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Louse-borne relapsing fever (*Borrelia recurrentis*) in an Eritrean refugee arriving in Switzerland, August 2015

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We report an imported case of louse-borne relapsing fever in a young adult Eritrean refugee who presented with fever shortly after arriving in Switzerland. Analysis of blood smears revealed spirochetes identified as *Borrelia recurrentis* by 16S rRNA gene sequencing. We believe that louse-borne relapsing fever may be seen more frequently in Europe as a consequence of a recent increase in refugees from East Africa travelling to Europe under poor hygienic conditions in confined spaces.

Here we communicate a case of louse-borne relapsing fever (LBRF) in an Eritrean refugee after arrival in Switzerland in August 2015. *Borrelia recurrentis*, the causative agent of LBRF, was identified by 16S rRNA gene sequencing. In addition to diagnostic and therapeutic aspects, we discuss the epidemiology of this potentially re-emerging and serious disease in the context of a recent increase in refugees from East Africa travelling to Europe.

Case description

In August 2015, a refugee from Eritrea in their late 20s presented to our emergency department with fever for two days, nausea, headache, dysuria and bilateral flank pain. After leaving Eritrea about two months earlier with stopovers in Sudan (two weeks) and Libya (three weeks), the patient arrived in Italy ca 12 days before presenting to our hospital (Figure 1). On their way through Sudan, the patient had experienced a similar episode of dysuria and flank pain without fever that was not treated. Others travelling with them had febrile illnesses that were diagnosed as malaria. All of the patient's clothes were exchanged once in Libya and a second time after arrival in Italy.

On presentation, the patient was afebrile and blood pressure and heart rate were within normal limits. Physical examination was significant for suprapubic and right flank tenderness, whereas skin examination was unremarkable. In particular, lice were not detected on the patient's clothes. Routine blood tests demonstrated mild microcytic anaemia (haemoglobin 101 g/L; norm: 140–180), thrombocytopenia (108 x 10^9/L; norm: 150–450) and elevated C-reactive protein (CRP: 108 mg/L; norm: <10). Urine analysis revealed mild pyuria (266 /µL; norm 0–40) and abundant squamous epithelial cells (45/µL; norm 0–9). Blood smears demonstrated a large number of extracellular spirochetes (Figure 2). Blood cultures remained negative and only bifidobacteria grew in urine culture. Serological screening tests for human immunodeficiency virus (HIV), syphilis and Lyme disease were negative.

The patient was initially treated with doxycycline 100 mg twice per day over two days and ceftriaxone 2 g per day to cover *Borrelia*-associated relapsing fever and suspected pyelonephritis. We did not observe a Jarisch–Herxheimer reaction [1]. They remained afebrile throughout the admission, and their pain and dysuria settled immediately after initiation of antibiotic treatment. Ceftriaxone was stopped after five days of treatment and the patient was discharged for outpatient follow-up.

Investigation of the disease-causing agent

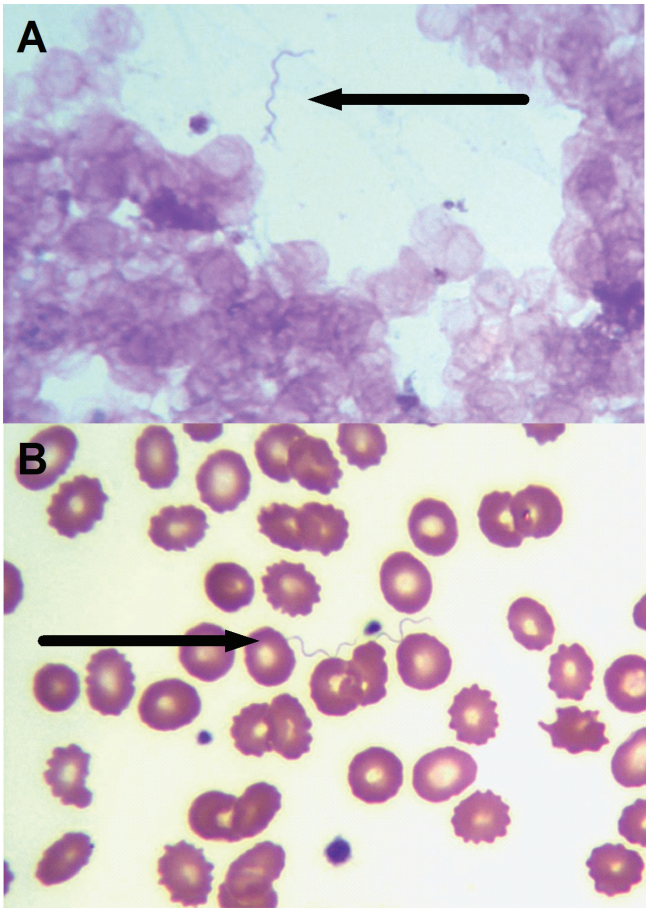
Subsequently, analyses were performed to further characterise the causative agent of the patients' relapsing fever syndrome. The detection and identification of *B. recurrentis* was performed with broad-range bacterial PCR followed by partial sequencing of the 16S rRNA gene applied to two EDTA blood specimens drawn two hours (before treatment, microscopy-positive) and 18 hours (after the first dose of ceftriaxone, microscopy-negative) after presentation to our hospital [2]. PCR

Figure 1

Travel route from Eritrea to Europe, louse-borne relapsing fever case, Switzerland, August 2015

Figure 2

Microscopic detection of spirochetes in blood, louse-borne relapsing fever case, Switzerland, August 2015



Panel A: Giemsa pH 7.2, stained thick film, 1,000-fold magnification.

Panel B: May-Grünwald Giemsa (MGG)-stained blood smear, 1,000-fold magnification.

was positive only on the first sample (100% identity with the *B. recurrentis* reference strain A1).

In a very recent LBRF patient, we additionally analysed the entire 16S rRNA gene, which yielded a 1,475 bp sequence (GenBank accession number: KT221542). Nucleotide sequence database analysis using the Basic Local Alignment Search Tool (BLAST, National Library of Medicine) showed 100% identity to the *B. recurrentis* reference strain A1 [3].

Background

LBRF once was a major worldwide endemic disease causing significant mortality in untreated patients (10–70%) [4-6]. The incubation period is usually short (four to eight days with a range of two to 15 days). In contrast to other *Borrelia*-associated relapsing fever syndromes, LBRF is considered a disease specific to the human host caused by *B. recurrentis* and transmitted by the human body louse *Pediculus humanus humanus* as the vector. In parallel with the markedly reduced incidence of body louse infestations after the 1940s, LBRF cases declined significantly worldwide with the exception of East African countries, in particular Ethiopia, where LBRF remains a common cause of hospital admission and death [7]. Interestingly, cases of LBRF have not recently been reported outside of Africa, including in refugees from affected countries, which may be indicative of a true decline in incidence or a lack of outbreaks in affected countries [8] rather than of difficulties in diagnosis, although microscopy of blood films has a low sensitivity and cannot differentiate between *Borrelia* species [9]. Hence, duration of treatment for *Borrelia*-associated relapsing fever syndromes, which differs according to the species, is usually determined based on geographical location and the respective predominant vector. Whereas single-dose procaine penicillin, tetracycline (500 mg) or doxycycline (200 mg) are effective treatment regimens for LBRF, treatment duration with doxycycline, penicillin G or ceftriaxone is much longer (10 to 14 days) in the case of TBRF.

Discussion

The current arrival of refugees from East Africa to Europe poses challenges for clinicians in European countries as they may be faced with tropical diseases including malaria [10] and diseases nowadays rarely diagnosed in Europe; for example, cases of cutaneous diphtheria have recently been diagnosed in refugees from East Africa at our institution (data not shown). In addition, some of these diseases have the potential to cause small outbreaks in refugee camps [11,12]. We present a case of LBRF imported into Europe in the context of the ongoing migration of refugees from Africa.

Taking into account the short incubation period and the patient's travel dates with a possible episode while residing in Sudan, infection with LBRF probably occurred in Eritrea, Sudan or Libya. Apart from Ethiopia, LBRF has previously been reported from Eritrea and

Sudan [6], the former being the predominant country of origin and the latter a common transit country of many refugees arriving in Europe [13]. Given the poor hygienic conditions and crowding on the way to Europe that might facilitate spread of *B. recurrentis* via body lice, we expect that cases of LBRF will be diagnosed more frequently in Europe. We can only speculate that despite a sustained high prevalence in neighbouring Ethiopia [7], LBRF is not a common disease in Eritrea or Sudan. However, this may change with the continuing increase in refugees from this area, in particular if Ethiopian foci of LBRF infection reach neighbouring countries such as Sudan [14] or if migrants from LBRF-endemic Ethiopia live or travel together with other refugees in confined spaces. Of note, two similar cases have just recently been diagnosed in Eritrean refugees arriving in the Netherlands [19].

While the human body louse has been considered the principal vector of LBRF, *B. recurrentis* DNA was recently detected in head lice (*Pediculus humanus capitis*) [15], although it remains to be determined whether *B. recurrentis* can be transmitted via head lice from person to person. In any case, we consider screening arriving refugees for lice useful in order to prevent spreading of louse-borne diseases in refugee camps. Given that our case's clothes had been exchanged twice before arrival in Switzerland and the fact that lice were not detected in their present clothes, neither formal contact tracing nor additional screening of refugees living in the same asylum seeker camp for lice or preventive delousing were conducted. Comprehensive source tracing is difficult, in our opinion, as it is impossible to locate and investigate all refugees that have previously travelled with a case in crowded conditions.

