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Gonococcal infections and emergence of gonococcal decreased susceptibility to cephalosporins in France, 2001 to 2012

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Resistance to cephalosporins may lead to untreatable gonococcal infections. We describe the results of the sentinel surveillance of gonococcal infections and the evolution of the resistance of *Neisseria gonorrhoeae* to antibiotics in France from 2001 to 2012. We also analyse the factors associated with decreased susceptibility to third generation cephalosporins. In France, surveillance of gonococcal infections is conducted through a network of voluntarily participating laboratories. Strains are sent to the national reference laboratory to determine the minimum inhibitory concentration (MIC) for six antibiotics. During the study period, the number of gonococcal infections increased steadily. The susceptibility of 8,649 strains was studied for this period. The proportion of strains with decreased susceptibility to cefixime (MIC>0.125 mg/L) quadrupled between 2011 (0.7%:10/1,521) and 2012 (3.0%: 33/1,093; p<0.001). Between 2001 and 2012, only two of the 8,649 strains, both collected in 2010, had a MIC>0.125 mg/L for ceftriaxone. Decreased susceptibility to cephalosporins increased with older age and was more common in pharyngeal strains. Decreased susceptibility to cefixime may indicate that the national recommendation to use ceftriaxone as a first line treatment for cases of urethritis and cervicitis has not been fully implemented. Enhanced surveillance of pharyngeal strains is strongly suggested.

Introduction

Although gonorrhoea is a common sexually transmitted infection (STI), normally responsible for uncomplicated genital infections, the disease can sometimes lead to severe complications (salpingitis, epididymo-orchitis, septicaemia) [1,2]. It also increases the risk of transmission by human immunodeficiency virus (HIV) [2]. Because of its short incubation time (mainly 2 to 5 days) [3] and characteristic symptoms in men, the incidence of gonorrhoea can be used as a very sensitive indicator of the relaxation of safe sexual behaviours, and as an early warning signal for increased risk of HIV transmission [2].

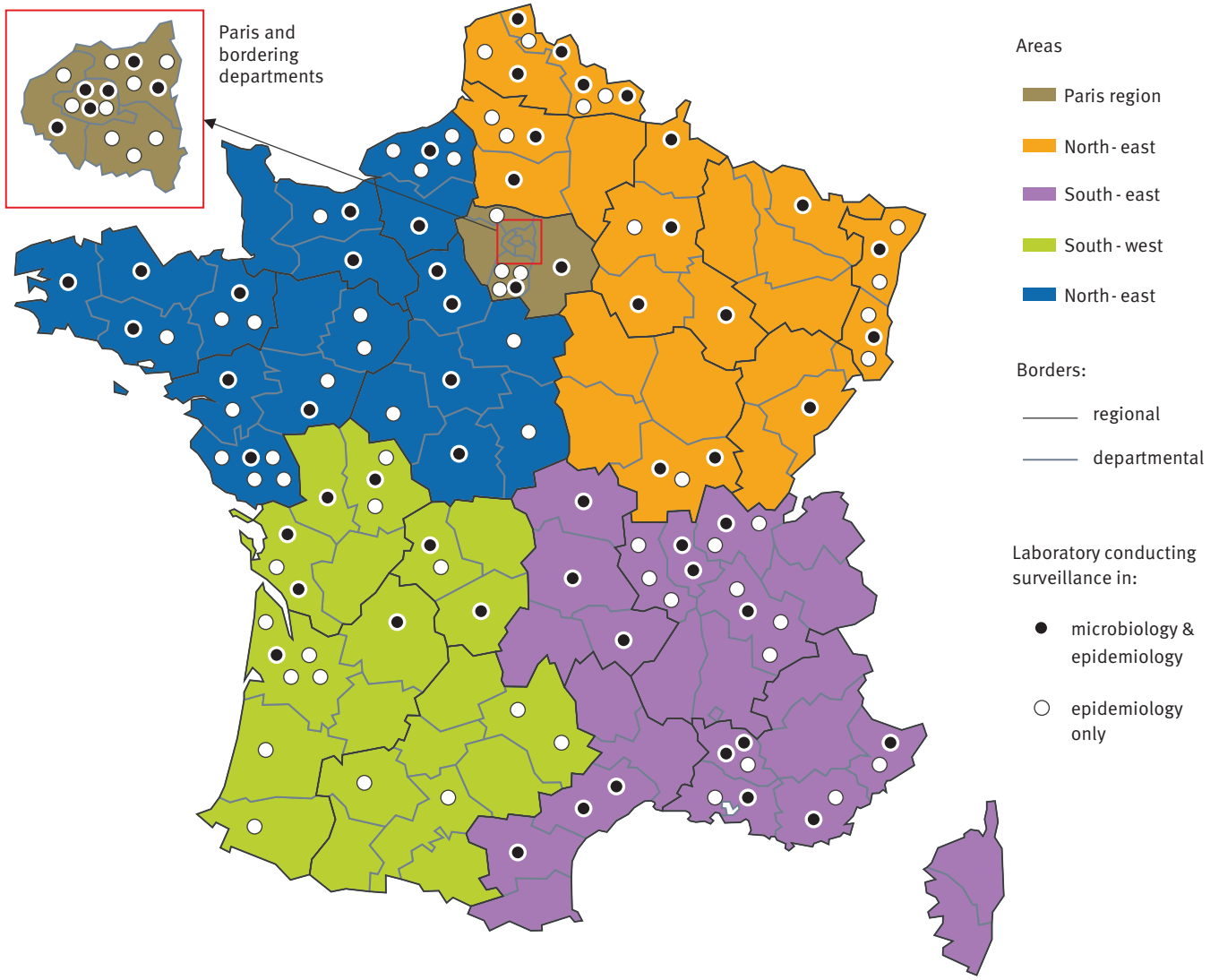
Following acquired immunodeficiency syndrome (AIDS) prevention campaigns in the 1980s and at the beginning of the 1990s, the number of cases of gonorrhoea in certain European Union (EU) and European Free Trade Association (EFTA) countries [4], including France [5], dropped significantly. This drop was the result of a reduction of at-risk sexual behaviours [6]. The advent of antiretroviral multitherapy in the mid-1990s brought with it a resurgence of these behaviours in the general population, especially in men who have sex with men (MSM). It also led to a resurgence in cases of gonorrhoea in some EU/EFTA countries [5,6] as well as in the United States [7].

In France, the epidemiological surveillance of gonorrhoea is based on two voluntary sentinel networks relying on clinicians (the RésIST network) or public and private laboratories (the Rénago network – National Gonorrhoea Network –) [8,9]. Since the beginning of the 2000s, an increase in gonococcal infections has been observed by these networks, both in men and women [9]. The RésIST network also highlighted that this increase exists irrespective of sexual orientation [8,9].

At the end of the 1990s and the beginning of the 2000s, concomitantly with the resurgence of gonoccocal infections, several countries reported increasing levels of gonococcal resistance to ciprofloxacin [2,10], a fluoroquinolone antibiotic, which was used at the time as a single-dose first-line treatment for uncomplicated urogenital gonorrhoea. Since the mid-2000s, single-dose third generation cephalosporins (TGC), specifically oral cefixime and injectable ceftriaxone, became the only remaining recommendable antimicrobial class [11]. These treatments were officially recommended

Figure 1

Geographical distribution of the 133 laboratories in the Rénago[a] network according to participation in microbiological surveillance, France, 2012



[a] Rénago is the national gonorrhoea network, a sentinel network based on voluntarily participating public and private laboratories.

in France at the end of 2005 [12]. However, the subsequent development of clinical and biological resistance to TGC, especially to cefixime, and, since the end of the 2000s, to ceftriaxone, may lead to a therapeutic dead end in the coming years [2,11]. Surveillance of gonococcal susceptibility to these antibiotics is therefore essential.

