might not have been substantially different with IVD ELISA. On the other hand, this change entailed the lack of a third serology test, therefore patients with discordant results had to be classified as uncertain. PCR was not available at our Centre before 2014, hence we could not use this method, that showed good accuracy compared with APC and Baermann technique [27,28]. PCR is less cumbersome than the traditional faecal-based methods and the samples can be stored (either frozen or with ethanol), therefore it could have been a useful tool considering the high number of individuals screened.

Conclusions

The improvement of hygienic conditions and sanitation, and the availability of deworming drugs are likely to successfully control most helminth infections in endemic areas in the forthcoming years. However, the lack of mass drug administration programmes specifically targeting *S. stercoralis* (using ivermectin) might lead to long-term persistence of this infection in some individuals. It is also important to note that, due to the peculiarity of the auto-infective cycle of *S. stercoralis*, this parasite may remain once the other helminth infections have disappeared. This has been observed in Italy. Physicians should be aware of the categories of patients that would require screening for *S. stercoralis* infection.

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Conflict of interest

None declared.

Authors' contributions

D Buonfrate, Z Bisoffi, and M Baldissera drafted the manuscript. Z Bisoffi and F Abrescia conceived the study. D Buonfrate, Z Bisoffi, F Abrescia and G Napoletano coordinated the investigations and supervised the study. M Baldissera performed the statistical analysis. M Bassetti, F Castelli, N Scattolo were responsible for the coordination of local investigations. Epidemiological investigations were conducted by D Buonfrate, M Mascarello, M Giobbia, G Caramaschi. All authors commented and agreed upon the final manuscript.

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Research article

Estimating the economic impact of a possible equine and human epidemic of West Nile virus infection in Belgium

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This study aimed at estimating, in a prospective scenario, the potential economic impact of a possible epidemic of WNV infection in Belgium, based on 2012 values for the equine and human health sectors, in order to increase preparedness and help decision-makers. Modelling of risk areas, based on the habitat suitable for *Culex pipiens*, the main vector of the virus, allowed us to determine equine and human populations at risk. Characteristics of the different clinical forms of the disease based on past epidemics in Europe allowed morbidity among horses and humans to be estimated. The main costs for the equine sector were vaccination and replacement value of dead or euthanised horses. The choice of the vaccination strategy would have important consequences in terms of cost. Vaccination of the country's whole population of horses, based on a worst-case scenario, would cost more than EUR 30 million; for areas at risk, the cost would be around EUR 16–17 million. Regarding the impact on human health, short-term costs and socio-economic losses were estimated for patients who developed the neuroinvasive form of the disease, as no vaccine is available yet for humans. Hospital charges of around EUR 3,600 for a case of West Nile neuroinvasive disease and EUR 4,500 for a case of acute flaccid paralysis would be the major financial consequence of an epidemic of West Nile virus infection in humans in Belgium.

Introduction

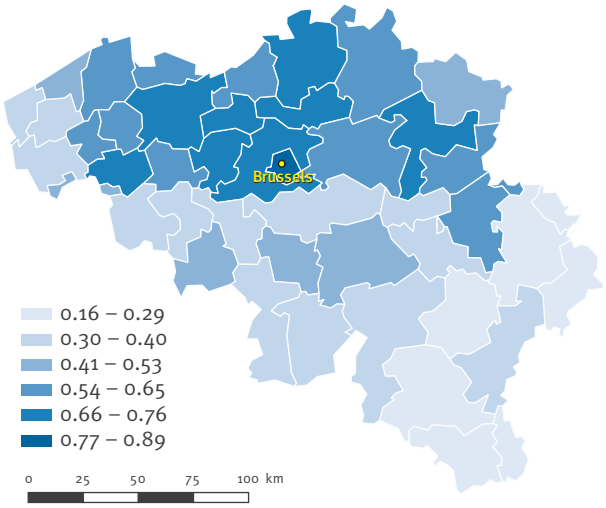
West Nile virus (WNV) is a vector-borne pathogen, member of the genus *Flavivirus* (family *Flaviviridae*); its main vectors are mosquitoes belonging to the *Culicidae* family, genus *Culex* [1,2]. The infection is maintained in a bird–mosquito enzootic cycle, and birds, especially passerines, are the primary reservoir hosts. Horses and humans are considered as accidental dead-end hosts, and are thought not to transmit the virus to other mosquitoes [3]. The disease generates clinical signs mainly in horses and humans, while most infected birds in Europe are not clinically affected [4]. The majority of horses remain asymptomatic, and about 10% of clinical cases develop neurological signs [5]. In humans, after an incubation period of 2 to 14 days, two main clinical pictures can be observed: an influenza-like syndrome (West Nile fever, WNF) and a neuroinvasive form (West Nile neuroinvasive disease, WNND) [4].

The virus is considered as an emerging pathogen in numerous parts of the world. In the United States (US), it has been responsible for substantial socio-economic losses, both in the equine industry and in the human health sector, since its emergence in 1999 [6]. In Europe, the virus is constantly expanding its geographical distribution [7] and even re-emerging in some areas: indeed, several equine cases have been reported since September 2015 in France, which also registered its first autochthonous human case since 2003 [8]. To date, no autochthonous human case has been reported in Belgium, but the first imported case was described in 2013, an elderly woman who had travelled to Greece [9]. As *Cx. pipiens*, a common mosquito vector of WNV in Europe [10,11], is endemic in Belgium, the risk of emergence in the near future should be seriously considered. There is thus a need to get prepared in advance of such an emergence, in terms of management strategies and their respective socio-economic impact. Indeed, western Europe has a recent history of severe economic losses associated with animal diseases, e.g. with bluetongue disease epidemics in 2006 [12] and Schmallenberg virus disease in 2011–12 [13].