Patients with LBRF usually present with non-specific symptoms such as high fever, headache or pain in other parts of the body [16]. Hence, presentation of LBRF may resemble many other serious infections such as malaria, viral haemorrhagic fever, leptospirosis, typhus, TBRF, meningococcal meningitis or typhoid fever [17]. In addition, co-infection with malaria is common [16], although not detected in our patient. Most patients with LBRF are diagnosed using microscopy. This method lacks interspecies differentiation of *Borrelia* species causing relapsing fever syndromes, which is important for determining treatment duration (see below). Molecular diagnostic tests such as multiplex PCR or 16S rRNA gene analysis or are not universally available but can aid in the diagnosis of LBRF [18]. Our case demonstrates that the detection and identification of the aetiological agent can be performed by 16S rRNA gene analysis directly from blood samples. Physicians should consider LBRF as differential diagnosis in refugees from East Africa presenting with fever of unknown origin, as mortality in untreated patients is high. Single-dose procaine penicillin, tetracycline (500 mg) or doxycycline (200 mg) are effective treatments for LBRF; the evidence for the superiority of tetracyclines is weak with regards to the risk of relapse and time to defervescence [1]. On the other hand, physicians should be aware of higher rates of the Jarisch–Herxheimer reaction with the use of tetracyclines [1].

Conclusion

Physicians in countries currently hosting Eritrean refugees need to consider LBRF in febrile migrants in addition to more common diseases like malaria.

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Conflict of interest

None declared.

Authors' contributions

Wrote the manuscript: DG, GJC, AE, MO; performed laboratory investigations: DG, MM, AB, BS; revised manuscript: DG, GJC, CB, TB, MM, AN, AB, AE, MO; managed the patient: GJC, CB, TB, MO.

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Incidence of gonococcal and chlamydial infections and coverage of two laboratory surveillance networks, France, 2012

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Surveillance of sexually transmitted diseases in France is based on voluntary networks of laboratories and clinicians. Despite the importance of incidence data in improving knowledge about the national context and in international comparisons, such data were not previously available. During nationwide quality control of laboratories, mandatory for all laboratories, we conducted a survey in June 2013 to estimate the incidence rates of gonococcal and chlamydial infections for 2012 and to estimate the proportion of diagnoses performed (coverage) by the country's two laboratory-based sentinel networks for these diseases. Estimated incidence rates for 2012 were 39 per 100,000 persons aged 15 to 59 years for gonorrhoea and 257 per 100,000 persons aged 15 to 49 years for chlamydia. These rates were consistent with the average levels for a group of other Western countries. However, different estimates between countries may reflect disparate sources of surveillance data and diverse screening strategies. Better comparability between countries requires harmonising data sources and the presentation of results. Estimated coverage rates of the gonococcal and chlamydial infection surveillance networks in France in 2012 were 23% and 18%, respectively, with substantial regional variations. These variations justify improving the representativeness of these networks by adding laboratories in insufficiently covered areas.

Introduction

Sexually transmitted infections (STIs) are a public health issue because of their frequency, the risk of sequelae (e.g. infertility) and transmission of human immunodeficiency virus [1]. The World Health Organization estimates that ca 450 million new cases of curable STIs (gonorrhoea, syphilis, chlamydia and trichomoniasis) occur annually worldwide [2]. The burden of these diseases is often underestimated as asymptomatic cases are likely to be undiagnosed and therefore remain untreated.

In France, the epidemiology of STIs has evolved considerably over the past two decades as a result of a rise in chlamydial infections and gonorrhoea since the end of the 1990s [3], a resurgence of early syphilis since 2000 [4,5] and the emergence of rectal lymphogranuloma venereum since 2002 [6].

Surveillance of STIs is based on voluntary networks of clinicians and public and private laboratories located throughout metropolitan France. Since 1986, gonococcal infections have been monitored by the Renago network (national laboratory network of gonorrhoea) [3,7]. Since the beginning of the 2000s, the annual number of gonococcal infections in men and women reported by this network has increased gradually. This increase has been due both to a real increase in infections and, since 2009, a sharp increase in the use of a nucleic acid amplification test (NAAT) to diagnose gonorrhoea. The surveillance of chlamydial infections has been performed since 1989 through the Renachla network (national laboratory network of chlamydia) [8]. Since 1997, the annual number of reported chlamydial infections by the Renachla network has increased gradually in men and women. This increase in asymptomatic cases is higher than in symptomatic cases for both sexes [9], reflecting increased screening with NAAT [8].

Table 1

Incidence rate of gonococcal infections by geographical area, ANSM-InVS survey, France, 2012 (n = 12,833 positive tests)

Geographical area	Reported number of cases	Estimated number of cases(95% CI)	Population 15–59 years[a]	Estimated incidence rate[b] (95% CI)
Paris area[c]	4,836	5,472 (4,785–6,159)	7,363,127	72.8 (63.7–82.0)
North-west area	1,970	2,237 (2,013–2,463)	7,171,146	30.6 (27.5–33.7)
North-east area	1,705	2,013 (1,648–2,378)	8,304,229	23.8 (19.4–28.1)
South-east area[c]	1,991	2,479 (2,097–2,861)	8,900,793	27.3 (23.1–31.5)
South-west area	1,287	1,503 (1,270–1,736)	4,889,397	30.1 (25.4–34.8)
Total Metropolitan France	**11,968**	**13,951 (13,007–14,895)**	**36,628,692**	**37.3 (34.8–39.9)**
French West Indies-French Guiana	353	495 (393–596)	605,245	80.1 (63.7–96.5)
Reunion Island-Mayotte	512	621 (445–798)	632,279	96.3 (68.9–123.6)
French overseas departments	**865**	**1,116 (935–1,297)**	**1,237,524**	**88.4 (74.0–102.7)**
Total France	**12,833**	**15,067 (14,105–16,028)**	**37,866,216**	**39.0 (36.5–41.5)**

ANSM: French National Agency for Medicines and Health Products Safety; CI: confidence interval; InVS: Institut de Veille Sanitaire.

[a] As of 1 January 2013. Census data updated in January 2014 [12].

[b] Per 100,000 persons aged 15 to 59 years.

[c] Excluding two private laboratories, one in the Paris area and another in the south-east area, which operate at a national level. Their data are included in the metropolitan and total results.

The two networks were set up and are managed by the French Institute for Public Health Surveillance (InVS). Participation of laboratories in the networks is on a voluntary basis, without special benefit apart from obtaining, for about half of the laboratories of Renago network, the results of susceptibility tests performed on the gonococcal strains. In 2012, respectively 202 and 70 laboratories participated in the Renago and Renachla networks (among them, 37 laboratories were part of both networks). Data collected from these two surveillance systems are used to estimate the evolution over time of gonococcal and chlamydial infections.

To complement this sentinel surveillance, it is essential to have incidence data in order to improve knowledge of the national context and for the purpose of international comparisons. French data on the incidence of STIs have not been available until the present work. Furthermore, French data on the prevalence of STIs are limited. The only national survey studying the prevalence of chlamydial infection in the general population (NatChla survey) was conducted in 2006 and estimated a prevalence of 1.6% and 1.4%, respectively, in women and men aged between 18 and 44 years, and of 3.2% and 2.5%, respectively, in women and men between 18 and 29 years [10,11]. During nationwide quality control of laboratories in June 2013, we conducted a survey to estimate the incidence of gonococcal and chlamydial infections in France for 2012 and the proportion of diagnoses performed by our two laboratory surveillance networks (for the purposes of this manuscript referred to as 'coverage').

Methods

The French National Agency for Medicines and Health Products Safety (ANSM) is responsible for national quality control of medical laboratories. It uses external quality assessment (EQA) programmes covering all laboratory disciplines, including bacteriology. Registration and participation of laboratories for quality control are mandatory. In June 2013, an EQA survey was performed involving the 1,399 registered bacteriology laboratories: 1,335 located in metropolitan France and 64 in the French overseas departments (FODs). FODs include the French West Indies (Guadeloupe and Martinique) and French Guiana in the Americas, and Reunion Island and Mayotte in the Indian Ocean. Each laboratory received a strain of *Neisseria gonorrhoeae* to perform susceptibility testing. By agreement between the ANSM and the InVS, a short questionnaire on the activity of laboratories on gonococcal and chlamydial diagnoses was added. For each laboratory in metropolitan France and the FODs, we recorded the number of gonococcal infections diagnosed by culture and by NAAT, and the number of chlamydial urogenital infections diagnosed by NAAT from 1 January to 31 December 2012. A positive

Table 2

Incidence rate of chlamydial infection by geographical area, ANSM-InVS survey, France, 2012 (n = 58,155 reported cases)

Geographical area	Reported number of cases	Estimated number of cases (95% CI)	Population 15–49 years [a]	Estimated incidence rate [b] (95% CI)
Paris area [c]	12,985	17,312 (12,658–21,965)	5,900,616	288 (210–365)
North-west area	6,395	7,687 (5,846–9,528)	5,473,758	138 (105–171)
North-east area	6,217	7,770 (5,058–10,482)	6,384,158	119 (78–161)
South-east area [c]	10,497	12,049 (491–23,608)	6,869,673	172 (7–338)
South-west area	3,915	4,753 (2,913–6,594)	3,704,377	126 (77–174)
Total Metropolitan France	**54,051**	**71,657 (41,522–101,792)**	**28,332,582**	**249 (144–352)**
French West Indies-French Guiana	2,529	3,367 (1,099–5,635)	467,288	706 (231–1,182)
Reunion Island-Mayotte	1,575	1,895 (247–3,542)	519,708	357 (47–668)
French overseas departments	**4,104**	**5,261 (2,996–7,527)**	**986,996**	**522 (297–747)**
Total France	**58,155**	**76,918 (46,711–107,126)**	**29,319,578**	**257 (156–358)**

ANSM: French National Agency for Medicines and Health Products Safety; CI: confidence interval; InVS: Institut de Veille Sanitaire.