Given this worrying context, the objective of this study was to describe the evolution of gonococcal infections and the changes in the susceptibility of gonorrhoea strains to antibiotics in France between 2001 and 2012. Factors associated with decreased susceptibility to TGC are also assessed.

Methods

The laboratories in the Rénago network are distributed throughout all of metropolitan France. This network helps monitor the resistance of *Neisseria gonorrhoeae* strains to antibiotics. Laboratory participation to surveillance is voluntary.

The laboratories receive patients referred by their doctor who prescribed a microbiological diagnosis for gonorrhoea. An anonymous epidemiological file, collected by the network laboratory microbiologist, provided the following information for each patient: sex, age, date of sample, anatomical site of sample, presence of symptoms, co-infection with another STI, probable country of infection, information on partner's infection, the place of consultation and specialisation of treating physician, type of laboratory and geographical area to which it belongs. The sexual orientation of the patient is not reported. Information is consolidated and analysed by the Institut de Veille Sanitaire (InVS).

Because of the large proportion of missing data for four variables (presence of symptoms, co-infection with another STI, probable country of infection, and information on patient's partner infection status), the latter were used only for univariate analysis for descriptive purposes. All other variables were included in the multivariate analysis; p<0.05 was used as the level for significance. Only the results of multivariate analysis are presented here, unadjusted and adjusted odds-ratios being very similar. For the analyses, the data concerning the years from 2001 to 2009 were pooled to highlight recent developments of the characteristics of gonococcal infections and of antibiotic susceptibility of strains in the period from 2010 to 2012.

Gonococcal infection is laboratory confirmed by a positive culture or a positive nucleic-acid amplification test (NAAT) for *N. gonorrhoeae* by the network laboratories. Strains isolated by culture by the network laboratories are sent for antibiotic susceptibility testing to the Alfred Fournier Institute in Paris, which is the national reference laboratory (NRL) for gonorrhoea.

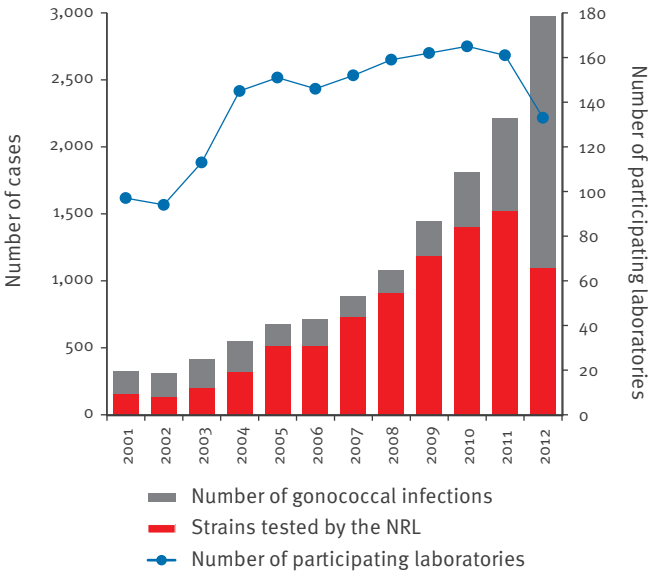
Because of the very high number of strains received by the NRL as well as budget constraints, the InVS and the NRL decided to separate epidemiological and microbiological surveillance. Although the epidemiological data collection continued for all laboratories in the network, the number of laboratories sending their strains to the NRL was reduced by about fifty per cent from the beginning of January 2012 (in 2012, 63 of the 133 laboratories in the network participated to the microbiological surveillance). Nevertheless, a homogeneous geographical distribution of laboratories continuing this microbiological surveillance was ensured (Figure 1).

The NLR determines the minimum inhibitory concentration (MIC) for six antibiotics (penicillin G, tetracycline, ciprofloxacin, spectinomycin, ceftriaxone, and, since 2008, cefixime) using Etest strips (first from AB Biodisk, Solna, Sweden; then from AES Laboratory, Combourg, France, and from May 2011 from i2a, Pérols, France). These antibiotics were or are still recommended for the treatment of gonorrhoea [11]. The presence of beta-lactamases is detected using Api NH (bioMérieux, Marcy-l'Etoile, France) identification test kits and, when there is any doubt, using a chromogenic cephalosporin test (cefinase, bioMérieux). The criteria used to define resistance are those used by the European Committee on Antimicrobial Susceptibility Testing –EUCAST 2013 [13]. For TGC, no defined threshold exists for resistance; strains with a MIC>0.125 mg/L for cefixime and for ceftriaxone are regarded as having a decreased susceptibility. A strain was considered multi-resistant if it was resistant to penicillin G, tetracycline and ciprofloxacin, and if the MIC for cefixime or for ceftriaxone was >0.125 mg/L.

The factors associated with a reduction in susceptibility of gonorrhoea to TGC in patients aged at least 15 years-old were determined through univariate and multivariate analyses using Stata v.11.0 software, excluding missing values. As the number of strains with MIC>0.125 mg/L for TGC was low, we studied the factors associated with a MIC≥0.094 mg/L for cefixime and a MIC≥0.032 mg/L for ceftriaxone. These values were chosen arbitrarily to have a sufficient number of strains with MICs above these cut-offs to allow comparison analyses. Taking into account such cut-offs, 6% of strains had decreased susceptibility to these two antibiotics.

Figure 2

Annual number of reported gonococcal infections and strains tested by the national reference laboratory for gonorrhoea, Rénago network[a], France, 2001–2012



NRL: national reference laboratory.

[a] Rénago is the national gonorrhoea network, a sentinel network based on voluntarily participating public and private laboratories. All laboratories participate to the epidemiological surveillance. Until 2011, all laboratories participated to the microbiological surveillance. In 2012, only 63 of the 133 laboratories of the network participated to the microbiological surveillance.

Results

Evolution of the number of gonococcal infections and analysed strains

Between 2001 and 2012, 13,400 cases of gonorrhoea were reported by the network's laboratories. From these cases, a total of 10,501 strains were sent to the NLR. The NLR succeeded in recultivating 8,649 (82%) of them to determine the susceptibility of *N. gonorrhoeae* to antibiotics. The proportion of strains sent to the NLR and then recultivated remained quite stable from 2005 to 2012 (84% on average for this period, range 82 to 87%).

Figure 2 shows the increase in the number of reported cases and the number of strains studied by the NLR

Table 1

Characteristics of cases of gonorrhoea, Rénago[a] network, France 2001–2012 (n=13,400)