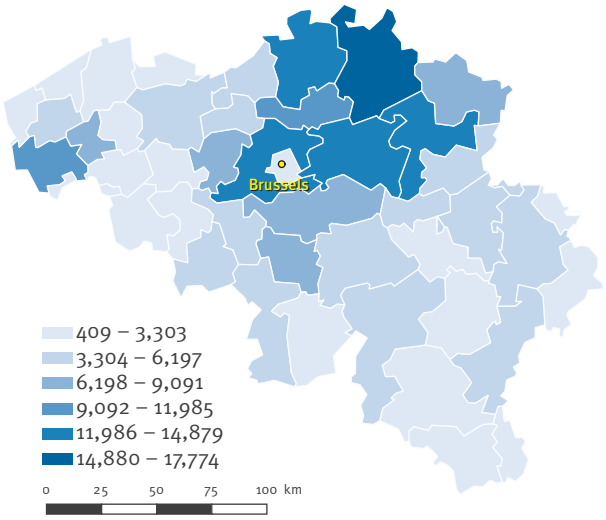
Figure 1

Proportion of land compatible with *Culex pipiens* ecology (A), horse population (B) and human density per km² (C), per district in Belgium, 2012

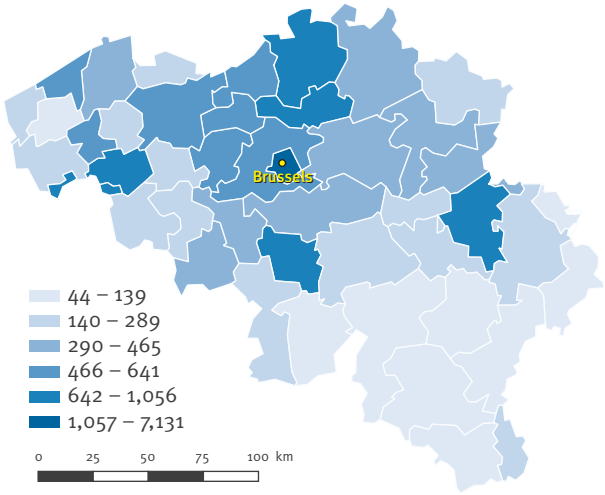
A. Proportion of land compatible with *Culex pipiens* ecology



B. Population of horses



C. Human density per square kilometre



The objective of the study presented here was to estimate, in predictive scenarios, the economic impact on both the equine and human health sectors of the spread of WNV in Belgium during an epidemic. When dealing with an unpredictable occurrence of a disease (limited knowledge about likelihoods) but with good knowledge of outcomes, a scenario analysis is recommended [14,15]. Estimating the cost of an illness is a useful aid to policy decision-making. It identifies the different components of cost and the size of the contribution of each sector, which can help determining mitigation measures, research and funding priorities by highlighting areas where inefficiencies may exist and savings could be made [16].

Methods

Determination of risk areas and populations at risk

In order to determine the proportion of Belgian territory representing a habitat suitable for *Cx. pipiens* (the main potential vector for WNV in Belgium), land cover data were extracted from the CORINE (Coordination de l'information sur l'environnement) land cover (CLC) database [17]; suitability of different land covers was further determined for *Cx. pipiens* and the proportion of these suitable land covers was estimated at the district level [18]. This first step allowed us to determine the equine (using information from the Belgian Horse Confederacy) and human [19] populations at risk, at the district level. It was assumed that the density of WNV-competent birds was high and homogeneously

distributed across the whole territory. The distribution of equestrian centres at district level was used to estimate their potential loss of earnings as a result of an epidemic; data were provided by regional and provincial equestrian leagues.

Equine industry

In Belgium, contrary to what is observed in many parts of the US, such as Texas, Colorado or Nebraska [20], horses are mostly used for recreational purposes, and their agricultural importance is very limited. A 2010 study assessed the economic weight of equine industry in southern Belgium and distinguished three categories of horses [21]: (i) high-value horses (20%), mostly show horses that are cared for intensively; (ii) leisure horses (40%), which usually spend the winter indoors and the summer in pastures (horses of equestrian centres also fall into this category); and (iii) semi-feral horses (40%), which spend most of their time in pastures, and are occasionally used for leisure (they are owned by individuals but are often untrained).

Economic impacts of WNV on the equine sector were estimated on the basis of the disease characteristics observed in previous European epidemics of WNV infection. Two scenarios based on infection rates previously estimated during outbreaks in France were included in the model: 8.5% infection rate [22] vs 34% infection rate [23]. A 10% morbidity rate was assumed, according to French data [5], and the number of equine cases was determined as follows:

Formula 1

$$N_{\text{horse cases}} = [a \times 0.1 \times b \times c]$$

a = infection rate = 8.5% vs 34% (proportion of horses infected by the virus, but not necessarily showing clinical signs) [22,23];

0.1 = morbidity rate (10% of infected horses will develop symptoms of disease) [5];

b = proportion of the district (in terms of land cover) suitable for *Cx. pipiens* (used to determine the whole population of horses living in risk areas);

c = district total horse population.

The hospitalisation rate of neurological equine cases was fixed at 35% of clinically affected horses [24], and a 28% case fatality rate, as observed in France, was applied [25]. The mean length of the clinical disease was considered to be seven days (as was duration of hospital stay) [26]. The duration of an epidemic was estimated to be 2.5 months, with the first case reported on 1 August and the last case resolved on 21 October. This is in agreement with findings of most European equine cases, which are reported between August and October [27].

In terms of Belgian legislation on animal movement in case of epidemics of WNV infection, restrictions only apply for suspected and confirmed cases of WNF, which cannot be moved, except to be transported to a veterinary healthcare facility [28].