[a] As of 1 January 2013. Census data updated in January 2014 [12].

[b] Per 100,000 persons aged 15 to 49 years.

[c] Excluding two private laboratories, one in the Paris area and another in the south-east area, which operate at a national level. Their data are included in the metropolitan and total results.

culture or a positive NAAT defined gonococcal infection while a positive NAAT defined chlamydial infection. The survey did not collect data on individual characteristics (age, sex, reason for testing etc). The number of tests performed was not collected; therefore the proportion of positive samples is unknown. Here we report only the results of the questionnaire-based survey, not the quality control susceptibility testing results.

In France, as a result of new regulations for medical laboratories, multi-site laboratories are becoming more common, i.e. groups of laboratories which remain urogenital sampling sites but centralise their bacteriological activity on one of the sites. For those laboratories, quality control and the questionnaire-based survey were performed with the specific laboratory's administrative structure providing combined information for all its sites using a single questionnaire for the whole group.

Estimation of the total number of gonococcal and chlamydial infections diagnosed in France in 2012

To estimate the total number of gonococcal and chlamydial infections diagnosed by all French laboratories, we built 67 strata by crossing, when possible, each geographical area with each sector of activity (private or public). We then assumed that infection diagnosis activity (i.e. the proportion of laboratories performing tests and the number of diagnosed infections) within each stratum was the same for non-respondent laboratories (i.e. laboratories not replying to calls for quality control checks, laboratories providing no information on the availability of tests, and laboratories not specifying the number of diagnosed infections) as for their responding counterparts. We assigned a sampling weight to each laboratory for all estimates. These weights were equal to the inverse of the participation rates observed in each stratum. Variances were estimated using Taylor linearisation.

For gonococcal infections, we were not able to identify the number of diagnoses made using NAAT and culture tests simultaneously for the same patient in each laboratory at the national level. These data were available however for the laboratories in the Renago network. Among private laboratories of the network where both NAAT and culture tests were available in 2012, 41% of gonococcal infections (444/1,072) were diagnosed using both techniques simultaneously. This percentage was 18% (119/644) in the public sector. Consequently, in order to estimate the total number of gonococcal infections diagnosed by using both techniques simultaneously in all French public and private laboratories, we applied the Renago network's percentages. Within the Renago network, nearly 98% of cases of patients

Table 3

Coverage of the Renago network for gonococcal infections, by geographical area and sector of activity, ANSM-InVS survey, metropolitan France, 2012 (n = 3,276 cases)

	Renago network (InVS data)		All laboratories (ANSM-InVS survey)		Coverage (A/B) (95% CI)
	Number of laboratories	Number of cases (A)	Number of laboratories	Estimated number of cases (B)	
Geographical area					
Paris area	22	1,060	254 [a]	5,472	19.4% (16.9–21.8)
North-west area	38	708	205	2,238	31.6% (28.5–34.8)
North-east area	34	498	232	2,013	24.7% (20.3–29.2)
South-east area	33	541	266 [a]	2,479	21.8% (18.5–25.2)
South-west area	23	469	149	1,503	31.2% (26.4–36.0)
Total Metropolitan France	**150**	**3,276**	**1,108**	**13,951**	**23.5% (21.9–25.1)**
Sector of activity					
Public laboratories	37	911	319	4,058	22.4% (20.4–24.5)
Private laboratories	113	2,365	789	9,893	23.9% (21.8–26.0)

ANSM: French National Agency for Medicines and Health Products Safety; CI: confidence interval; InVS: Institut de Veille Sanitaire.

[a] Excluding two private laboratories, one in the Paris area and another in the south-east area, which operate at a national level. Their data are included in the metropolitan results.

with gonococcal infections were aged 15 to 59 (97.5% in 2010, 97.8% in 2011 and 2012). We therefore also assumed that 98% of gonococcal cases reported by all French laboratories were in this age group. Similarly, ca 98% of cases of chlamydial infections reported within the Renachla network concerned patients aged 15 to 49 (98.2% in 2010 and 2011, 98.1% in 2012). Accordingly, we assumed that 98% of chlamydial cases reported by all French laboratories were in this age group.

Estimation of incidence rates for gonococcal and chlamydial infections in France in 2012

The estimated national incidence rate for each infection type equalled the total estimated number of infections diagnosed in 2012 divided by the size of the population aged 15 to 59 for gonococcal infections and aged 15 to 49 for chlamydial infections. National census data of the French population estimated on 1 January 2013 were used to evaluate both population sizes [12]. For Mayotte, the age distribution of the 2007 census [13] is extrapolated to the 2012 population census [14]. Incidence rates and their 95% confidence intervals (CIs) were also estimated for each of the five areas in metropolitan France defined by telephone area codes (01, 02, 03, 04 and 05) and for the FODs (differentiating areas in the Americas and in the Indian Ocean). Unlike other laboratories which receive local specimens, two private laboratories, one located in the Paris area (01) and the other in the south-east area (04), received specimens from all over France and were therefore excluded from regional calculations.

Estimation of coverage of Renago and Renachla networks in metropolitan France

Gonococcal and chlamydial infections are reported on a continuous basis by the two sentinel networks to the InVS. The coverage of the two networks in 2012 was estimated by dividing the number of infections these networks reported during 2012 by the estimated total number of gonococcal and chlamydial infections diagnosed in all laboratories in metropolitan France. Coverage rates and their 95% CIs were also estimated for each of the five areas and according to whether laboratories were in the public or private sector. All statistical analyses were performed using Stata v.12.1 software.

Results

Characteristics of French laboratories and participation in quality control

Of the 1,399 French bacteriology laboratories, 921 (66%) were based in one site while 478 (34%) were multi-site. Almost half of the private laboratories were multi-site (477/1,033; 46%). Conversely, only one public laboratory was multi-site (1/366:<1%). The 1,399 laboratories consisted of a total of 4,057 sites (3,942 in metropolitan France and 115 in the FODs). Among multi-site laboratories, the median number of sites was 5 (range: 2–60).

The rate of participation in quality control was only 96% (1,342/1,399) despite its mandatory nature. Among the 1,342 respondent laboratories, 1,279 (938 private and 341 public) were located in metropolitan

Table 4

Coverage of Renachla network for chlamydial infections, by geographical area and sector of activity, ANSM-InVS survey, metropolitan France, 2012 (n = 13,074 cases)

	Renachla network (InVS data)		All laboratories (ANSM-InVS survey)		Coverage (A/B) (95% CI)
	Number of laboratories	Number of cases (A)	Number of laboratories	Estimated number of cases (B)	
Geographical area					
Paris area	11	3,719	96 [a]	17,312	21.5% (15.7–27.2)
North-west area	12	1,961	48	7,687	25.5% (19.4–31.6)
North-east area	11	2,065	46	7,770	26.6% (17.4–35.8)
South-east area	7	3,016	36 [a]	12,049	25.0% (1.2–48.9)
South-west area	11	2,313	42	4,753	48.7% (29.9–67.4)
Total Metropolitan France	**52**	**13,074**	**270**	**71,657**	**18.2% (10.6–25.9)**
Sector of activity					
Public laboratories	26	5,389	92	20,848	25.8% (11.6–40.1)
Private laboratories	25	7,685	178	50,809	15.1% (6.9–23.4)

ANSM: French National Agency for Medicines and Health Products Safety; CI: confidence interval; InVS: Institut de Veille Sanitaire.

[a] Excluding two private laboratories, one in the Paris area and another in the south-east area, which operate at a national level. Their data are included in the metropolitan results.

France and 63 (49 private and 14 public) in the FODs. The response rate by private and public laboratories was similar between metropolitan France and the FODs, and between the five metropolitan areas. Of the 1,342 laboratories participating in quality control, 127 (9%) did not answer the additional questionnaire about their activity.

Estimated number of diagnoses and incidence rate of gonococcal infections in France in 2012

Of the 1,342 laboratories which participated in quality control, 1,147 (85%) reported performing gonococcal cultures in the laboratory or, in the case of multi-site laboratories, in at least one laboratory site. Sixty-three (5%) laboratories declared that they did not perform this test and 132 (10%) did not answer this question. Among the 1,147 laboratories performing cultures, 1,061 (93%) indicated the number of positive specimens diagnosed in 2012: a total of 8,820 positive specimens (8,235 in metropolitan France and 585 in the FODs). The average number of positive specimens per laboratory was higher in the FODs than in metropolitan France (14.3 (range: 0–207) vs 8.1 (range: 0–114); $p<0.001$) and slightly higher in private than in public laboratories (8.5 (range: 0–207) vs 7.9 (range 0–178); $p<0.001$). In metropolitan France, taking into account the number of sites, an average of 2.6 positive specimens per site were diagnosed in 2012.