Variables	2001–2009 N (%)	2010 N (%)	2011 N (%)	2012 N (%)	Total 2001–2012 N (%)
Total	**6,407 (100)**	**1,807 (100)**	**2,211 (100)**	**2,975 (100)**	**13,400 (100)**
Sex					
Male	5,552 (87)	1,506 (83)	1,718 (78)	2,044 (69)	10,820 (81)
Female	846 (13)	299 (17)	493 (22)	928 (31)	2,566 (19)
Not provided	9 (<1)	2 (<1)	0 (0)	3 (<1)	14 (<1)
Age (in years)					
0–14	12 (<1)	8 (<1)	5 (<1)	21 (1)	46 (<1)
15–24	1,838 (29)	673 (37)	916 (41)	1,387 (47)	4,814 (36)
25–34	2,323 (36)	598 (33)	687 (31)	904 (30)	4,512 (34)
35–44	1,318 (21)	305 (17)	318 (14)	347 (12)	2,288 (17)
≥45 years	688 (11)	180 (10)	228 (10)	254 (9)	1,350 (10)
Not provided	228 (4)	43 (2)	57 (3)	62 (2)	390 (3)
Anatomical site					
Urethra or urine	4,905 (77)	1,394 (77)	1,598 (72)	1,972 (66)	9,869 (74)
Cervix or vagina	745 (12)	278 (15)	459 (21)	839 (28)	2,321 (17)
Anus	588 (9)	98 (5)	105 (5)	77 (3)	868 (6)
Pharynx	35 (1)	8 (<1)	23 (1)	33 (1)	99 (1)
Other sites	112 (2)	24 (1)	25 (1)	35 (1)	196 (1)
Not provided	22 (<1)	5 (<1)	1 (<1)	19 (1)	47 (<1)
Presence of symptoms					
Yes	4,336 (68)	1,099 (61)	1,365 (62)	1,739 (58)	8,539 (64)
No	127 (2)	97 (5)	93 (4)	160 (5)	477 (4)
Not provided	1,944 (30)	611 (34)	753 (34)	1,076 (36)	4,384 (33)
Co-infection with another STI					
Yes	951 (15)	287 (16)	425 (19)	715 (24)	2,378 (18)
No	1,941 (30)	437 (24)	568 (26)	868 (29)	3,814 (28)
Not provided	3,515 (55)	1,083 (60)	1,218 (55)	1,392 (47)	7,208 (54)
Probable country of infection					
France	1,691 (26)	502 (28)	576 (26)	719 (24)	3,488 (26)
Abroad	134 (2)	27 (1)	35 (2)	27 (1)	223 (2)
Not provided	4,582 (72)	1,278 (71)	1,600 (72)	2,229 (75)	9,689 (72)
Patient's partner infected					
Yes	212 (3)	76 (4)	102 (5)	124 (4)	514 (4)
No	122 (2)	38 (2)	47 (2)	90 (3)	297 (2)
Not provided	6,073 (95)	1,693 (94)	2,062 (93)	2,761 (93)	12,589 (94)
Place of consultation					
Private sector or clinic	3,410 (53)	1,175 (65)	1,425 (64)	1,581 (53)	7,591 (57)
Hospital	1,203 (19)	274 (15)	316 (14)	462 (16)	2,255 (17)
Specialised facilities for STIs[b]	831 (13)	321 (18)	398 (18)	751 (25)	2,301 (17)
Other structures	0 (0)	9 (<1)	47 (2)	93 (3)	149 (1)
Not provided	963 (15)	28 (2)	25 (1)	88 (3)	1,104 (8)
Prescribing doctor					
General practitioner	3,660 (57)	1,260 (70)	1,458 (66)	1,814 (61)	8,192 (61)
Gynaecologist	634 (10)	156 (9)	262 (12)	386 (13)	1,438 (11)
Dermatologist-venereologist	860 (13)	100 (6)	86 (4)	168 (6)	1,214 (9)
Other medical specialisation	877 (14)	256 (14)	320 (14)	381 (13)	1,834 (14)
Not provided	376 (6)	35 (2)	85 (4)	226 (8)	722 (5)
Type of Laboratory					
Private	4,612 (72)	1,376 (76)	1,617 (73)	1,984 (67)	9,589 (72)
Hospital	1,610 (25)	352 (19)	500 (23)	804 (27)	3,266 (24)
Community clinic	185 (3)	79 (4)	94 (4)	187 (6)	545 (4)
Laboratory location (area in France)					
Paris region	2,820 (44)	677 (37)	751 (34)	1,021 (34)	5,269 (39)
North-west	1,119 (17)	421 (23)	508 (23)	672 (23)	2,720 (20)
North-east	966 (15)	304 (17)	384 (17)	412 (14)	2,066 (15)
South-east	938 (15)	232 (13)	332 (15)	494 (17)	1,996 (15)
South-west	564 (9)	173 (10)	236 (11)	376 (13)	1,349 (10)

STI: sexually transmitted infection.

[a] Rénago is the national gonorrhoea network, a sentinel network based on voluntarily participating public and private laboratories.

[b] Specialised facilities for the management of STI: STI clinics, free anonymous counselling and testing services and family planning centres.

between 2001 and 2012. During the first five years of the study, the number of laboratories participating in epidemiological surveillance greatly increased, from 97 in 2001 to 151 in 2005. This number subsequently remained quite stable before decreasing between 2011 (n=161) and 2012 (n=133). The yearly number of strains whose susceptibility was examined by the NLR increased tenfold from 150 in 2001 to 1,521 in 2011. In 2012, with the number of participating laboratories to the microbiological surveillance decreasing by half, 1,093 strains were tested.

Since 2009, larger numbers of diagnoses are being made using NAAT. NAAT accounted for only 6% (84/1,444) of microbiological diagnoses of gonorrhoea in 2009, this figure increased to 13% (237/1,807) in 2010, 25% (554/2,211) in 2011 and 47% (1,408/2,975) in 2012. NAAT was exclusively used to diagnose gonorrhoea in 3% (40/1,444), 8% (136/1,807), 14% (302/2,211) and 28% (839/2,975) of patients, for each of these years, respectively (p<0.001, chi-square test for trend).

Epidemiological and clinical characteristics of cases of gonorrhoea

The clinical characteristics of reported cases of gonorrhoea are shown in Table 1. Most were male (81% over the whole study period: 10,820/13,400), yet the proportion of female constantly increased, reaching 31% (928/2,975) of cases in 2012. The 15- to 24-year-old age group was most affected, and saw a constant increase over time for both sexes. Median age was higher in men (29 years compared with 22 years in women; p<0.001, analysis of variance) over the whole study period, but decreased over time both in men and women (from, respectively, 30 and 24 years between 2001 and 2009, to 26 and 21 years in 2012; p<0.001 for both comparisons).

Information on the presence of symptoms was missing for 33% (4,384/13,400) of cases. Considering those with such information available, 98% (7,587/7,720) of men and 73% (942/1,285) of women presented with symptoms (p<0.001). Information on simultaneous infection with another STI was missing in 54% (7,208/13,400) of cases. For cases with data, 32% (1,456/4,622) of men and 59% (918/1,561) of women had another STI (p<0.001), mainly co-infection with chlamydia (19%: 862/4,622 of co-infection in men and 48%: 744/1,561 in women; p<0.001). Information on probable country of infection (i.e. in France or abroad) was missing in 72% (9,689/13,400) of cases. When it was available, infection had occurred outside of France in 7% (206/3,146) and 3% (17/561) of men and women, respectively (p=0.001). The partner's infection status was hardly ever recorded by the laboratories, as the information was missing for 94% (12,589/13,400) of cases.

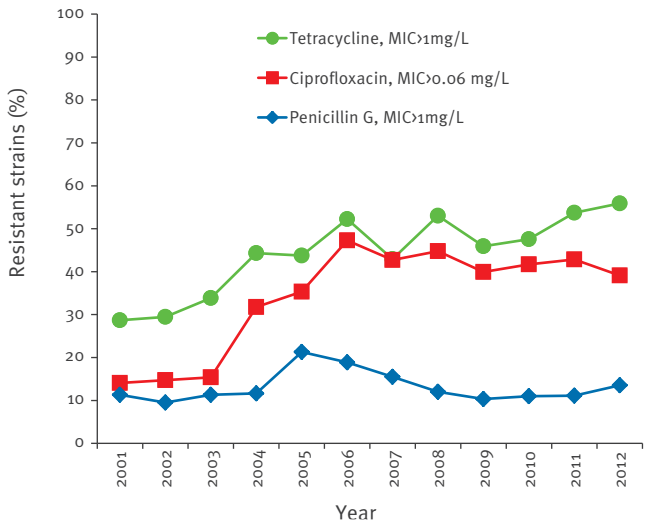
In men, samples were taken mainly from the urethra or urine (90%: 9,741/10,820). Samples from the anus (8%: 842/10,820 over the whole study period, diminishing in recent years to 4%: 72/2,044 in 2012) and pharynx (1%: 93/10,820) were much rarer. In women, samples were taken mainly from the cervix or vagina (90%: 2,320/2,566). Urethral (5%: 119/2,566) and anal (1%: 24/2,566) samples were also much rarer.