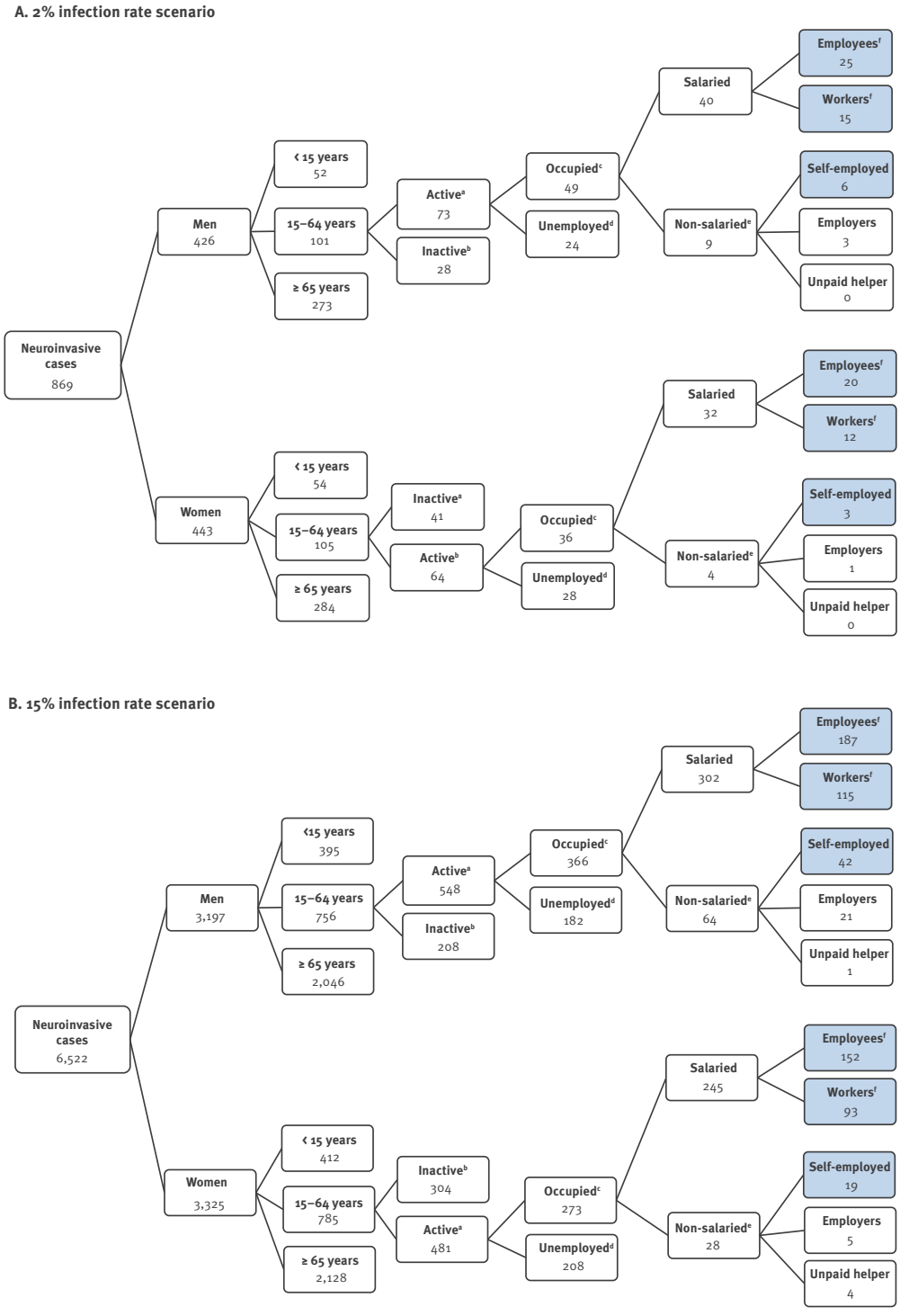
A distinction was made between non-hospitalised and hospitalised horses to estimate economic costs. The following costs were included for non-hospitalised horses: visits of a veterinary practitioner, serological diagnosis (ELISA) and reverse transcription (RT)-PCR, as well as treatment (non-steroidal anti-inflammatory medicine). Hospitalised horses were considered to be seen first by a veterinary practitioner before being referred to a veterinary hospital. The estimation of costs included also: costs of a seven-day stay, complementary examinations (blood analysis, radiography, puncture of cerebrospinal fluid, diagnosis and neurology) and seven-day treatment (steroidal and non-steroidal anti-inflammatory medicine, intravenous fluids) [24]. For horses that died or had to be euthanised, transport and destruction of cadavers, as well as replacement value, were considered for the three categories of horses (i.e. high-value, leisure and semi-feral).

The cost of vaccination was also included in the estimations. According to current Belgian legislation, the vaccination of equids against WNV is not mandatory; nevertheless, the Minister of Agriculture could modify that decision in case of epidemics [29]. Two vaccination scenarios were thus applied in our model in order to investigate the potential impact of such a preventive measure on the estimation of costs: the first scenario relies on the vaccination of the entire equine population (except sick horses), while the second scenario considered the vaccination of horses in risk areas only. Primary vaccination consists of two doses, the second dose being administered 3–5 or 4–6 weeks later, depending on the vaccine used; indeed, in Belgium, two vaccines are registered for horses older than 5–6 months [30].

According to the Belgian Federal Authorities, an outbreak is confirmed when there is proof that the WNV is effectively transmitted by a local infected vector population, competent for transmitting the virus [28,29]. Consecutively, passive clinical surveillance is enhanced and active serological surveillance is implemented for horses in the area where the epidemiological investigation is carried out (within a 50 km radius) [28,29]. Active surveillance in horses consists of an ELISA (detection of IgG and IgM) performed on blood samples, by the National Reference Laboratory in Brussels. In order to determine the number of blood samples that would need to be taken, estimation was made through Win Episcope 2.0 software, considering a sample size needed to detect the disease, with a 5% expected prevalence and 95% confidence interval.

Figure 2

Estimated number of patients with West Nile neuroinvasive disease, by employment category and infection rate scenario, following an epidemic of West Nile virus infection in Belgium, 2012



These estimates were based on statistics related to the labour market, e.g. employment rate (according to sex or status) of working patients. Costs and losses were not estimated for children under 15 years of age and students.

Shaded boxes show the number of patients estimated for each professional category considered in our estimations of costs (related to productivity losses).

[a] Active individuals include all persons of working age, who carry out a paid activity (population active occupied) and job seekers (population active unoccupied/ unemployed) [61].

[b] Inactive individuals include all persons, even those under 15 years of age, who are not economically active [61].

[c] Occupied individuals include all persons, aged 15 years and above, who have a paid job (self-employed, employees and workers).

[d] We considered unemployed people not only to be those who did not work (and were entitled to unemployment benefit), but also people not on the labour market, such as housewives/house husbands.