Of the 1,342 respondent laboratories, 111 (8%) reported performing NAAT for gonococcal infections in the laboratory or, in the case of multi-site laboratories, in at least one laboratory site. In addition, 1,092 (81%) declared they did not perform this test and 139 (10%) did not answer this question. Among the 111 laboratories performing gonococcal infection diagnosis by NAAT, 94 (85%) indicated the number of diagnoses made in 2012: a total of 4,013 cases of gonococcal infection were diagnosed by NAAT (3,733 in metropolitan France and 280 in the FODs). The average number of gonococcal infections diagnosed by NAAT per laboratory was similar between the FODs and metropolitan France (35.0 vs 43.4; $p=0.67$), and between private and public laboratories (41.1 vs 45.3; $p=0.90$). In metropolitan France, an average of 8.1 cases per site were diagnosed by NAAT in 2012.

Finally, among the 1,342 respondent laboratories, 1,213 (90%) provided information on gonococcal infection diagnosis by culture or NAAT: 1,042 laboratories (86%) performed cultures only, six (<1%) performed NAAT only, 105 (9%) performed both tests and 60 (5%) did not perform either of the two tests (and accordingly, they did not diagnose any cases). Thus, 95% (1,108/1,164) of the laboratories in metropolitan France and 92% (45/49) in the FODs performed one of the two tests. In 2012, a total of 12,833 positive tests for gonococcal infection were reported by the 1,069 laboratories (1,027 in metropolitan France and 42 in the FODs) which reported the number of diagnoses they made by culture or NAAT: 11,968 cases in metropolitan France and 865 cases in the FODs.

Table 1 shows the estimated incidence rate of gonococcal infection in France in 2012 at the national level and by geographical area. The overall incidence rate was

Table 5

Incidence rates of gonococcal infections in selected countries, 2012

Country [reference]	Sources of data	Incidence rate in the population[a]	Age group (years)	Incidence rate in the age group[a]
United States [16]	Notifiable diseases, prevalence studies, network for STI surveillance, national surveys	108	15–54 15–64	191 158
England [19,20]	Genitourinary medicine clinics	48	15–44 15–64	108 72
Canada: Quebec [21]	Network of hospital laboratories	28	15–59	44
Sweden [17]	Notification by doctors	11	15–59	19
Belgium [22]	Sentinel network of laboratories	NA[b]	15–59	14
France	Bacteriology laboratories	NA	15–59	39

NA: not available.

[a] Rates per 100,000 persons. For the recalculation of rates using age data, documents referenced in the first column were used, except for Quebec and Sweden where population estimates were used ([30] and [31], respectively).

[b] Rate in the reference study seems to have been calculated in the age group 15–59 years.

estimated at 39.0 per 100,000 persons aged 15 to 59 years (95% CI: 36.5–41.5). It was significantly higher in the FODs (88.4 per 100,000 persons aged 15 to 59 years; 95% CI: 74.0–102.7) than in metropolitan France (37.3 per 100,000 persons aged 15 to 59 years; 95% CI: 34.8–39.9). Within metropolitan France, the incidence rate was higher in the Paris area than in the other four areas (Table 1).

Estimated number of diagnoses and incidence rate of chlamydial infections in France in 2012

Of the 1,342 laboratories who participated in quality control, 290 (22%) reported that they performed NAAT for chlamydial infections in the laboratory or, in the case of multi-site laboratories, in at least one laboratory site. In addition, 912 (68%) declared they did not perform this test and 140 (10%) did not answer this question. Among the 290 laboratories which diagnosed chlamydial infections, 255 (88%) indicated the number of diagnoses in 2012: a total of 58,155 cases of chlamydial infection were diagnosed (54,051 in metropolitan France and 4,104 in the FODs). The average number of chlamydial infections per laboratory was not statistically different between the FODs and metropolitan France (216.0 vs 229.0; $p = 0.07$) or between private and public laboratories (234.8 vs 216.3; $p = 0.34$). In metropolitan France, an average of 56.9 cases per site were diagnosed in 2012.

Table 2 shows the estimated incidence rate of chlamydial infections in France in 2012 at national level and by geographical area. The overall incidence rate was estimated at 257 per 100,000 persons aged 15 to 49 years (95% CI: 156–358). As was the case for gonorrhoea, it was higher in the FODs (522 per 100,000 persons aged 15 to 49 years; 95% CI: 297–747) than in metropolitan France (249 per 100,000 persons aged 15 to 49 years; 95% CI: 144–352). Likewise, within metropolitan France the incidence rate was higher in the Paris area than in the other four areas (Table 2).

Characteristics and coverage of the Renago network in metropolitan France in 2012

Of the 1,335 laboratories registered by the ANSM for bacteriology quality control in metropolitan France, 199 (15%) were part of the Renago network in 2012. Three other laboratories in the Renago network did not participate in quality control that year as they were not registered at the time by the ANSM. The response rate to quality control was not statistically different between laboratories in the Renago network and other metropolitan laboratories (98% vs 95%; $p = 0.10$).

The average number of gonococcal infections diagnosed per laboratory either by culture or NAAT was 3.3 times higher in the Renago network than in other metropolitan laboratories (27.9 vs 8.4; $p < 0.001$). Nearly three times more laboratories in the Renago network than in other metropolitan laboratories performed NAAT testing (18% vs 7%; $p < 0.001$). Multi-site laboratories were more common in the Renago network than in other laboratories (55% vs 32%; $p < 0.001$). There were on average 5.0 cases per site diagnosed in 2012 in the Renago network compared with 3.3 cases per site in all other metropolitan laboratories.

Table 6

Incidence rates of chlamydial infections in selected countries, 2012

Country [reference]	Sources of data	Incidence rate in the population[a]	Age group (years)	Incidence rate in the age group[a]
United States [16]	Notifiable diseases, prevalence studies, network for STI surveillance, national surveys	457	15–44 15–54	1,091 817
England [19,23]	Genitourinary medicine and other community services	390	15–44 15–64	930 586
Canada: Quebec [21]	Notifiable diseases	252	15–49	528
Sweden [18]	Notification by doctors	395	15–49	866
Belgium [22]	Sentinel network of laboratories	70[b]	15–49	88
France	Bacteriology laboratories	NA	15–49	257

NA: not available.

[a] Rates per 100,000 persons. For the recalculation of rates using age data, documents referenced in the first column were used, except for Quebec and Sweden where population estimates were used ([30] and [31], respectively).

[b] Rate in the reference study seems to have been calculated in the age group 15–59 years.

The overall coverage of the Renago network was 23% (95% CI: 22–25%); coverage in the Paris area (area code 01) was 19% and lower than in the other four areas (Table 3). Furthermore, coverage rates in the two eastern areas (area codes 03 and 04) were ca 23% and lower than those in the two western areas (area codes 02 and 05) with ca 31%. Coverage by private laboratories (24%) was slightly higher than that by their public counterparts (22%).

Characteristics and coverage of the Renachla network in metropolitan France in 2012

Of the 1,335 laboratories registered by the ANSM for bacteriology quality control in metropolitan France, 65 (5%) were part of the Renachla network in 2012. Five other laboratories belonging to the Renachla network did not participate in quality control that year as they were not registered at the time by the ANSM. The response rate to the quality control was similar between the Renachla network and other metropolitan laboratories (98% vs 96%, $p = 0.23$).

The average number of chlamydial infections diagnosed per laboratory was slightly higher in the Renachla network than in the other metropolitan laboratories (255.9 vs 223.6; $p < 0.001$). The proportion of multi-site laboratories was not different between the Renachla network and other metropolitan laboratories (35% vs 40%; $p = 0.42$). On average, 55.3 cases per site were diagnosed in 2012 in the Renachla network compared with 57.3 cases per site in other metropolitan laboratories.

The overall coverage of the Renachla network was 18% (95% CI: 11–26%). Coverage in the south-west area (area code 05) (49%) was higher than those of the other four areas (Table 4). Furthermore, regional coverage rates in the north (area codes 02 and 03) of ca 26% were lower than those in the Paris and south-east areas (ca 29% each). The coverage of private laboratories was 15% and lower than that of their public counterparts with 26%.

Discussion

This study presents, for the first time, estimates of the incidence of gonococcal and chlamydial infections in France from a comprehensive laboratory survey. Incidence rates for 2012 were estimated at 39 per 100,000 persons aged 15 to 59 years for gonococcal infections and at 257 per 100,000 persons aged 15 to 49 years for chlamydial infections. We found disparities for both infections' incidence rates between metropolitan France and the FODs, rates being twice as high in the latter. Differences within metropolitan France were also observed, with the Paris area having higher rates than its four counterparts.

Incidence data help create international comparisons. However, differences in the estimation of incidence rates between countries are difficult to interpret for two major reasons. Firstly, data sources may differ greatly from one country to the next. Such sources include mandatory notification, laboratory data, sentinel networks of laboratories, clinicians etc. Aggregate data from several countries should be used with caution because of the heterogeneity in reporting and

healthcare systems [15]. Secondly, incidence rates calculated on the basis of the entire population do not give a true picture of reality since only a portion of the population is at risk of STIs. Approximately 98% of gonococcal and chlamydial infections diagnosed by the two French laboratory networks, Renago and Renachla, affect people aged 15 to 59 years and 15 to 49 years, respectively. Similar age distributions have also been found in other Western countries, for example in the United States (US) [16] and Sweden [17,18]. Tables 5 and 6 show the incidence of gonococcal and chlamydial infections in a group of Western countries, after recalculation for specific age groups.

The incidence rate of gonococcal infections estimated for 2012 in France from laboratory data was about four times lower than that in the US, obtained by combining several sources of data [16], about two times lower than that in England [19,20] and close to that in Quebec [21]. However it was two to three times higher than that in Sweden [17] and Belgium [22] (Table 5).

The incidence rate of chlamydial infections estimated for 2012 in France was approximately three times lower than that in the US [16], Sweden [16] and England [19,23], two times lower than that in Quebec [21], but approximately three times higher than that in Belgium [22] (Table 6).