Patients had mainly consulted in private general practitioner offices or in clinics (67%: 6,588/9,859 and 41%: 1,000/2,424 of the men and women, respectively, for whom information was available; p<0.001). Consultation had occurred less frequently in hospitals (17%: 1,632/9,859 and 26%: 621/2,424 of men and women, respectively; p<0.001) and less frequently in the following specialised facilities: STI clinics, free Anonymous Counselling and Testing Services and Family Planning Centres (15%: 1,521/9,859 and 32%: 772/2,424 of men and women, respectively; p<0.001). The prescribing doctor was a general practitioner in most cases (71%: 7,236/10,244 of men and 39%: 950/2,421 of women for whom information was available; p<0.001), a gynaecologist (50%: 1,209/2,421 of women) or a specialist in dermato-venereology (11%: 1,127/10,244 and 3%: 83/2,421 of men and women, respectively; p<0.001).

Finally, bacteriological samples were mainly taken in private laboratories (74%: 7,992/10,820 of men and 62%: 1,586/2,566 of women; p<0.001). They were less frequently collected in hospital laboratories (22%: 2,384/10,820 and 34%: 879/2,566 of men and women, respectively; p<0.001) and rarely at a community clinic (4% of men: 444/10,820 and of women: 101/2,566; p=0.70).

Figure 3

Evolution of the resistance of gonococcal strains to penicillin G, tetracycline and ciprofloxacin, Rénago[a] network, 2001–2012 (n=8,649 strains)



MIC: minimum inhibitory concentration.

[a] Rénago is the national gonorrhoea network, a sentinel network based on voluntarily participating public and private laboratories.

Table 2

Evolution of the susceptibility of gonoccocal strains to antibiotics, Rénago network[a], 2001–2012 (n=8,649 strains)

Antibiotics	2001–2009 N (%)	2010 N (%)	2011[b] N (%)	2012 N (%)
Penicillin G				
Susceptible strains (MIC≤0.064mg/L)	1,251 (27)	383 (27)	130 (23)	284 (26)
Decreased susceptibility (0.064<MIC≤1mg/L)	2,741 (59)	863 (62)	373 (66)	661 (60)
Low-level chromosomal resistance (MIC>1mg/L and beta-lactamase negative)	161 (3)	46 (3)	11 (2)	52 (5)
High-level plasmid-mediated resistance (beta-lactamase positive)	477 (10)	108 (8)	52 (9)	96 (9)
Total number of strains tested (N=7,689 strains)	4,630 (100)	1,400 (100)	566 (100)	1,093 (100)
Tetracycline				
Susceptible strains (MIC≤0.5mg/L)	1,000 (22)	328 (23)	104 (18)	188 (17)
Decreased susceptibility (0.5<MIC≤1mg/L)	1,516 (33)	406 (29)	157 (28)	294 (27)
Low-level chromosomal resistance (1<MIC<16mg/L)	1,445 (31)	449 (32)	171 (30)	366 (33)
High-level plasmid-mediated resistance (MIC≥16mg/L)	670 (14)	217 (16)	132 (23)	245 (22)
Total number of strains tested (N=7,688 strains)	4,631 (100)	1,400 (100)	564 (100)	1,093 (100)
Ciprofloxacin				
Susceptible strains (MIC≤0.064mg/L)	2,831 (61)	814 (58)	861 (57)	664 (61)
Decreased susceptibility (0.032<MIC≤0.064mg/L)	19 (<1)	2 (<1)	7 (<1)	1 (<1)
Low-level resistance (0.064<MIC<1mg/L)	200 (4)	53 (4)	117 (8)	19 (2)
High-level resistance (MIC≥1mg/L)	1,584 (34)	531 (38)	536 (35)	409 (37)
Total number of strains tested (N=8,648 strains)	4,634 (100)	1,400 (100)	1,521 (100)	1,093 (100)
Spectinomycin[c]				
Susceptible strains (MIC≤64mg/L)	4,631 (100)	1,400 (100)	1,426 (100)	1,093 (100)
Total number of strains tested (N=8,550 strains)	4,631 (100)	1,400 (100)	1,426 (100)	1,093 (100)
Cefixime[d]				
Susceptible strains (MIC≤0.125mg/L)	1,983 (100)	1,391 (99)	1,511 (99)	1,060 (97)
Decreased susceptibility (MIC>0.125mg/L)	8 (<1)	8 (1)	10 (1)	33 (3)
Total number of strains tested (N=6,004 strains)	1,991 (100)	1,399 (100)	1,521 (100)	1,093 (100)
Ceftriaxone				
Susceptible strains (MIC≤0.125mg/L)	4,631 (100)	1,398 (100)	1,521 (100)	1,093 (100)
Decreased susceptibility (MIC>0.125mg/L)	0 (0)	2 (<1)	0 (0)	0 (0)
Total number of strains tested (N=8,645 strains)	4,631 (100)	1,400 (100)	1,521 (100)	1,093 (100)
Multidrug resistance[e]				
No multidrug resistance	1,989 (100)	1,396 (100)	563 (100)	1,075 (98)
Multidrug resistant strains	1 (<1)	3 (<1)	1 (<1)	18 (2)
Total number of strains tested (N=5,046 strains)	1,990 (100)	1,399 (100)	564 (100)	1,093 (100)

EUCAST: European Committee on Antimicrobial Susceptibility Testing; MIC: minimum inhibitory concentration.
EUCAST 2013 criteria were used for the definition of resistance [13].

[a] Rénago is the national gonorrhoea network, a sentinel network based on voluntarily participating public and private laboratories.

[b] In 2011, only 37% of strains were tested for penicillin G and tetracycline because of budgetary constraints.

[c] For spectinomycin, precise analysis of MIC values was restricted to the period when the new E-test was used (from May 2011).

[d] Cefixime was tested from 2008 onwards. There is no official threshold defining gonococcal resistance to third generation cephalosporins but 0.125 mg/L is used in practice [25].

[e] Defined as resistance to penicillin G (MIC>1mg/L or beta-lactamase positive), tetracycline (MIC>1mg/L), and ciprofloxacin (MIC>0.064mg/L) and demonstration of elevated MICs of cefixime or ceftriaxone (MIC>0.125mg/L) [25].

Evolution of the susceptibility of gonococcal strains to antibiotics

The analysis is based on 8,649 gonococcal strains tested by the NLR. Proportionally, more strains were tested from men (70%: 7,569/10,815) than women (42%: 1,070/2,564; p<0.001). Furthermore anal samples were tested proportionally more often (84%: 730/868) than samples from the other anatomical sites (63%: 7,898/12,478; p<0.001).

The majority of strains (73%:5,641/7,689) presented decreased susceptibility to penicillin G (MIC>0.064 mg/L). The proportion of strains resistant to penicillin G (MIC>1 mg/L) fluctuated between 10 (12/126 in 2002) and 21% (109/512 in 2005; Figure 3, 13% on average: 1,003/7,689) during the study period, with, in 2012, 14% of strains resistant (148/1,093; 95% confidence interval (CI): 11.6–15.7). Nearly three quarters of resistant strains (733/1,003) had a high level of plasmid-mediated penicillin resistance (Table 2).

The proportion of strains resistant to tetracycline increased from 29% (43/150) in 2001 to 56% (611/1,093) in 2012 (Figure 3). In 2012, 33% (366/1,093) of the

strains (95% CI: 31–36) had low-level chromosomal resistance and 22% (245/1,093; 95% CI: 20–25) had high-level plasmid-mediated resistance.