[e] In the 'non-salaried' category, only self-employed people were considered for the estimate of losses. Indeed, no losses can be estimated for unpaid helpers; losses for employers were those estimated for occupied people in our study.

[f] We made a distinction between employees (who generally carry out intellectual work) and workers (who mainly carry out manual tasks).

For equestrian centres, serology was also performed on asymptomatic horses located in the same centre, as suggested in the WNV scenario elaborated by the Belgian Federal Agency for the Safety of the Food Chain [28]. Active surveillance was considered to be implemented throughout the epidemic (13 weeks), with a 15-day frequency [31], which means six sampling periods.

In Belgium, any movement of a live animal suspected to have viral encephalitis is prohibited [29]. As it is also mandatory to isolate horses that are suspected or confirmed to have WNV infection [29], associated additional costs of feed (cereals, hay and water) and litter (straw) were estimated for the duration of the epidemic for all equine cases (hospitalised and non-hospitalised horses), because suspected cases are assumed not to be left out on pastures.

The loss of income for affected equestrian centres (mean of 20 horses for public use per centre [21]), of the 830 registered in the country, was also estimated, considering the mean number of days of lost-use for equids clinically sick and recovering [32]. All assumptions made in our study are compiled in Table 1.

Public health

In humans, two main clinical disease forms are described: WNF and WNND [33]. Our study only included patients affected by WNND for the estimation of costs.

The potential consequence of WNF would logically be for the affected person to visit to a general practitioner (GP) and miss five days at work [34]. It seems unlikely, however, that all affected people would consult their GP. The impact on productivity should be limited, as these people would not be replaced for such a short length of absence, and they would have to deal with the backlog on returning to work. Alternatively, many people with WNF could miss work for less than five days (e.g. self-employed people who lose a net salary when missing a working day).

Three main syndromes may be observed in case of neuroinvasive disease: meningitis, encephalitis and acute flaccid paralysis (AFP) [4]. As a previous study estimated the costs for meningitis and encephalitis to be quite similar [35], our work distinguished between two main syndromes: meningoencephalitis and AFP.

The human population at risk was determined per district according to the same procedure as for equids. Two scenarios were tested, based on infection rates estimated during an equine WNV outbreak in southern France in 2000 [36].

The number of WNND cases was calculated as follows:

Formula 2

$$N_{\text{WNND cases}} = [a \times b \times c \times 0.007]$$

a = district total population;

b = proportion of the district (in terms of land cover) suitable for *Cx. pipiens*;

c = infection rate (2%, as estimated among 1,104 blood donors living outside the region of the 2000 equine epidemic of WNV infection in southern France, or 15%, determined among 1,053 blood donors living in the epidemic zone [36]);

0.007 = percentage of patients develop WNND [37].

A 0.7% morbidity rate for WNND was considered, which means that among infected patients, 0.7% will develop the neuroinvasive form requiring hospitalisation [37]. Infection rates were assumed to be uniformly distributed in the population, without considering criteria such as age and sex, as they have not been identified as risk factors in an important case–control study performed in Greece in 2010 [38]. Of 197 WNND cases reported in Greece in 2010, 3% were classified as AFP cases [39]. An 11% case fatality rate was applied, as reported for the whole of the European Union (EU) in 2012 [7]. All WNND cases were assumed to be hospitalised, as considered in a previous work [40]. The mean hospitalisation length of stay was nine days (Philippe Leonard, personal communication, September 2011). No vaccine is currently available for humans and the only treatment is supportive care [4]. All assumptions made in our study are shown in Table 1.

As for the equine sector, economic impacts were estimated taking into account several aspects, such as medical and hospital costs, costs for home care, compensation paid for the death of a patient and costs associated with work absenteeism. Short-term initial costs included a visit to a GP. In Belgium, the healthcare system is divided into state and private sectors. It is funded by a combination of social security contributions and health insurance funds. Healthcare insurance is mandatory: patients generally pay the costs and are then reimbursed for part of the charges. Individuals can also improve their cover by taking out private insurance, which allows them to be fully refunded for all medical costs [41]. Inpatient costs for acute care and rehabilitation were also considered: hospital stay (room and board charges), complementary tests and examinations (e.g. electroencephalogram, cerebrospinal fluid analysis, visit of a neurologist, heart monitoring, imaging, laboratory investigation and serology) and pharmacy/medical supplies (treatment charges: pharmacy, drugs such as antibiotics and antiviral medicine, and anti-epileptics, injections, medical supplies, intravenous fluids and intravenous therapy).

Costs related to hospital charges were indexed, as information was collected from hospital records concerning a patient hospitalised in Belgium for WNND in 2004 (encephalitis with epilepsy) (Philippe Leonard, personal communication, September 2011).

Table 1a

Model parameters for estimating economic impact of an epidemic of West Nile virus infection in Belgium, based on 2012 values

Parameter	Value	Unit	Source
Vectors			
Proportion of territory (land cover) representing a habitat suitable for *Culex pipiens*	Variable	%	[18]
Duration of the epidemic	2.5	Months	[7]
Horses			
District horse population	Variable	Number	Belgian Horse Confederacy, Jean-Pierre Devos, personal communication, July 2013
Equestrian centres (per district)	Variable	Number	Belgian Regional and Provincial Equestrian leagues, Jan Deboitselier, personal communication, July 2013
Horse infection rate (proportion of the horse population living in the risk areas infected by the virus)	8.5 vs 34	%	[22,23]
Horse morbidity rate (will develop clinical signs of disease)	10	%	[5]
Hospitalisation rate for neurological cases	35	%	[24]
Horse case fatality rate (mortality among neurological cases; the most severe cases being hospitalised)	28	%	[25]
Mean length of the clinical disease (duration of hospital stay for hospitalised horses)	7	Days	[26]
Active surveillance (screening)	ELISA results	NA	[28,29]
Duration of active surveillance (whole epidemics)	13	Weeks	[28]
Frequency of sampling – active surveillance	15	Days	[31]
Detection of the disease – 5% expected prevalence (95% confidence interval)[a]	Variable	Number	Win Episcope 2.0; [24]
Mean number of horses per equestrian centre (for public use; not privately owned)	20	Number	[21]
Public health			
District human population	Variable	Number	[19]
Human infection rate (proportion of the population living in the risk areas infected by the virus)	2 vs 15	%	[36]
Human morbidity rate for WNND (all patients assumed to be hospitalised)	0.7	%	[37]
Proportion of AFP among WNND cases (all assumed to be›65 years-old)	3	%	[39]
Human case fatality rate (mortality among patients with WNND)	11	%	[7]
Mean age of deceased patients	78	Years	[58]
Mean hospitalisation length of stay	9	Days	Philippe Leonard, personal communication, July 2011
Home recovery			
Duration (working days)	20	Days	Philippe Leonard, personal communication, July 2011
Daily cost for a home nurse (two visits a day – one hour a day)	16	Euros	[59]
Daily cost for a caregiver (eight hours a day)	62	Euros	[60]