The different estimates between countries may reflect a combination of true differences in incidence, disparate sources of surveillance data and diversity of testing and screening strategies. In particular for chlamydia, screening policies for young adult populations vary in different countries. Availability and affordability of testing are also key issues. In France, only 8% of laboratories performed NAAT for gonorrhoea because this test is not yet reimbursed by health insurance.

Perennial surveillance of STIs in France is mainly based on sentinel networks, including voluntary laboratory networks. The higher average number of gonococcal infections diagnosed in laboratories in the Renago network than in other metropolitan laboratories reflects the initial selection of active and experienced laboratories in gonococcal culture to join the network when it was first established. The Renago and Renachla networks' coverage rates for 2012 were estimated at 23% and 18%, respectively, with substantial regional variations. In the coming years, efforts should be made to improve the representativeness of these networks, notably by adding laboratories in some geographical areas (e.g. in the Paris area for gonococcal infection surveillance and in northern France for chlamydial infection surveillance) and balance the regional coverage rates.

Survey limitations

The incidence rates of gonococcal and chlamydial infections presented here should be considered as underestimations of the true incidence rates in the population. Biologically confirmed cases represent only a portion of infections for a number of interrelated reasons. A significant proportion of infections are asymptomatic, particularly in women (55 to 95% [24,25]) or in cases of extragenital (oral or anal) localisations [26,27], and therefore remain unrecognised. Symptomatic patients do not always consult a doctor. Physicians do not systematically prescribe a microbiological test, whether or not the patients receive presumptive treatment. When tests are prescribed, patients may decide not to go to a laboratory. When tests are performed, they can be negative in infected patients. This is particularly true for gonococcal cultures in women, although the increasing use of NAAT reduces the proportion of false negative tests and allows diagnosis in asymptomatic individuals [28].

Other reasons for underestimation of incidence rates are more specific to the present study. This survey did not account for laboratories that were not recorded in the national process of bacteriology quality control in 2013. However, the number of such laboratories was probably very small, given the compulsory nature of the registration to quality control. Moreover, in the case of multi-site laboratories, it is possible that some central administrative structures may have provided information on gonococcal and chlamydial infections for only a portion of all sites. Finally, regional rates were underestimated due to the exclusion of data from two laboratories active at the national level, where cases had not been assigned to a specific geographic area.

Another limitation of our study is the extrapolation of missing data from respondents. However, our extrapolation seems reasonable insofar as it is unlikely that non-participation in quality control was related to the activity of a laboratory in the diagnosis of gonococcal or chlamydial infections.

Recommendations

STI surveillance allows us to monitor the trends of these infections and to assess the impact of public policies aiming to control them. Each surveillance system has its advantages, disadvantages and limitations. However, to make indicators comparable between countries, more information on testing policies must be collected and harmonisation is needed. The comparability of the data provided by European countries is not optimal in the absence of standardisation. The European Centre for Disease Prevention and Control recognises the need to improve harmonisation of surveillance systems, case definitions and presentation of data [29]. Based on our study, we recommend for example the use of appropriate age groups to calculate the incidence of gonococcal (15–59 years) and chlamydial (15–49 years) infections to avoid an underestimation of the incidence and ensure comparability of results.

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Conflict of interest

None declared.

Authors' contributions

GLR conceived and wrote the paper. YLS, MF, BB, AG, BDB, PS, EC and FL participated in the design of the analysis, commented on the first draft of the paper and approved the final version.

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Age-related prevalence of cross-reactive antibodies against influenza A(H3N2) variant virus, Germany, 2003 to 2010

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To estimate susceptibility to the swine-origin influenza A(H3N2) variant virus (A(H3N2)v) in the German population, we investigated cross-reactive antibodies against this virus and factors associated with seroprotective titre using sera from representative health examination surveys of children and adolescents (n = 815, 2003–06) and adults (n = 600, 2008–10). Antibodies were assessed by haemagglutination inhibition assay (HI); in our study an HI titre ≥ 40 was defined as seroprotective. We investigated associated factors by multivariable logistic regression. Overall, 41% (95% confidence interval (CI): 37–45) of children and adolescents and 39% (95% CI: 34–44) of adults had seroprotective titres. The proportion of people with seroprotective titre was lowest among children younger than 10 years (15%; 95% CI: 7–30) and highest among adults aged 18 to 29 years (59%; 95% CI: 49–67). Prior influenza vaccination was associated with higher odds of having seroprotective titre (odds ratio (OR) for children and adolescents: 3.4; 95% CI: 1.8–6.5; OR for adults: 2.4; 95% CI: 1.7–3.4). Young children showed the highest and young adults the lowest susceptibility to the A(H3N2)v virus. Our results suggest that initial exposure to circulating seasonal influenza viruses may predict long-term cross-reactivity that may be enhanced by seasonal influenza vaccination.

Introduction

Pigs can serve as hosts for the reassortment of influenza viruses of different origins (avian, human and swine) which involves the risk of emergence of new, genetically different influenza viruses that may infect humans. In addition, influenza viruses in pigs do not develop as much genetic diversity in a given time period as those in humans, resulting over time in divergent antigenic characteristics between viruses circulating in swine and humans that may make humans more susceptible to swine viruses [1]. When such zoonotic swine influenza viruses are isolated from humans, they are called variant viruses [2]. The emergence of the pandemic influenza A(H1N1)pdm09 virus in 2009 confirmed the risk posed by zoonotic influenza viruses emerging from pigs.

More than 300 infections with a variant influenza A(H3N2) virus (A(H3N2)v) have been recorded in the United States (US) since 2011, and most cases occurred in 2012 [3]. Most influenza A(H3N2)v infections occurred outside of the influenza season in children, and the course and severity of illness were similar to that of seasonal influenza. Most of the infected persons reported to have attended agricultural fairs where they had either direct or indirect contact with pigs. Limited human-to-human transmission of the A(H3N2)v virus may have occurred as some cases in 2011 did not have any recent history of contact with pigs [4].

The A(H3N2)v virus is a triple reassortant A(H3N2) virus that acquired the matrix protein gene of the A(H1N1) pdm09 virus [5,6]. It is closely related to human A(H3N2) virus strains, represented by the vaccine reference strain A/Wuhan/359/95, that circulated among humans in the mid-1990s until 1997 [5]. The observed age-related pattern of influenza A(H3N2)v infections in the US is most likely due to the similarity of the haemagglutinin of the A(H3N2)v virus to that of A/Wuhan/359/95-like viruses as most cases were born after 1997 and not exposed to such human A(H3N2) viruses. The acquisition of the matrix gene after the

Figure 1

Reverse cumulative distribution curve of haemagglutination inhibition antibody titres against influenza A/Wisconsin/12/2010, by age group, adults, Germany, 2008–2010 (n = 600)