The proportion of strains resistant to ciprofloxacin (MIC>0.06 mg/L) increased sharply between 2003 (15%: 30/195, 13%: 25/195 of which with high-level resistance (MIC≥1mg/L) and 2006 (47%: 243/514, 43%: 221/514 of which with high-level resistance). It then remained quite high at around 42% (Figure 3). In 2012, 2% (19/1,093; 95% CI: 1.0–2.7) of the strains had low-level resistance while 37% (409/1,093; 95% CI: 35–40) had high-level resistance.

No strain resistant to spectinomycin was detected throughout the whole study period. The change in E-test type from May 2011 means that a comparison of the evolution of the distribution of MIC values before and after this date could not be made for this antibiotic. Nevertheless, the median MIC between 2001 (6 mg/L) and 2011 until April (8 mg/L) remained relatively stable. Similarly, the median MIC remained stable between 2011 after May (16 mg/L) and 2012 (16 mg/L).

The proportion of strains with decreased susceptibility to cefixime (MIC>0.125 mg/L) was less than 1% until 2011 (Table 2). In 2011 it was 0.7% (10/1,521; 95% CI: 0.3–1.2) and increased significantly to 3.0% in 2012 (33/1,093; 95% CI: 2.1–4.2; p<0.001). The proportion of strains with MIC values>0.094 mg/L increased consistently between 2008 and 2012 (Figure 4).

In 2010, for the first time two strains of 1,400 (0.14%; 95% CI: 0.02–0.57) showed decreased susceptibility to ceftriaxone, and both of these also showed decreased susceptibility to cefixime. Both cases were in men. One was about twenty years of age (MIC of 0.19 mg/L for ceftriaxone and 0.5 mg/L for cefixime). The other was a MSM in his fifties (MIC of 0.75 mg/L for ceftriaxone and 2 mg/L for cefixime) [14]. No decreased susceptibility to ceftriaxone was subsequently found in 2011 or in 2012. The upward trend in MIC values between 2008 and 2010 was not observed in the 2011 to 2012 period (Figure 5).

Finally, multidrug resistant strains were rare over the 2001 to 2011 period (5/3,953: 0.1%; 95% CI: 0–0.3) but their proportion increased significantly in 2012 (18/1,093: 1.6%; 95% CI: 1.0–2.6; p<0.001).

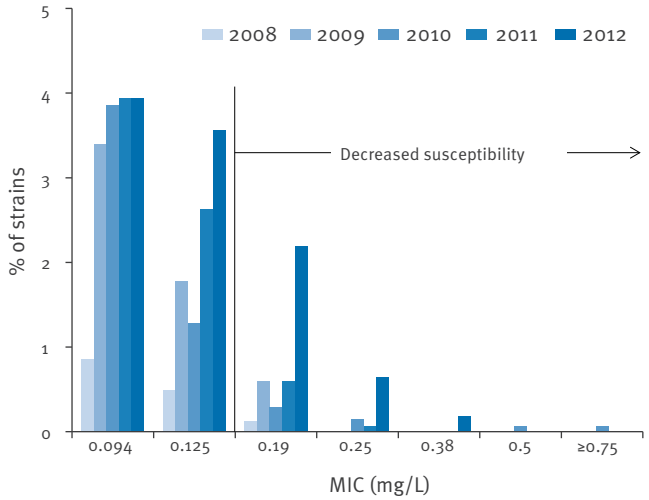
Restricted analysis of those laboratories which reported throughout the whole study period and continued to participate in microbiological surveillance during 2012 provided similar results in terms of the evolution of the susceptibility of gonococcal strains to antibiotics between 2001 and 2012 (data not shown).

Factors associated with a reduction in the susceptibility of gonococcal strains to third generation cephalosporins in patients 15 years and older

Univariate analysis was performed using the variables from Table 1. The proportion of strains with decreased susceptibility to cefixime (MIC≥0.094 mg/L) and to ceftriaxone (MIC≥0.032 mg/L) (Table 3) was significantly associated with age group, year of surveillance and

Figure 4

Evolution of the distribution of minimum inhibitory concentration values for cefixime, Rénago[a] network, 2008–2012

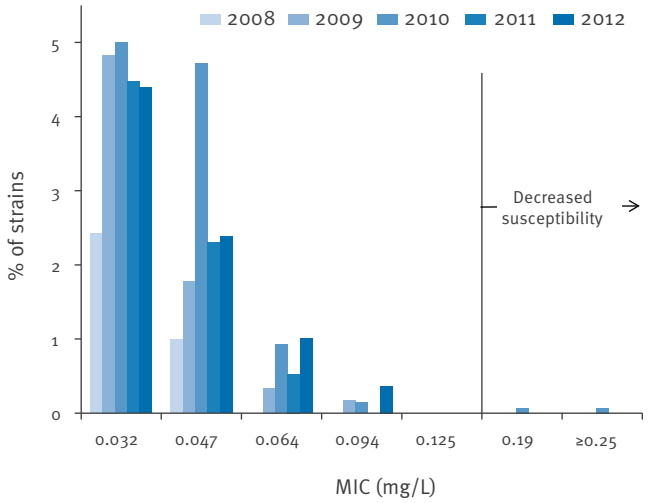


MIC: minimum inhibitory concentration.

[a] Rénago is the national gonorrhoea network, a sentinel network based on voluntarily participating public and private laboratories.

Figure 5

Evolution of the distribution of minimum inhibitory concentration values for ceftriaxone, Rénago[a] network, 2008–2012



MIC: minimum inhibitory concentration.

[a] Rénago is the national gonorrhoea network, a sentinel network based on voluntarily participating public and private laboratories.

Table 3

Factors associated with decreased susceptibility of gonococcal strains to third generation cephalosporins in patients 15 years-old and over, Rénago network[a], 2001–2012