AFP: acute flaccid paralysis; NA: not applicable; WNND: West Nile neuroinvasive disease.

a The decision to select 5% expected prevalence arose from the results of Durand et al. in France [22] who estimated an 8.5% prevalence in a West Nile virus outbreak in horses in 2000; thus, a 5% threshold seemed realistic.

b Active individuals include all persons of working age, who carry out a paid activity (population active occupied) and job seekers (population active unoccupied/unemployed) [61].

c We considered unemployed people not only to be those who did not work (and were entitled to unemployment benefit), but also people not on the labour market ,such as housewives/house husbands.

d We made a distinction between employees (who generally carry out intellectual work) and workers (who mainly carry out manual tasks).

e All information regarding costs for a hospitalised case was obtained from hospital records regarding a patient hospitalised in Belgium for WNND in 2004: we therefore adapted the costs to 2012 values, taking into account the evolution of the consumer price index.

Table 1B

Model parameters for estimating economic impact of an epidemic of West Nile virus infection in Belgium, based on 2012 values

Parameter	Value	Unit	Source
Productivity lost			
Percentage of men in the population	49.06	%	[19]
Percentage of women in the population	50.94	%	[19]
Activity rate[b] in people aged 15–64 years – men	72.5	%	[19]
Activity rate[b] in people aged 15–64 years – women	61.3	%	[19]
Employment rate[c] – men	66.9	%	[19]
Employment rate[c] – women	56.8	%	[19]
Proportion of employees[d]	62.0	%	[19]
Proportion of workers[d]	38.0	%	[19]
Mean annual growth (to adapt 2004 healthcare prices[e] to 2012 values)	1.7	%	[42]
Costs associated with the death of a patient (insurance claims paid to beneficiaries)	9,800	Euros	Belgian insurance company, personal communication, November 2014
Mean occupational interruption to estimate productivity lost (working days)	30	Days	[40]
Mean gross monthly salary – Men (2012) – Employee	3,668	Euros	[19]
Mean gross monthly salary – Men (2012) – Worker	2,749	Euros	[19]
Mean gross monthly income – Men (2012) – Self-employed	3,700	Euros	[19]
Mean gross monthly salary – Women (2012) – Employee	3,372	Euros	[19]
Mean gross monthly salary – Women (2012) – Worker	2,527	Euros	[19]
Mean gross monthly income – Women (2012) – Self-employed	3,413	Euros	[19]

AFP: acute flaccid paralysis; NA: not applicable; WNND: West Nile neuroinvasive disease.

[a] The decision to select 5% expected prevalence arose from the results of Durand et al. in France [22] who estimated an 8.5% prevalence in a West Nile virus outbreak in horses in 2000; thus, a 5% threshold seemed realistic.

[b] Active individuals include all persons of working age, who carry out a paid activity (population active occupied) and job seekers (population active unoccupied/unemployed) [61].

[c] We considered unemployed people not only to be those who did not work (and were entitled to unemployment benefit), but also people not on the labour market ,such as housewives/house husbands.

[d] We made a distinction between employees (who generally carry out intellectual work) and workers (who mainly carry out manual tasks).

[e] All information regarding costs for a hospitalised case was obtained from hospital records regarding a patient hospitalised in Belgium for WNND in 2004: we therefore adapted the costs to 2012 values, taking into account the evolution of the consumer price index.

We estimated the costs in 2012: thus a mean annual growth rate of 1.7% was applied to adapt healthcare prices to the evolution of the consumer price index for the eight-year period and convert them into 2012 values [42]. Hospitalisation costs were estimated to be 1.25 times higher for AFP cases than the costs for patients affected by WNND, based on estimates used in a study in the US [35].

Costs associated with the death of patients were estimated through an ex post approach, as part of human capital theory [43], and consisted of insurance claims paid to beneficiaries. In order to obtain representative data, a directory of accidents from a car insurance branch of a Belgian insurance company was analysed over a five-year period (2009–13). The insurance claim considered only covered the economic loss, and not the suffering of close relatives (i.e. pretium doloris). Considering a 9.78% annuity conversion rate, estimated according to the life expectancy of a 78 year-old person, the insurance claim reached EUR 9,800, on the basis of a 1.5% annual discount rate.

Additionally, a mean occupational interruption of 30 working days (for symptomatic disease and recovery) was taken into account to estimate productivity lost [40]. To establish the distribution of patients in different employment categories, statistics relative to the labour market – such as employment rate according to age and sex, status of working patients (self-employed vs employed) and mean gross monthly wages – were provided by the Belgian Federal Public Service of Economy, Small and Medium Enterprises, Self-employed and Energy, for 2012. We made a distinction between employees (who generally carry out intellectual work) and workers (who mainly carry out manual tasks).

Results

Habitat suitability in Belgium for *Cx. pipiens* is illustrated at the district level in Figure 1 (panel A). The proportion of suitable habitat was the basis for estimating the number of individuals at risk (in terms of proportions of the whole population of the district). Horse and human populations are shown in panels B and C, respectively.

Equine industry

Estimates of economic costs associated with WNF per horse are summarised in Table 2.

When considering national estimations (Table 3), the main costs would be related to the replacement of dead or euthanised horses, followed by hospitalisation charges. If vaccination was implemented (whole territory or areas at risk), associated costs would then represent the major expense.