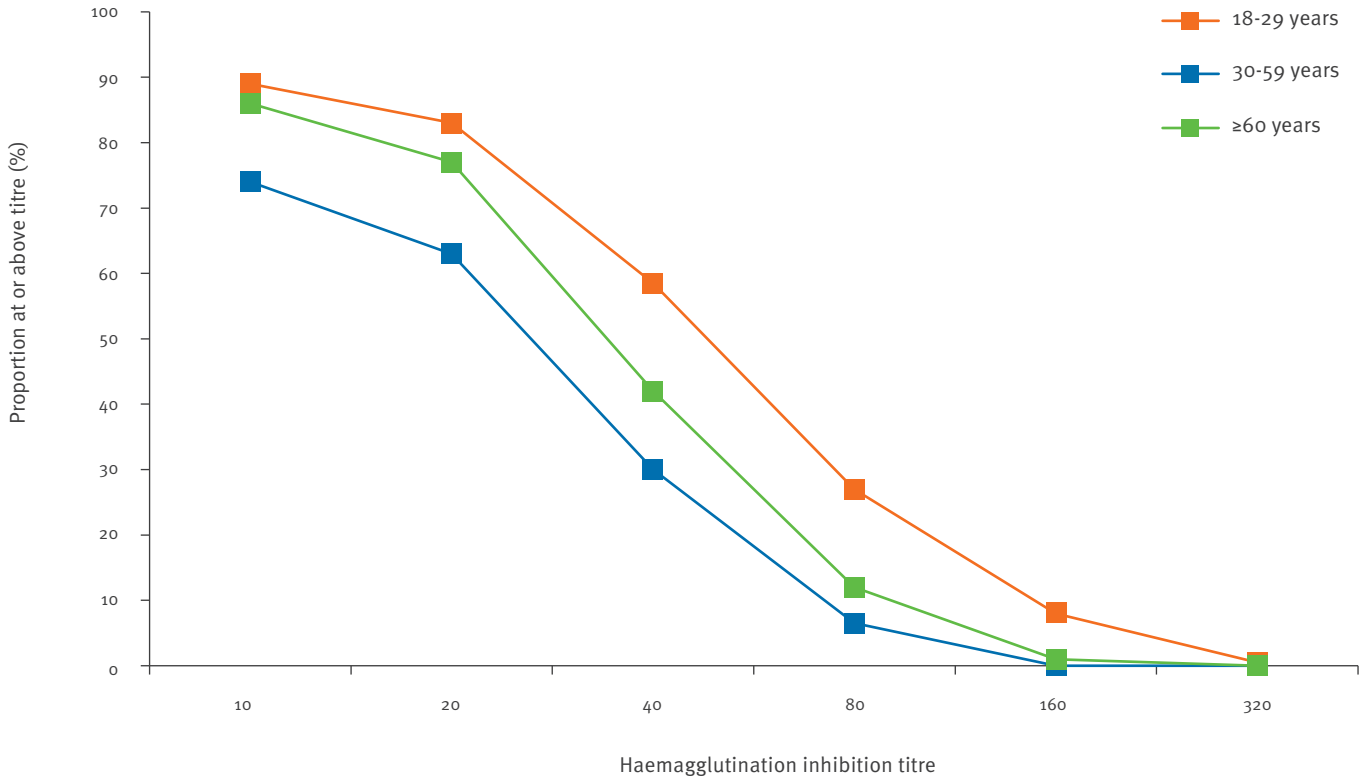
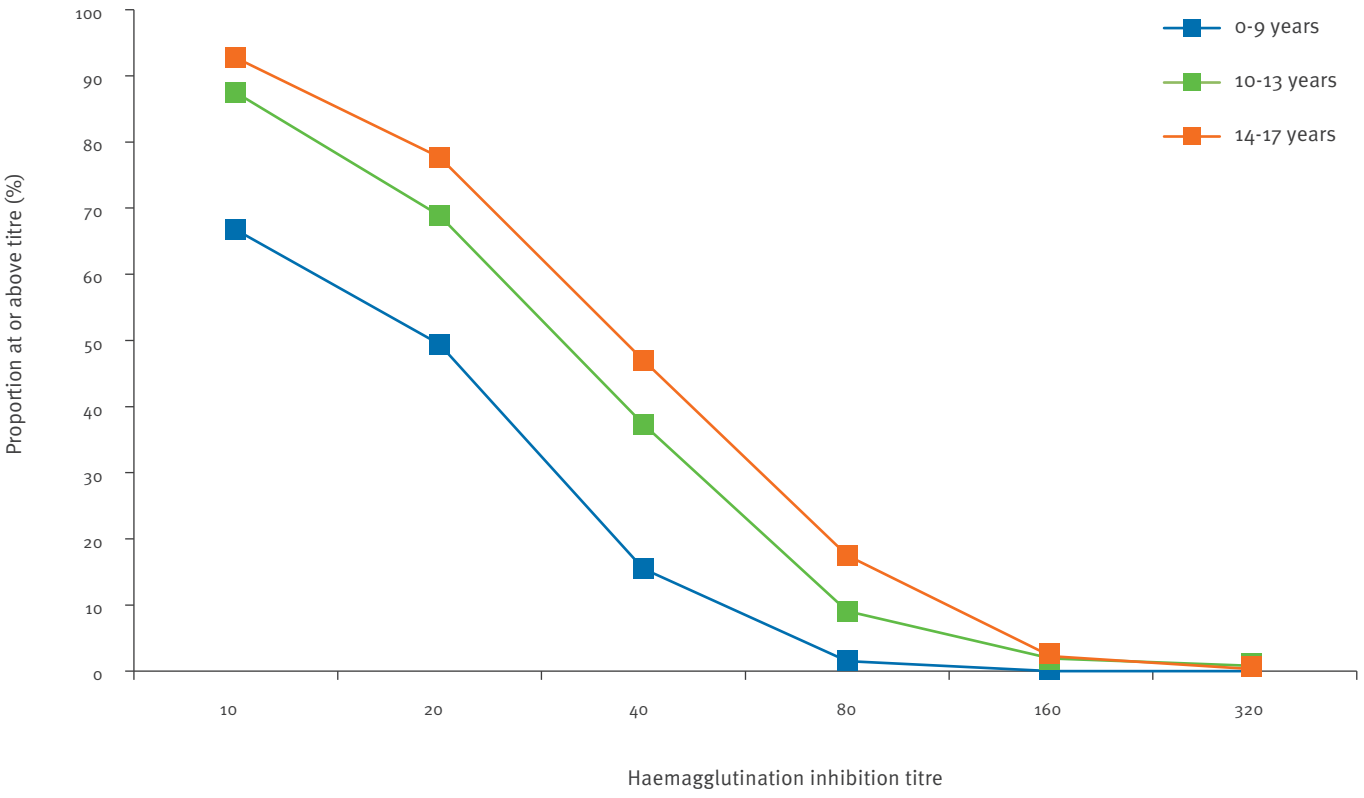


Figure 2

Reverse cumulative distribution curve of haemagglutination inhibition antibody titres against influenza A/Wisconsin/12/2010, by age group, children and adolescents, Germany, 2003–2006 (n = 815)



Proportions are weighted (see Methods).

pandemic in 2009, possibly resulting in increased swine–human transmissibility of the virus, may explain the increasing number of A(H3N2)v infections. The low level of cross-reactive antibodies observed among young children and some adult age groups in seroepidemiologic studies in Canada, Japan, Norway, the United Kingdom (UK), and the US, may pose a risk of pandemic emergence of the A(H3N2)v virus [7-11]. A vaccine virus has been developed in case an influenza A(H3N2)v virus vaccine needs to be produced [7,12].

No data are available on the prevalence of cross-reactive antibodies against the A(H3N2)v virus in the population of Germany and most of Western Europe. While infections with the A(H3N2)v virus have not been reported in Europe to date, this cannot be excluded in the future, and further adaptation of the virus to humans, involving increased ability of human-to-human spread, is possible. In a seroepidemiological study among adults, we showed that age-related seroprevalence patterns of antibodies against the A(H1N1) pdm09 virus in Germany did not follow those observed in other countries in Europe and North America [13]. Such variability may, among other reasons, be due to the circulation of different seasonal influenza virus subtypes in the different countries or to different vaccination histories in the studied populations.

To estimate the susceptibility to influenza A(H3N2)v in the German population, we assessed the prevalence of cross-reactive antibodies against this virus as a correlate of pre-existing protection and investigated the association between characteristics of the study population and the prevalence of cross-reactive antibodies.

Methods

Serum samples

The serum samples used for this study originated from two population-wide, representative health examination surveys conducted in Germany. For the adult population we used sera from the first wave of the German Health Interview and Examination Survey for Adults (DEGS), conducted from November 2008 through December 2011 [13,14]. DEGS is part of the national health monitoring and combines periodic health surveys with a longitudinal study of adults 18 years or older. Participants (8,152 persons) were randomly selected from the general population by using a stratified two-stage cluster sample approach. The study included completion of standardised questionnaires, physical examinations and tests, and a blood sample (available for 7,116 of the 8,152 participants). For our adult study population, we used an age-stratified (three age groups) random sub-sample of DEGS sera (n = 600, 200 in each age group) collected from November 2008 through April 2010 [13].

To assess the seroprevalence in children and adolescents we used sera from the nationwide, population-based German Health Interview and Examination Survey for Children and Adolescents (KiGGS) that assessed persons between 0 and 17 years of age. Data and blood samples (1–17 years of age) were collected from 2003 through 2006 using an approach similar to DEGS [15]. From the 17,641 participants in the original study, a simple random sub-sample of those for whom blood samples were available (n = 815 of 14,387 participants) was used for our study. The reason why the sub-sample for children and adolescents was larger than the adult sample was that these sera were also used in a study of another research group and were thawed and aliquoted at the same time; this allowed us to increase our power for the analysis.

Serological analysis

We performed haemagglutination inhibition (HI) testing to analyse sera for cross-reactive antibodies against three influenza A(H3N2) virus strains. Influenza A/Wisconsin/12/2010 (A/Wisconsin) was used as representative for the A(H3N2)v virus, the primary target of the study. To confirm the specificity of measured antibody titres against A/Wisconsin, we also tested sera for antibodies against two other A(H3N2) virus strains. One of them, A/Niedersachsen/59/2007 (A/Niedersachsen), was a swine A(H3N2) virus circulating among pigs in Germany that caused one human infection in Germany in 2009 [16] and is closely related to the A(H3N2) swine viruses that emerged in Europe around 1980 after reassortment between human A(H3N2) viruses and swine viruses of subtype A(H1N1). The second strain used for comparison, A/Perth/16/2009 (A/Perth), was the A(H3N2) vaccine strain used in the influenza seasons 2010/11 and 2011/12, representing human influenza A(H3N2) viruses circulating recently [16]. The reference strains A/Wisconsin/12/2010 and A/Perth/16/2009 were obtained from the World Health Organization (WHO) Collaborating Centre in London, UK. A/Niedersachsen was isolated in Lower Saxony and belongs to the influenza virus strain selection of the German National Influenza Reference Centre.

The HI tests were performed according to standard WHO protocol as described before [13]. Prior to testing, each serum was treated with receptor-destroying enzyme (Cholera filtrate, Sigma, Germany) to inactivate non-specific inhibitors, achieving a final serum dilution of 1:10. The sera were then diluted serially twofold to detect titres up to ≥ 1,280. All samples were tested twice to account for the variability of results in the HI assay.