Variables	Cefixime[b] MIC ≥0.094 mg/L			Ceftriaxone[c] MIC ≥0.032 mg/L		
	n/N (%)	ORa (95% CI)	p	n/N (%)	ORa (95% CI)	p
Years						
2001–2009	80/1,988 (4)	Ref.	–	201/4,622 (4)	Ref.	–
2010	79/1,394 (6)	**1.43 (1.03–1.99)**	**0.034**	152/1,395 (11)	**2.52 (2.00–3.18)**	**<0.001**
2011	110/1,520 (7)	**1.78 (1.31–2.43)**	**<0.001**	111/1,520 (7)	**1.54 (1.19–1.98)**	**0.001**
2012	115/1,090 (11)	**2.65 (1.94–3.62)**	**<0.001**	89/1,090 (8)	**1.66 (1.26–2.19)**	**<0.001**
Sex						
Men	332/5,217 (6)	Ref.	–	485/7,556 (6)	Ref.	–
Women	52/768 (7)	1.73 (0.69–4.35)	0.24	67/1,061 (6)	1.49 (0.66–3.35)	0.34
Age group in years						
15–24	98/2,048 (5)	Ref.	–	147/2,717 (5)	Ref.	–
25–34	140/2,113 (7)	**1.41 (1.08–1.86)**	**0.013**	203/3,103 (7)	**1.28 (1.02–1.60)**	**0.036**
35–44	79/1,048 (8)	**1.78 (1.30–2.45)**	**<0.001**	120/1,629 (7)	**1.55 (1.19–2.01**	**0.001**
≥45	60/659 (9)	**1.97 (1.39–2.78)**	**<0.001**	72/951 (8)	**1.49 (1.10–2.02)**	**0.010**
Anatomical site						
Urethra or urine	309/4,784 (6)	Ref.	–	435/6,763 (6)	Ref.	–
Cervix or vagina	45/690 (7)	0.56 (0.21–1.47)	0.24	58/949 (6)	0.69 (0.29–1.61)	0.39
Anus	19/392 (5)	0.88 (0.51–1.52)	0.65	43/729 (6)	1.19 (0.81–1.73)	0.37
Pharynx	7/35 (20)	**4.21 (1.62–10.91)**	**0.003**	7/52 (13)	**2.86 (1.17–6.99)**	**0.021**
Other sites	4/78 (5)	0.71 (0.22–2.28)	0.56	8/113 (7)	0.96 (0.41–2.26)	0.93
Place of consultation						
Private sector or clinic	286/3,994 (7)	Ref.	–	382/5,365 (7)	Ref.	–
Hospital	39/883 (4)	0.55 (0.30–1.00)	0.052	89/1,459 (6)	1.13 (0.71–1.79)	0.60
Specialised facilities for STIs[d]	51/983 (5)	0.77 (0.48–1.23)	0.28	68/1,276 (5)	0.86 (0.60–1.25)	0.44
Other structures	4/36 (11)	1.07 (0.32–3.58)	0.92	3/36 (8)	0.67 (0.16–2.85)	0.59
Prescribing doctor						
General practitioner	276/4,198 (7)	Ref.	–	379/5,634 (7)	Ref.	–
Gynaecologist	28/363 (8)	1.18 (0.68–2.07)	0.55	35/587 (6)	1.38 (0.90–2.11)	0.14
Dematologist-venereologist	20/358 (6)	1.43 (0.83–2.47)	0.20	37/760 (5)	0.98 (0.61–1.57)	0.93
Other medical specialisations	50/903 (6)	1.19 (0.76–1.84)	0.45	85/1,288 (7)	1.13 (0.79–1.61)	0.51
Type of laboratory						
Private	308/4,501 (7)	Ref.	–	428/6,349 (7)	Ref.	–
Hospital	63/1,251 (5)	1.19 (0.71–1.94)	0.52	107/1,966 (5)	0.72 (0.48–1.08)	0.12
Community clinic	13/240 (5)	1.24 (0.60–2.56)	0.56	18/312 (6)	0.98 (0.52–1.83)	0.94
Laboratory location (area)						
Paris region	96/2,073 (5)	Ref.	–	170/3,379 (5)	Ref.	–
North-west	86/1,324 (6)	1.36 (0.98–1.90)	0.07	111/1,747 (6)	1.27 (0.97–1.67)	0.09
North-east	107/1,095 (10)	**1.99 (1.45–2.73)**	**<0.001**	154/1,451 (11)	**2.23 (1.73–2.87)**	**<0.001**
South-east	54/881 (6)	1.06 (0.72–1.56)	0.76	70/1,226 (6)	1.08 (0.78–1.49)	0.65
South-west	41/619 (7)	1.23 (0.81–1.85)	0.33	48/824 (6)	1.11 (0.78–1.60)	0.56

MIC: minimum inhibitory concentration; ORa: odds ratio adjusted for the other variables in the table; 95% CI: 95% confidence interval; ref.: reference group; STIs: sexually transmitted infections.

For each variable, the number of strains with decreased susceptibility (n) is given relative to the total tested for the antibiotic (N) as well as the percentage. The multivariate analysis includes all the variables in the Table. Significant results (p<0.05) are in bold.

[a] Rénago is the national gonorrhoea network, a sentinel network based on voluntarily participating public and private laboratories.

[b] Cefixime tested from 2008 onwards; MIC≥0.094 mg/L for 385 strains of 6,004 (6.4%).

[c] Ceftriaxone: MIC≥0.032 mg/L for 554 strains of 8,645 (6.4%).

[d] Specialised facilities for the management of STI: STI clinics, free anonymous counselling and testing services and family planning centres.

geographical area. This proportion was also greater in the absence of gonorrhoea-associated STI, notably in the absence of co-infection with chlamydia. Furthermore the proportion of strains with decreased susceptibility to cefixime was associated with anatomical site of sample, presence of symptoms and place of consultation.

Multivariate analysis (Table 3) was performed using the variables from Table 1 excluding four variables with a large proportion of missing data (as mentioned in the methods section). The proportion of strains with decreased susceptibility to cefixime (MIC≥0.094 mg/L) increased in the 2010 to 2012 period. Indeed, this proportion was 2.7 times greater in 2012 than for the period between 2001 and 2009. This reduced susceptibility increased with the patients' age. It was almost four times higher for strains derived from the pharynx than for those derived from the urethra, even when analysis was restricted to male patients (adjusted odds ratio 4.2; 95% CI: 1.5–11.6; p=0.006). Finally, the decrease in susceptibility to cefixime was found to be higher in the north-eastern area of France.

The proportion of strains with decreased susceptibility to ceftriaxone increased in the 2010 to 2012 period, this increase being particularly sharp in 2010. Taking 2010 as the reference year, the proportion of strains with decreased susceptibility to ceftriaxone (MIC≥0.032 mg/L) significantly diminished in the 2011 to 2012 period. As was the case for cefixime, decreased susceptibility for ceftriaxone increased with patients' age, was higher for pharyngeal-based strains (even when restricting the analysis to male patients) (adjusted odds ratio: 3.4; 95% CI: 1.4–8.2; p=0.008) and was higher in the north-eastern area of France.

Multivariate analysis of the 2001 to 2012 period, restricted to the laboratories which continued microbiological surveillance in 2012, provided similar results (data not shown).

Discussion

The microbiological surveillance network in France shows that the proportion of resistant strains to antibiotics fluctuated around 13% for penicillin G over the whole study period. It increased throughout the study for tetracycline, reaching 56% in 2012. For ciprofloxacin it increased between 2003 and 2006 after which it remained high at around 42%. These three families of antibiotics have a resistance level which prevents them from being used in current practice for the treatment of gonorrhoea. Indeed, according to the World Health Organization, first-line treatment must cure at least 95% of patients and must not be used if more than 5% of strains are resistant [2].

Gonorrhoea was susceptible to spectinomycin throughout the study period. The marketing of this antibiotic in France stopped in 2008 and then started again since 2011. However, therapeutic failures for pharyngeal-based infections have led it to become a second-line treatment when there are contraindications to beta-lactam antibiotics [15].

The proportion of strains with decreased susceptibility to cefixime (MIC>0.125 mg/L) remained moderate, less than 5%, but it quadrupled between 2011 and 2012. Furthermore, the proportion of isolates with high MIC values (MIC≥0.094 mg/L) increased consistently in recent years (Figure 4 and Table 3). The only two strains presenting MICs>0.125 mg/L to ceftriaxone were both detected in France in 2010, and one of these was associated with therapeutic failure using cefixime in an infected patient [14]. With respect to ceftriaxone, the number or proportion of isolates with high MIC values (MIC≥0.032 mg/L) did not increase in 2011 or 2012. The very rare cases of decreased susceptibility to ceftriaxone [14,16-19] and cases of therapeutic failure with cefixime described in Japan, Canada and Europe [14,20-23], confirm the recommendations made by the French Agency for Medicine published in 2005 [12], which were reaffirmed in 2008 [15]. These guidelines recommend the use of ceftriaxone (500 mg single-dose injection) in first-line treatment, and that the use of cefixime (400 mg single-dose oral) be reserved for cases of patient refusal or when parenteral treatment is not possible.

Nevertheless, the increase in gonococcal decreased susceptibility to cefixime observed in France suggests that clinicians may have continued to prescribe this antibiotic as a first-line treatment after the recommendations of 2005 were published (and reaffirmed in 2008). Indeed, a study performed in 2008 on general practitioners in the 'Sentinelles' network showed that only a minority of prescribing doctors were aware of the 2005 recommendation to abandon ciprofloxacin in favour of TGC [24]. By analogy, it is possible that in 2012 doctors had not yet sufficiently implemented the recommendation reiterated in 2008 regarding the use of TGCs [15]. A new study on doctors' prescriptions could help support this hypothesis. Another hypothesis to explain the recent increase in gonococcal resistance to cefixime is the spread of a resistant clone [25].