At national level, the lost revenue for equestrian centres following an outbreak would reach between EUR 113,022 and EUR 450,450 for the 8.5% and 34% infection rate scenarios, respectively, assuming that two clinically sick horses per equestrian centre were not used during the time of clinical disease/treatment and recovery. The occurrence of WNV infection in these equestrian centres also implied the sampling of all horses located in the same centre (mean of 20 horses per centre). Detailed estimations can be consulted in supplementary material [44-47].

Public health aspects

The estimated distribution of WNND cases, for both infection rate scenarios, according to the criteria used is shown in Figure 2. No productivity loss was estimated for people aged over 65 years and for patients with AFP, as they are all assumed to be retired. Nor did we estimate productivity lost for caregivers (often another family member), who might miss work to care for the recovering patient after hospital discharge. Home care costs were nevertheless estimated for surviving hospitalised patients during their recovery (20-day period).

The costs incurred per patient with WNND, as well as the associated productivity losses, are summarised in Table 4.

National estimates, as shown in Table 5, highlight the importance of hospital costs (around 50% of total costs), compared with those for home care during recovery (about 30% of total costs). Detailed estimations can be consulted in supplementary material [44-47].

Discussion

We estimated, in a prospective scenario, the economic impact of a possible epidemic of WNV infection in Belgium, according to different infection rate scenarios in horses and humans. Considering the whole territory would be concerned might be an overestimation (worst-case scenario). In view of epidemics reported in the EU to date, spatial extension might be less pronounced. All conditions for epidemic spread are met in the northern part of the country, as *Cx. pipiens* habitat and areas of human density are closely related, as are areas with horse populations. Our selected infection rates might seem low compared with those in WNV-endemic areas of Africa, where seroprevalence of more than 90% was detected in horses in some places [48], but they reflect better the dynamics of the virus in Europe. Applying infection rates reported in epidemic situations allowed us to identify substantial costs and losses associated with the disease, both in horses and humans.

Impact on the equine industry

The total estimated cost for treating a horse affected by neurological disease due to WNV in 2012 would be more than estimated in the US, in Colorado and Nebraska, in 2002 [32]. Intensive care for moderate cases (wobbly gait, difficulty eating, signs of colic, reluctance to move, hypersensitivity to noise and touch, and altered awareness) was estimated to cost USD 400 (EUR 381) (equivalent to USD 539 (EUR 408) in 2012). For hospitalised horses showing severe neurological disease, we estimated such costs to reach EUR 741 (USD 917). Our estimates are more in line with a later survey performed in Texas, US, for 2002, in which veterinarians estimated the cost of treatment of a severe WNND equine case to be over USD 701 (EUR 668) (when considering a mean annual growth rate of 1.7%, as mentioned above) [49].

Vaccination is the main cost associated with epidemic spread of WNV in horses: the strategy, if implemented, would have a major economic impact, as vaccinating the whole equine population (100% coverage scenario) would cost over EUR 30 million. The time needed to produce and commercialise enough vaccine doses would not be less than six to nine months. Thus we assume that complete vaccination of the whole population of horses could be achieved by the following vector season. The vaccine should be administered to horses aged five to six months and above [30]: unfortunately, the proportion of animals falling into this age category was not available, which would have allowed us estimate more precisely the costs. Thus the vaccination costs detailed here are probably overestimates (based on a worst-case scenario).

A recommendation to keep horses indoors would not be very effective against *Cx. pipiens* if additional measures such as mosquito nets or fans are not installed, as these mosquitoes are also active indoors. Nevertheless, from a practical point of view, it is probable all horse owners would not have the possibility or willingness to follow such a recommendation and to invest in adequate protective equipment. Furthermore, once horses are completely vaccinated, such a recommendation would no longer be relevant. The lost revenue for equestrian centres, due to affected horses not

Table 2

Economic losses and costs associated with West Nile virus fever in the equine industry following an epidemic of West Nile virus infection in Belgium, estimated per horse, 2012 values

Economic impact	Value in euros
Disease in horse	
Visit of veterinary practitioner	42
Hospitalisation (7 days' duration)[a]	
Stay of the horse	69
Veterinarian specialists (internal medicine, neurology)	85
Complementary examinations (blood sampling and analysis, X-rays, CSF puncture and analysis)	191
Medical treatment (NSAID, SAID, supportive treatment)	396
No hospitalisation	
Medical treatment (NSAID)	27
Diagnosis (serology, RT-PCR)	76
Indirect costs – containment of cases in stables	
Extra feed	
Hospitalised surviving horse[b]	
High-value horse	33
Leisure horse	19
Semi-feral horse	12
Non hospitalised horse[c]	
High-value horse	39
Leisure horse	22
Semi-feral horse	14
Extra bedding	
Hospitalised surviving horse[b]	
High-value horse	44
Leisure and semi-feral horse	22
Non-hospitalised horse[c]	
High-value horse	53
Leisure and semi-feral horse	26
Management of horse mortality	
Transport, destruction of cadaver	70
Replacement value for dead/euthanised horse	
High-value horse	10,000
Leisure horse	4,000
Semi-feral horse	2,000
Loss of earnings	
Per affected horse for rent[d]	1,638
Vaccination	
Two doses of vaccine, veterinary costs	144

CSF: cerebrospinal fluid; NSAID: non-steroidal anti-inflammatory drug; RT: reverse transcription; SAID: steroidal anti-inflammatory drug.

[a] Hospitalised horses were assumed to be the most severely affected, when considering nervous clinical signs.

[b] Mean income per horse (39 workdays during clinical disease and recovery; three hours of work a day and one day off per week).

[c] A 42-day recovery period was considered for a horse that was not hospitalised.

[d] Regarding indirect costs and containment of cases indoors, a duration of 35 days was considered for recovery of a horse after its discharge from a veterinary hospital.

being used during the time of clinical disease/treatment and recovery, is very similar to the losses Gavlan et al. estimated in Texas, US, for 2002 [49].