Statistical analysis

For statistical analyses, the following age groups were used: 18–29 (born 1979–92), 30–59 (born 1949–80) and ≥ 60 years (born before 1951) for adults and 0–9 (born 1994–2006), 10–13 (born 1990–96) and 14–17 years (born 1986–92) for children and adolescents. As end point we used the geometric mean of two antibody measurements as a single observation. We defined a titre ≥ 40 as seroprotective in line with other studies and with definitions used for evaluating influenza vaccines

Table 1

Independent variables investigated in the univariable logistic regression analysis among adults and children and adolescents, Germany, 2003–10

Variable name	Description	Population
Sex	Female, male	Adults, children and adolescents
Age group	18–29, 30–59, ≥ 60 years	Adults
	0–9, 10–13, 14–17 years	Children and adolescents
Sampling year	2008, 2009, 2010; 2008-09, 2010	Adults
	2003, 2004, 2005, 2006	Children and adolescents
Prior influenza vaccination	Seasonal or pandemic 2009 (based on vaccination card or self-reporting)	Adults
	Seasonal (based on vaccination card)	Children and adolescents
Number of influenza vaccinations	Based on vaccination card	Children and adolescents
Body weight	Body weight in kg	Adults, children and adolescents
Body mass index	Cut-off values: > 25, > 30, > 33, > 40, < 18	Adults
Nationality	German vs non-German	Adults
Migrant	Migrant vs non-migrant	Children and adolescents
Geographical area	Former East-, West Germany (Berlin included in East Germany)	Children and adolescents
Smoking	Smoker; amount of smoking	Adults
	Participant is smoker; at least one parent is smoker; friends are smokers	Children and adolescents
Community size	Four categories for number of inhabitants; two categories for urban vs rural	Adults, children and adolescents
Social status	Three categories	Adults, children and adolescents
Education	Three categories	Adults
	Education of parents in three categories	Children and adolescents
Health status	Based on self-reporting	Adults, children and adolescents
Any chronic disease	Having any chronic disease	Adults, children and adolescents
Alcohol intake	Risk drinking; amount of alcohol	Adults
Number of persons living in household	Living alone vs 2, 3, 4, 5 or 6 persons	Children and adolescents
Sibling(s) in household	0 vs 1, 2, 3 or 4 siblings	Children and adolescents

(the 50% seroprotective threshold) [17]. Titres < 10 were considered negative and were assigned a value of 5 for further calculations.

We calculated the proportion of persons with seroprotective titre and the geometric mean titre (GMT) with 95% confidence intervals (CI) in each age group for each influenza strain. For A/Wisconsin, we also calculated proportions with titres ≥ 10, ≥ 20, ≥ 80, ≥ 160 and ≥ 320. We examined the effect of different variables on the dichotomous variable 'titre ≥40', using logistic regression to obtain odds ratios (OR) with 95% CI for each influenza strain. As a first step, we conducted univariable regression analysis to identify potential factors associated with seropositivity. Independent variables with a p value < 0.25 based on the Wald test were considered for the multivariable analysis [18]. Manual stepwise forward selection based on p values from the Wald test was then used to obtain the final model. A p value < 0.05 was considered statistically significant. All variables in the final model were tested for possible two-way interactions. The variables investigated in the logistic regression analysis included demographic characteristics, history of influenza vaccination, year when blood sample was taken, region of residence in Germany, community size, factors related to health and social status and to living conditions (Table 1).

Statistical analyses for children and adolescents were conducted by using survey weights based on cross-classifications by age, sex, residence in Eastern or Western Germany and nationality (German vs non-German) to

Table 2

Haemagglutination inhibition antibody titres against influenza A(H3N2), by age group and virus strain, adults, Germany, 2008–2010 (n = 600)

Age group (years)	n	Proportion with titre ≥ 40 (95% CI)			GMT (95% CI)		
		A/Wisconsin	A/Perth	A/Niedersachsen	A/Wisconsin	A/Perth	A/Niedersachsen
18–29	200	59 (49–67)	24 (17–31)	23 (16–31)	33 (28–39)	12 (10–15)	15 (13–17)
30–59	200	30 (24–38)	16 (13–21)	49 (40–57)	17 (15–20)	9 (8–10)	27 (23–32)
≥ 60	200	42 (35–49)	23 (18–29)	26 (19–35)	23 (21–26)	14 (11–16)	18 (16–21)
Overall	**600**	**39 (34–44)**	**19 (17–22)**	**37 (31–43)**	**21 (19–23)**	**11 (10–12)**	**22 (19–24)**

CI: confidence interval; GMT: geometric mean titre.
Overall proportions and overall GMTs are weighted (see Methods).

account for differences between the design-weighted net sample and German population characteristics [15]. Original survey weights could not be used for the adult sample as the sera for our study were selected from a subgroup of the adult study population for which the survey weights were calculated. Based on German population characteristics of 2010 (data source: German Federal Statistical Office), we calculated weights based on sex and age group for adults, and these were used for the calculation of overall proportions and GMTs. In our study, 43 of the 180 study locations (sample points) of the original adult study were included. Two of 16 German states were not represented in our sample (the city states Bremen and Hamburg). As those city states comprise less than 5% of the total German population, we can assume that the adult sample was sufficient to represent German geographic characteristics [13]. The analyses accounted for the two-stage cluster design of the surveys.

All analyses were carried out with STATA software version 12.1 (StataCorp, College Station, TX, US). The study was approved by the Ethics Committee of Charité University Medicine, Berlin, Germany.

Results

Adults

The unweighted median age of the 600 participants included in the adult study was 47 years (range: 18–84) and 50% were female. Overall, 43% of participants (unweighted proportion) had been vaccinated at least once in their lifetime with an influenza vaccine (seasonal or pandemic 2009). Among the 600 adults, 44% had cross-reactive antibodies at titre ≥ 40 against A/Wisconsin. The overall prevalence of cross-reactive antibodies at titre ≥ 40, weighted for sex and age group, was 39% (95% CI: 34–44) (Table 2).

For A/Wisconsin, the highest proportion of persons with titre ≥ 40 and the highest GMT were seen in the youngest age group (18–29 years). In the age group 30–59 years, the proportion was lower, whereas among persons aged 60 years and older, the proportion was again higher, but did not reach the level of the youngest age group. For A/Perth, the pattern across the age groups was similar to A/Wisconsin with lower proportions in all age groups. For A/Niedersachsen, the proportions with seroprotective titre and the GMTs were highest in the age group 30–59 years.

The distribution of different cut-off values for antibody titres against A/Wisconsin in the three age groups was explored in reverse cumulative distribution curves (Figure 1). The pattern of difference between age groups was observed at all cut-off values. Titres declined rapidly after titre 40, and no titres › 320 were measured.

The final multivariable logistic regression model for all three strains contained age group, sex and prior influenza vaccination as independent variables (Table 3). Sex was included in the final model to show that it was not associated with seroprotective titres; inclusion did not relevantly change ORs for other variables. Those 60 years and older had higher odds of having a seroprotective titre compared with the 30–59 year-olds in the univariable analysis for A/Wisconsin (OR: 1.6; 95% CI: 1.1–2.4; p = 0.014). This difference was not significant after adjustment for prior influenza vaccination. Prior influenza vaccination was shown to be a factor associated with seroprotective titre for A/Wisconsin and A/Perth with the highest OR for A/Perth and lower for A/Wisconsin; the association for A/Niedersachsen was not significant. There were no significant interactions between the variables of the final models.

Children and adolescents

The unweighted median age of persons in this study was 13 years (range: 2–17) and 46% were female. Overall, 9.6% of the study participants (unweighted proportion) had been vaccinated at least once in their lifetime with a seasonal influenza vaccine. Among the 815 children and adolescents, 42% had cross-reactive antibodies at titre ≥ 40 against A/Wisconsin. The weighted overall prevalence of cross-reactive antibodies at titre ≥ 40 was 41% (95% CI: 37–45) (Table 4). For the three influenza strains investigated, the lowest proportion with seroprotective titre and the lowest

Table 3

Adjusted effects of associated factors on the odds of haemagglutination inhibition antibody titre ≥ 40 against influenza A(H3N2), by virus strain, adults, Germany, 2008–2010 (n = 582)

Variable	A/Wisconsin			A/Perth			A/Niedersachsen		
	OR	95% CI	p value	OR	95% CI	p value	OR	95% CI	p value
Age group (years)									
18–29	REF	NA	NA	REF	NA	NA	REF	NA	NA
30–59	0.28	0.16–0.50	<0.001	0.53	0.32–0.90	0.019	3.2	1.9–5.4	<0.001
≥ 60	0.36	0.20–0.64	0.001	0.53	0.31–0.90	0.019	1.0	0.61–1.70	0.954
Sex									
Male	REF	NA	NA	REF	NA	NA	REF	NA	NA
Female	1.2	0.85–1.80	0.320	0.97	0.63–1.50	0.903	1.2	0.78–1.80	0.434
Prior influenza vaccination	2.4	1.7–3.4	<0.001	4.3	2.6–7.2	<0.001	1.5	0.99–2.20	0.055

CI: confidence interval; NA: not applicable; OR: odds ratio; REF: reference group.
Participants with missing data (n = 18) were excluded.

Table 4

Haemagglutination inhibition antibody titres against influenza A(H3N2), by age group and virus strain, children and adolescents, Germany, 2003–2006 (n = 815)

Age group (years)	n	Proportion with titre ≥ 40 (95% CI)			GMT (95% CI)		
		A/Wisconsin	A/Perth	A/Niedersachsen	A/Wisconsin	A/Perth	A/Niedersachsen
0–9	62	15 (7–30)	2.2 (0.5–9.4)	0.6 (0.0–4.2)	13 (10–17)	6.1 (5.5–6.8)	5.7 (5.1–6.3)
10–13	353	37 (31–44)	3.0 (1.6–5.5)	3.6 (2.0–6.5)	21 (19–24)	6.3 (6.0–6.7)	7.9 (7.4–8.5)
14–17	400	47 (42–52)	3.9 (2.3–6.5)	12.0 (9.0–16.0)	27 (24–29)	6.5 (6.1–6.9)	11 (10–12)
Overall	**815**	**41 (37–45)**	**3.4 (2.3–5.0)**	**8.1 (6.3–10.0)**	**23 (22–25)**	**6.4 (6.1–6.7)**	**9.3 (8.8–9.8)**

CI: confidence interval; GMT: geometric mean titre.
Proportions and GMTs are weighted (see Methods).