The levels of resistance to penicillin G, tetracycline and ciprofloxacin in France were higher than those found in the United States, but of the same order for TGC and multidrug resistance [26]. In contrast, the levels of resistance in France were of the same order as European levels for penicillin G and ciprofloxacin, but lower for TGC [27].

We currently do not have data on the susceptibility of gonorrhoea to azithromycin. In France this antibiotic is taken in a single, 1 g dose with ceftriaxone in suspected cases of urethritis and cervicitis [12,15]. Elsewhere, notably in the United States, azithromycin is recommended in monotherapy for gonococcal infections, in the case of allergies to TGC [28]. Because of resistance to azithromycin described in various countries [26,29]

this antibiotic is being tested within the Rénago network as of 2013.

Our study shows factors associated with a reduction in the susceptibility to TGC. We acknowledge that the combination of data from 2001 to 2009 only allowed to highlight the recent epidemiological and microbiological changes. The proportion of strains with decreased susceptibility to TGC increased with patients' age. This has already been observed for other antibiotics (penicillin and tetracycline) in Canada [29]. A lower proportion of gonococcal resistant strains in the case of co-infection with chlamydia was found for cefixime in the United Kingdom [30] and for ciprofloxacin at the European level [27], although the reason for this is not clearly established.

The proportion of strains with decreased susceptibility to TGC did not differ significantly according to sex. Anal-based strains did not present higher decreases in susceptibility than urethral-based strains. However, the proportion of pharyngeal-based strains with decreased susceptibility to TGC was three to four times higher than urethral-based strains. This may be explained by the horizontal transfer of gene mutations associated with decreased susceptibility to TGC from oral commensal Neisseria [31]. Anal and pharyngeal gonorrhoea in men was probably acquired through homosexual transmission, although the absence of information on sexual behaviours from the Rénago network prevents us from being certain about this. Several studies have shown that MSM are likely to be infected more frequently and more quickly than heterosexuals by resistant gonococcal strains [26,30,32]. This fact highlights the need for specific surveillance of antibiotic susceptibility in MSM. We found that the monitoring of anal strains brought information on resistance similar to those of urethral strains. Despite the absence of behavioural data, our study shows, however, that increased monitoring of pharyngeal-based strains with respect to decreased susceptibility to TGC seems essential.

Finally, we observed a higher level of decreased susceptibility in the north-eastern area of France. It is hard to interpret the reason for this. Obviously this area should remain under close scrutiny, while still keeping the national distribution of the network's laboratories homogeneous.

In the Rénago network, an increase in the number of declared gonococcal infections occurred over the 2001 to 2012 period. Several interlinked phenomena contributed to this: the real growth in infections, the increase in NAAT diagnoses, the increase in the number of participating laboratories and the growing shift towards the consolidation of laboratories. Despite this complex evolution, the Rénago network continued to help monitor gonococcal resistance to antibiotics. This microbiological surveillance network would appear to be reliable as the changes that have been made to it have (the reduction by half of the number of laboratories submitting strains to the NRL), a priori, had little or no effect on the proportion of resistant strains observed. Restricted analysis to those laboratories which continued to participate in microbiological surveillance in 2012 provides strong evidence for this.

The surveillance of gonococcal resistance by the Rénago network brings with it several limitations. Overseas French regions are not yet included in surveillance. Furthermore, the laboratories participating in the network do not provide a representative sample of metropolitan France. Extrapolation of the results therefore requires supplementary investigations. An assessment of the completeness of this laboratory surveillance system is ongoing but results are not yet available. Finally, this network does not collect behavioural data as microbiologists do not often have access to such information. This explains the large amount of missing values for certain variables. One way for us to have behavioural data, in particular sexual orientation, is to monitor the susceptibility of gonococcal strains in patients diagnosed through our second voluntary network, the RésIST network of clinicians.

The emergence of resistance to TGC is extremely worrying as the latter represent the last line of treatment, with no therapeutic alternatives currently available [31]. This fact justifies the continuation of microbiological surveillance of gonorrhoea at a collective level in order to adjust therapeutic recommendations. Meanwhile, surveillance of therapeutic failure with respect to TGC is currently being implemented at the European level [33]. Given the increase in NAAT diagnosis, it is essential that medical laboratories continue to perform gonococcal cultures which adapt care to the individual's needs while waiting for molecular tests on susceptibility to antibiotics to be developed and made accessible [2].

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

Conflict of interest

None declared.

Authors' contributions

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Alveolar echinococcosis in a highly endemic area of northern Slovakia between 2000 and 2013

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Long-term surveillance of *Echinococcus multilocularis* occurrence in red foxes in Slovakia revealed the existence of highly endemic areas, with an overall prevalence rate of 41.6 % in the northern part of the country. Between 2000 and 2013, 26 human cases of alveolar echinococcosis were detected and only three of them were not in endemic localities in northern Slovakia. Remarkable is the occurrence of the disease in eight people younger than 35 years, including three patients aged eight, 14 and 19 years. Occurrence of *E. multilocularis* in red foxes throughout the country and high incidence of alveolar echinococcosis in young people indicate high infectious pressure in the environment of northern Slovakia. It can be assumed that the real incidence of alveolar echinococcosis is significantly higher than recorded by official data due to the lack of existing registration and reporting system. For effective management of prevention and control strategies for this disease improvement of the national surveillance system and engagement of specialists outside the medical community are necessary. Our study presents a comprehensive picture of the epidemiological situation of *E. multilocularis* in northern Slovakia. In addition, we report the first list of confirmed human cases of this serious parasitosis in Slovakia.

Introduction

Echinococcus multilocularis, causative agent of alveolar echinococcosis in humans, has become the target of intensive research at the beginning of the 1980s [1]. In the past decades, the known range of the parasite in Europe has expanded. The formerly known geographical range of the parasite included regions of (by date of detection) Austria, Switzerland, France, Germany, Liechtenstein, Luxembourg, Denmark, the Baltic states, Belgium, the Netherlands, northern Italy, Poland, the Czech Republic, Slovakia, northern Hungary and Slovenia [2,3]. Recently, the occurrence of *E. multilocularis* was for the first time confirmed also in Sweden [4,5], and in southern Denmark, a new high-endemic focus was detected [6]. The importance of the issue is highlighted by a recent regulation issued by the European Commission in 2011 [7]. The aim of the measures outlined in the regulation is to minimise the risk of introducing the parasite into territories where it has not yet been detected, namely Finland, the United Kingdom, Ireland and Malta [7].

In Slovakia, *E. multilocularis* was detected for the first time in red foxes in 1999 [8] and intensive epidemiological studies have been carried out since. During the surveillance of *E. multilocularis* between 2000 and 2010, more than 4,700 red foxes (Vulpes vulpes) from all regions of the country have been examined. The total average prevalence of the parasite was 30.3% and the existence of highly endemic areas in northern regions of Slovakia was revealed [9].

The survey presents a comprehensive picture of the epidemiological situation of *E. multilocularis* in highly endemic regions of northern Slovakia from its first detection in 1999 until 2012 and includes results from the territory of the High Tatras National Park. Moreover, we report here the first list of confirmed human cases of this serious parasitic disease.

Methods

Collection and examination of red foxes

Monitoring of *E. multilocularis* in red foxes living in northern Slovakia (Žilina and Prešov Region including the High Tatras National Park) was conducted in the years 2000 to 2005, 2007, and 2010 to 2012. Red foxes were collected in the framework of the control of the effectiveness of anti-rabies vaccination carried out twice a year, in spring/summer and autumn/winter campaigns. The number of samples and the time schedule of campaigns were set by the State Veterinary and Food Administration of the Slovak Republic. From each of 79 Slovak districts, a representative sample of at least six foxes per campaign was shot. Animals originating from the protected area of the High Tatras

National Park were found dead, road-killed or legally hunted by workers of the Tatra National Park Research Station. Small intestines of the animals were deep-frozen at -80°C prior to necropsy and parasitological examination. Sedimentation and counting method [10] with modification of using 0.09 mm mesh analytic sieves for washed fraction filtration [9] was applied for *E. multilocularis* detection.