As a prospective study, our estimations have, of course, some limitations. Several aspects were not taken into account to estimate economic impact, such as: (i) the costs of surveillance in birds (wild avifauna and sentinel domestic poultry), as it has already been implemented since 2010, and would not generate additional costs [50]; (ii) entomological surveillance, as this has not been clearly defined in the Belgian context to date; (iii) the preventive action of applying repellents, as this is suspected to be difficult to implement in the field [51] as people's perceptions and behaviour towards protecting themselves and their horses

Table 3

Economic losses and costs associated with West Nile virus fever in the equine industry following an epidemic of West Nile virus infection in Belgium, by infection rate scenario, estimated at national level, 2012 values

Economic impact	8.5% infection rate scenario		34.0% infection rate scenario	
	Cost in euros	Number	Cost in euros	Number
General				
Hospitalisation (7 days)	278,748	356	1,116,558	1,426
No hospitalisation	145,640	662	582,560	2,648
Diagnosis (serology, RT-PCR)	77,368	1,018	309,624	4,074
Management of cadavers	7,000	100	27,930	399
Active surveillance[a] (serology)	147,888	24,648[b]	147,888	24,648[b]
Containment of cases indoors – maintenance costs	47,118	918	188,564	3,674
Replacement value				
Total	440,000	100	1,750,000	399
High-value horses	200,000	40	790,000	79
Leisure horses	160,000	40	640,000	160
Semi-feral horses	80,000	20	320,000	160
Total (general and replacement value)	1,143,762	NA	4,123,124	NA
Loss of earnings for equestrian centres				
Amount	113,022	69	450,450	275
Vaccination costs (2 doses of vaccine, veterinary costs)				
100% coverage	33,091,632	229,803	32,651,712	226,748
Areas at risk	17,105,040	118,785	16,665,264	115,731

NA: not applicable; RT: reverse transcription.

The population of horses was subdivided into three categories: 20% high-value horses, 40% leisure horses and 40% semi-feral horses [21].

[a] Expected 5% prevalence (set at this level to be realistic compared with that in [22]) determined according to the equine population per district (risk areas)). Six sampling periods would be implemented, with sampling planned every two weeks [31]. If a horse is affected in a rental centre, all other animals should be regularly tested as well (mean of 20 horses per rental centre) [21].

[b] Number of tests performed.

is unknown; and (iv) impact of epidemic spread of WNV on trade of horse semen, even though the World Organisation for Animal Health (OIE) recommends that its member states should not impose trade restrictions on dead-end hosts such as horses [52]. In addition, some horse owners might have their horses insured, particularly if they are high-value horses: that parameter was not considered in our study due to lack of data.

Impact on public health

Regarding the economic consequences for the human health sector, our estimations of hospital costs (EUR 3,553 for a WNND case and EUR 4,441 for an AFP case) are less than USD 8,274 (EUR 7,890), which was the median cost of inpatient treatment calculated for a WNV disease epidemic in Louisiana, US, in 2002 [53], and far below the USD 33,143 (EUR 28,094) estimated by Barber et al. in California, US, in 2005 [40]. In our study, several criteria were not taken into account, such as residual neurological after-effects.

Even if a diagnostic test would be performed systematically for WNND patients, results of analyses arrive late, sometimes after hospital discharge (Philippe Leonard, personal communication, July 2011). Furthermore, confirmation of diagnosis would not provide an alternative to effective palliative medical care.

It is not surprising that hospital costs were higher than productivity losses, as more elderly patients develop neuroinvasive disease [4]; no productivity loss was estimated for people aged 65 years and above, as they are not considered as part of the labour market any more. Home care of recovering patients was the second most expensive cost. It is important to note that, in Belgium, professional home care services are partly refundable through the healthcare system, so family members are not systematically obliged to miss work to provide care to the patient. They can turn to these professionals to take care of them. Nevertheless, it was decided to include home care costs in our estimations. Our WNND scenario was based on estimates related to the overall population of areas at risk, infection rate and morbidity rate for WNND. Some cases could pass unnoticed at the beginning of the epidemic. An mean nine-day hospitalisation stay was selected, which is slightly longer than the length reported in a five-year survey of initial and long-term medical and lost-productivity costs for patients hospitalised in Colorado, US, in 2003 [35]. Higher costs are expected for AFP

Table 4

Economic losses and costs of West Nile neuroinvasive disease in humans following an epidemic of West Nile virus infection in Belgium, estimated per human case, 2012 values

Economic impact	Cost in euros
Initial visit, to a general practitioner	23
Diagnostic tests (serology)	21
Hospitalisation: WNND case	
Total hospitalisation cost	3,553
Stay[a] (9 days hospitalisation)	739
Pharmaceutical expenses	926
Provision of services[b]	1,888
AFP case[c]	
Total hospitalisation cost	4,441
Home care[d] (during a 30-day home recovery period)	
Costs	2,340
Productivity lost due to work absenteeism	
Men	
Employee[e]	5,502
Worker[e]	4,124
Self-employed	5,550
Women	
Employee[e]	5,058
Worker[e]	3,791
Self-employed	5,120
Compensation	
Paid to beneficiaries after the death of a patient[f]	9,800

AFP: acute flaccid paralysis; WNND: West Nile neuroinvasive disease.

[a] Includes costs of hospital stay, medical services, patient's share of the costs, initial clinical tests and daily pharmacy costs.

[b] Includes monitoring of vital functions, medical imaging, clinical biology, neurology, etc.

[c] AFP occurs in 3% of all WNND cases [39]. Hospitalisation costs are 1.25 higher for AFP cases compared with those for WNND cases [35].

[d] Including costs for a nurse and caregiver. Home care costs are underestimated for AFP cases, as recovery can take several months [62].

[e] We made a distinction between employees (who generally carry out intellectual work) and workers (who mainly carry out manual tasks).

[f] Estimated from compensation paid to beneficiaries by a Belgian insurance company following the death of a 78 year-old person in a car accident (median age of patients who died from WNND being 78 years).

cases, due to longer hospitalisation and a higher lost productivity [35].