GMT were seen among those younger than 10 years, with proportions and GMTs increasing with increasing age. The overall proportion with seroprotective titre for A/Perth and A/Niedersachsen was less than 10%.

The distribution of different cut-off values for antibody titres against A/Wisconsin in the three age groups was explored using reverse cumulative distribution curves (Figure 2). The pattern of difference between age groups was observed at all cut-off values, with rapidly declining titres after titre 40 and no measurable titres > 320.

The final multivariable logistic regression model included age group, sex and prior influenza vaccination as independent variables for all three strains (Table 5). For A/Perth, there was no significant association with age. Significant associations between prior vaccination and seroprotective titres were shown for each tested strain, with the highest OR for A/Perth, followed by A/Wisconsin and A/Niedersachsen. Female sex was associated with higher odds of having a seroprotective titre for A/Wisconsin and A/Niedersachsen. There were no significant interactions between the variables of the final models.

Discussion

Using sera from two representative health examination surveys in the German population including adults, children and adolescents, we have shown that children younger than 10 years (in 2003–06, i.e. those born 1994–2006) had the lowest prevalence of cross-reactive antibodies against the A(H3N2)v virus representative A/Wisconsin. Among children aged 10 years and older, adolescents and adults, the prevalence was markedly higher. The pattern of differences between age groups could be observed at many titre cut-off values, not just at the level defined as seroprotective. The observed

Table 5

Adjusted effects of associated factors on the odds of haemagglutination inhibition antibody titre ≥ 40 against influenza A(H3N2), by virus strain, children and adolescents, Germany, 2003–2006 (n = 815)

Variable	A/Wisconsin			A/Perth			A/Niedersachsen		
	OR	95% CI	p value	OR	95% CI	p value	OR	95% CI	p value
Age group (years)									
0–9	REF	NA	NA	REF	NA	NA	REF	NA	NA
10–13	3.2	1.3–8.0	0.013	1.3	0.27–6.60	0.713	5.7	0.69–47.0	0.105
14–17	4.6	1.8–12.0	0.001	1.8	0.40–7.90	0.442	20.0	2.6–151	0.004
Sex									
Male	REF	NA	NA	REF	NA	NA	REF	NA	NA
Female	1.5	1.0–2.0	0.025	0.78	0.33–1.80	0.549	2.7	1.5–4.9	0.001
Prior influenza vaccination	3.4	1.8–6.5	< 0.001	5.4	2.2–14.0	< 0.001	2.2	1.1–4.6	0.028

CI: confidence interval; NA: not applicable; OR: odds ratio; REF: reference group.
The regression models are weighted (see Methods).

pattern of age-specific seroprevalence of cross-reactive antibodies against the A(H3N2)v virus is consistent with findings from countries in Asia (Japan), Europe (Norway, UK) and North America (Canada, US) [7-11]. The levels of cross-reactive antibody titres against the influenza A(H3N2)v virus in the different age groups were consistent with the likelihood of exposure to seasonal A/Wuhan/359/95-like influenza strains. Likewise, cross-reactive antibody titre levels against the contemporary human influenza A(H3N2) virus (A/Perth) and the swine influenza A(H3N2) virus (A/Niedersachsen) were in line with the likelihood of exposure to related circulating seasonal influenza strains and confirm the specificity of our results regarding the A(H3N2)v virus.

Several mechanisms may explain the observed prevalences of cross-reactive antibodies against the A(H3N2)v virus and the highest proportions with seroprotective titre among those aged 14–17 and 18–29 years. Young adults are at high risk for infection as their contact patterns result in frequent exposure to circulating seasonal influenza viruses. The 14–29 year-olds in our study are likely to have been infected at a young age with the A/Wuhan/359/95-like human A(H3N2) virus that is very similar to A(H3N2)v. This may have represented the initial exposure to influenza viruses, resulting in long-lasting immunity. The first infection with an influenza virus is believed to influence and dominate immune responses to later infections (original antigenic sin theory) [19,20]. Although they had the opportunity of exposure to A/Wuhan/359/95-like human A(H3N2) viruses, older adults had lower antibody levels against the A(H3N2)v virus. Lower frequency of influenza infections among older adults may explain this observation. In addition, the initial childhood exposure of these adults to influenza was to viruses different from the A(H3N2)v virus. Children younger than 10 years were least likely to have been exposed to influenza viruses similar to the A(H3N2)v virus, which explains why the antibody levels in this group were the lowest.

Any influenza vaccination in the lifetime of our study participants (after adjustment for age and sex) was positively associated with having a seroprotective titre of cross-reactive antibodies against all three investigated strains. This effect was strongest for the human influenza A(H3N2) virus A/Perth and weaker for the swine-related influenza A(H3N2) viruses A/Wisconsin and A/Niedersachsen, which is plausible considering that A/Wisconsin and A/Niedersachsen are less related to human vaccine strains than A/Perth.

In the final model, female children and adolescents had slightly higher odds of having a seroprotective titre against the A(H3N2)v and swine A(H3N2) virus. This may be explained by a generally stronger immune response to infections and vaccination in women, and sex-related differences in HI titres after seasonal influenza vaccination have been described before [21]. Such an effect was not observed among adults, nor with the human A(H3N2) virus strain among children and adolescents.

The proportions of adults with seroprotective titre against the A(H3N2)v virus differed significantly between the age groups 30–59 and ≥ 60 years. Similar observations have been made by others for the A(H3N2)v virus [10,11] and also for seasonal H3N2 and H1N1 subtypes where, among others possible causes, the priming infection of a person and prior influenza vaccination were suggested to be strong determinants of such a pattern [20,22-24]. Using information about prior influenza vaccination in a multivariable logistic regression model, we have shown that, in our

population, this difference was most probably a result of the higher proportion of elderly people with prior influenza vaccination (one of the risk groups for whom seasonal influenza vaccination is recommended in Germany).

Our study had several limitations. Serum samples originated from two different population surveys carried out in different time periods over several years (2003–06 and 2008–10) and therefore under the influence of different circulating human influenza viruses. Considering this and the exposure history of the population after the surveys were carried out, caution should be applied when using our results to predict age-related susceptibility in the population today. Some of the age groups of KiGGS and DEGS participants overlap when they are considered as birth cohorts. This overlap may explain the similar prevalence of seroprotective titres among those aged 14–17 and 18–29 years.

HI is used as a correlate for immunity against infection with influenza virus, but beside cross-reactive antibodies, many other biological mechanisms influence the actual immune response of a person (e.g. T-cell mediated immunity) [25,26] and thus susceptibility to influenza viruses. Increased HI titres may not necessarily prevent higher infection rates. We demonstrated this in an earlier study conducted in Germany, where the age group with the highest pre-pandemic titres against the A(H1N1)pdm09 virus had the highest infection rates with this virus [13]. It cannot be assumed that prior influenza vaccination necessarily provides true protection against infection with the A (H3N2)v virus as studies have shown that prior vaccination against seasonal influenza may be associated with a higher risk of becoming infected with a pandemic influenza strain (A(H1N1)pdm09) [27-30]. A recent study did not show the 2011/12 seasonal trivalent inactivated influenza vaccine to have a cross-protective effect against the A(H3N2)v virus in seronegative ferrets [31]. This may have implications for young children not yet infected by influenza virus because a seasonal influenza vaccine without an A(H3N2)v virus component would most probably not confer any immunity against the A(H3N2)v virus among them. In addition, the assumption that an HI titre ≥ 40 indicates 50% seroprotection is based on studies in adults. Recent studies suggest that a titre ≥ 40 or higher may be used as a correlate of 50% seroprotection for children aged 6–17 years [32], but higher titres may be needed to predict at least 50% protection in younger age groups [33]. Furthermore, compared to younger adults, HI titres seem to be less reliable in predicting protection in the elderly and in certain risk groups [34].

Our results indicate that young children have the highest susceptibility to infection with the A(H3N2)v virus. In case of increased sustained human-to-human transmission of the virus, these children may contribute to the rapid spread of the A(H3N2)v virus as transmission among children plays a major role in the propagation of the spread of influenza [35]. They are also at high risk for severe disease from influenza infections. A large proportion of the population 10 years and older has cross-reactive antibodies at potentially seroprotective level and thus, based on this correlate for immunity, may be at low risk for A(H3N2)v infection. Our results suggest that the first exposure to circulating seasonal influenza viruses in a person's lifetime may predict their long-term cross-reactive antibody response. These cross-reactive antibodies can be boosted by vaccination and possibly by exposure to seasonally circulating influenza viruses. Further studies may further our understanding of the effect of seasonal influenza viruses on the serological immune response.

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The study was approved by the Ethics Committee of Charité University Medicine, Berlin, Germany.

Conflict of interest

None declared.

Authors' contributions

B. Blümel contributed to the overall design of the study, analysed the data, and drafted the manuscript. B. Schweiger contributed to the study design, supervised the laboratory analysis, and reviewed the manuscript. M. Dehnert and I. Czogiel contributed to the design of the analysis, supervised data analysis, and reviewed the manuscript. S. Buda, A. Reuss contributed to the study design and reviewed the manuscript. P. Kamtsiuris, M. Schlaud, C. Poethko-Müller, and M. Thamm provided the data and sera for the study and reviewed the manuscript. W. Haas contributed to the overall design of the study and the design of the analysis, and reviewed the manuscript.

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