As for the equine part of the study, our estimates for the public health section have several limitations, as the study was performed prospectively. The control of vectors should be recommended to individuals and to public health authorities in case of a severe epidemic, but associated costs were not included in the estimations, in contrast to a previous study [35]. We did not consider impact of preventive use of chemical repellents or anti-mosquito infrastructural measures applied in houses (such as mosquito nets), as it is unpredictable which option people would choose, if any. We consider that emergency aerial spraying, even if proven to be effective in reducing mosquito populations and the number of human cases of WNV infection in the US [40], would not be the first option of vector control in Belgium, given the substantial environmental risks; furthermore, in our opinion, such a measure would not be easily accepted by the population. Also, the risk of mosquitoes developing resistance against insecticides should not be neglected. Furthermore, a recent study identified knowledge gaps concerning vector control in Europe and urged that the most appropriate and environmental friendly control strategies should be identified, given the reduced availability of products for mosquito control in recent years [54].

Neither outpatient costs (e.g. nursing home, transportation and child care) nor long-term costs (e.g. durable medical equipment, medication, medical appointments and institutional care) were taken into account, unlike previous estimations [35]. Actual costs might thus be higher. Inclusion of such costs would have been difficult, however, as it was a predictive study. Long-term costs of potential WNND after-effects, such as cost of treatment in rehabilitation facilities, were not evaluated, unlike a previous study in the US [53], as our scenario assumed the absence of after-effects in recovering patients (which is far from being the case when considering AFP).

The financial burden for the Belgian public health agency was assumed to be already included in its annual financing package. Communication about the epidemic by public agencies (e.g. production of brochures for the whole population or information aimed at horse owners) could be a cost to consider as well, but is difficult to estimate when taking a predictive approach, as it would probably be related to the importance of the epidemic.

The screening of blood and organ donors (most human infections being asymptomatic [4]) could be recommended, especially for those who return from an area with ongoing transmission of WNV in humans. Blood donations from WNV-positive donors should thus be deferred, as transmission of WNV through blood transfusion and organ donation has been well assessed [55]. The European Commission established a deferral period for prospective donors of 28 days after leaving an area with ongoing transmission of WNV in humans [56]. Blood and organ screening should be considered as an important contribution to epidemiosurveillance and epidemiovigilance systems [57] (useful to detect emergence of the disease).

The US healthcare system is quite different from that in western Europe. In Belgium, *in the event of absence for* medical reasons, a worker (at least an employee) does not lose their salary: the loss is thus borne by the employer. Nevertheless, for long periods of illness,

Table 5

Economic costs and productivity losses of West Nile neuroinvasive disease in humans following an epidemic of West Nile virus infection in Belgium, by infection rate scenario among people at risk, estimated at national level, 2012 values

Economic impact	2% infection rate scenario		15% infection rate scenario	
	Cost in euros	Number of patients	Cost in euros	Number of patients
Visits to a general practitioner	19,987	869[a]	150,006	6,522[a]
Hospital charges				
Total	3,110,645	869[a]	23,346,714.00	6,522[a]
WNND	2,995,179	843[b]	22,476,278	6,326[b]
AFP	115,466	26[c]	870,436	196[c]
Other				
Diagnosis (serology)	18,249	869[a]	136,962	6,522[a]
Home care (30-day recovery period after hospital discharge)	1,808,820	773[d]	13,583,700	5,805[d]
Work absenteeism (productivity losses)	495,924	120[e]	2,857,613	587[e]
Compensation paid to beneficiaries (after the death of a patient)	940,800	96[f]	7,026,600	717[f]
Total	**6,394,425**	**869[a]**	**47,101,595**	**6,522[a]**

AFP; acute flaccid paralysis; NA: not applicable; WNND; West Nile neuroinvasive disease.

AFP occurs in 3% of all WNND cases [39]. Hospitalisation costs are 1.25 higher for AFP cases compared with those for WNND cases [35].

[a] Number of patients consulting a general practitioner (WNND and AFP cases).

[b] Number of patients hospitalised for WNND.

[c] Number of patients hospitalised for AFP.

[d] Number of hospitalised patients who survived and needed homecare after discharge from hospital.

[e] Number of hospitalised patients, who are active on the labour market (and have a job) and for whom losses due to work absenteeism were estimated.

[f] Number of deceased patients.

it is possible for an employer to hire interim staff, to replace the worker on sick leave. Thus productivity losses could be attenuated, even if a replacement contract generates additional costs for the employer (especially if they rely on employment agencies).

Conclusion

The originality of our study is its prospective approach (preparedness) compared with previous works estimating economic impact of WNV in the US (retrospective studies). Our two scenarios relied on the variation of one parameter only, i.e. infection rate, while almost all parameters entered in the model are subject to uncertainty; a multifactor sensitivity analysis would have certainly widened the range of estimates. Better quality information is needed to predict the cost of a WNV outbreak in Belgium with more accuracy. In horses, if animal health authorities were to decide to recommend or to make vaccination compulsory, the choice of the strategy would have major consequences in terms of costs. Furthermore, animal health authorities would have to consider the delay involved in producing a high number of vaccine doses. It would thus not be possible to vaccinate the entire horse population during a first hypothetical epidemic. Targeted vaccination of horses at risk (living in habitat suitable for *Cx. pipiens*) could be a first-line preventive measure. A cost–benefit analysis of horse vaccination versus vector control is worth investigation. In humans, hospital charges would be the major financial consequences of an epidemic of WNV infection. It is thus essential to invest in research on preventive measures in the European context, e.g. through the development of a human vaccine (as none is commercialised to date) and integrated biological control of vectors, on a large scale. Integration of these impacts in healthcare plan/insurance schemes are of prime importance in terms of preparedness.

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Conflict of interest

None declared.

Authors' contributions

Marie-France Humblet was involved in the original methodological design of the study; she compiled all data needed to estimate the costs associated with the epidemics and led the writing of the article. Sébastien Vandeputte was partly involved in the original methodological design of the study and collaborated in the compilation of data. Fabienne Fecher-Bourgeois and Benoît Durand participated in the methodological design of the study. Philippe Léonard provided part

of data. Christiane Gosset and Thomas Balenghien participated in the methodological design of the study. Claude Saegerman was in charge of supervising the study.

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