Climatic data collection

Influence of climatic factors (mean annual air temperature and mean annual precipitation) on the prevalence rate of *E. multilocularis* in red foxes in individual years was assessed. The data were obtained from datasets of Statistical Office of the Slovak Republic [11]. Data measured at three weather observatories in northern Slovakia (Oravská Lesná – Žilina Region, Poprad and Stropkov – Prešov Region) were used for this purpose.

Collection and examination of dog samples for *Echinococcus multilocularis*

To determine the role of dogs in the spread of the disease in highly endemic areas, 138 animals originating from the northern areas of Slovakia (Prešov and Žilina Region) were collected between 2002 and 2005. Faecal samples came from stray and owned dogs that were not dewormed regularly with the last deworming treatment at least four months before examination. In order to reduce the risk of infection, all samples were deep-frozen at -80 °C for at least seven days prior to examination.

Data concerning the age, sex, locality and usage of dogs were gathered with the help of a questionnaire. Other questions were related to how often the dogs could move freely in rural areas, were fed with raw viscera or caught rodents. Animals aged up to 10 months were classified as young, older animals were identified as adults.

Nested PCR was used to detect the presence of *E. multilocularis* DNA in faecal samples [12].

Statistical analyses

The prevalence of *E. multilocularis* in the examined dog and red fox population was calculated. The seroprevalence values were given with 95% confidence intervals (CI), and odds ratios (OR) were calculated to estimate the association between variables included in the study (age, sex, utilisation, etc.) and the risk of echinococcosis. All statistical analyses were conducted using STATISTICA 6 Base (StatSoft, Inc, 2001).

Sampling and diagnostics of alveolar echinococcosis in humans

Human samples and data about the patients were collected in cooperation with medical doctors and hospitals, especially with infectious disease and surgical clinics from different regions in Slovakia. Sera from patients suspected for echinococcosis, e.g. patients with clinical symptoms of echinococcosis or patients with cystic lesions found during imaging examination (ultrasonography (USG), radiography, computed tomography (CT)) were sent to the Institute of Parasitology SAS (IP SAS) for serological examination. At the beginning, IP SAS was the only laboratory where antibodies to Echinococcus could be examined. After a few years, private laboratories started with serological diagnostics and it could not be estimated how many samples were sent elsewhere. Currently, IP SAS is the only laboratory performing Western blot and PCR tests. Specific antibodies to *E. multilocularis* were detected by modified ELISA [13,14] or commercial ELISA test kits (NovaLisa Echinococcus IgG, NovaTec Immunodiagnostica, Germany), and positive sera of patients who had not undergone surgery, i.e. for whom PCR samples or histology were not available, were examined also by Western blot (Echinococcus WB IgG, LDBIO Diagnostics, France). Imaging techniques (USG, CT, magnetic resonance imaging (MRI)) were performed to detect the presence of parasitic lesions in the liver and other organs, and material obtained during surgery was submitted to histological and/or PCR examination [15]. Some clinical cases were detected accidentally, during surgical intervention for another disease.

The diagnosis was confirmed if at least two of following four parameters were verified [16]: (i) typical organ lesions detected by imaging techniques, (ii) presence of antibodies to *Echinococcus multilocularis* (ELISA, Western Blot), (iii) histological findings compatible with *E. multilocularis* metacestode, or (iv) detection of *E. multilocularis* DNA by PCR methods.

Results

Surveillance of *Echinococcus multilocularis* in red foxes in northern Slovakia during 2000 to 2012

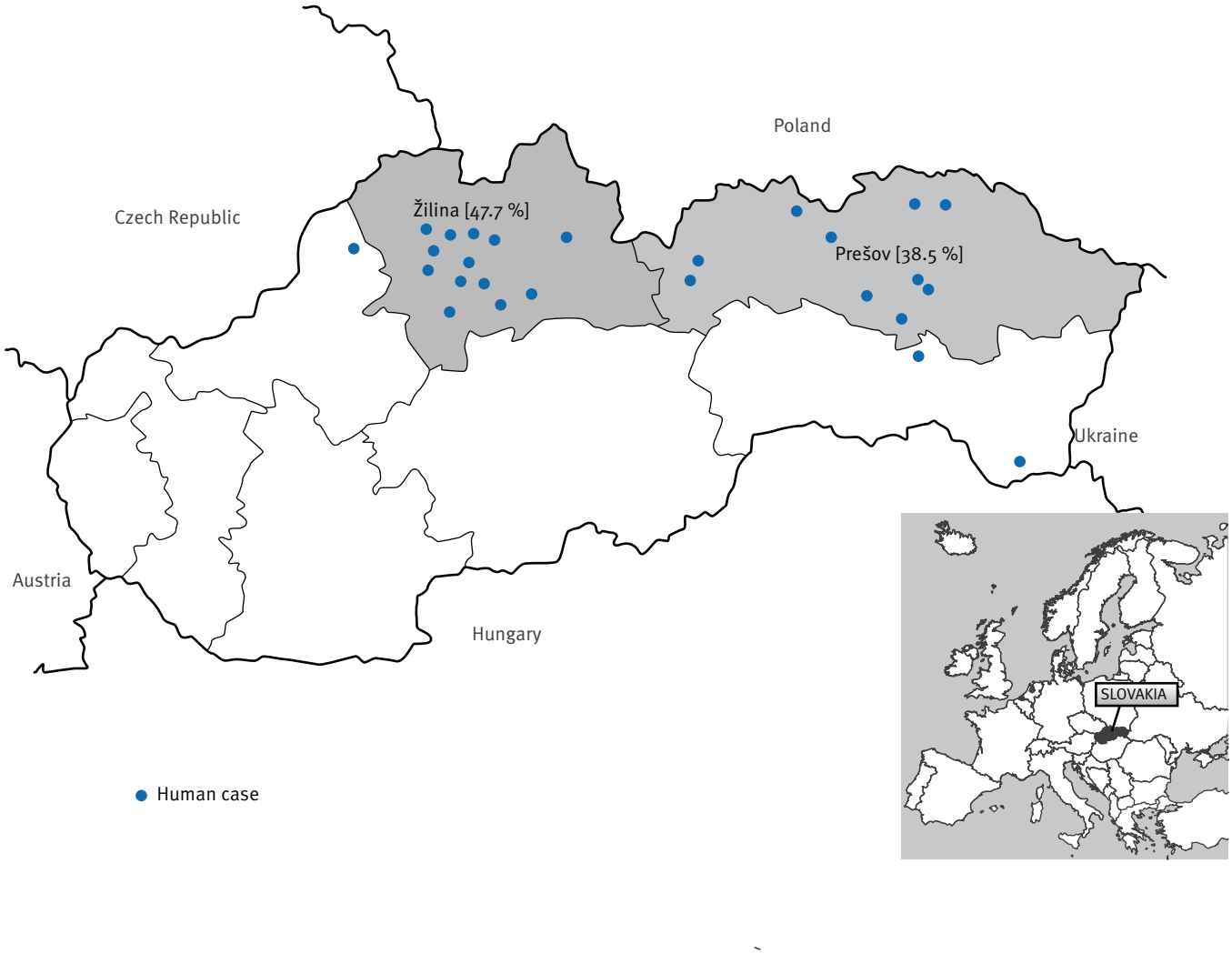
Since 2000, a total of 1,875 red foxes originating from the northern Slovak regions have been examined for *E. multilocularis* presence. The tapeworm was detected in the small intestines of 779 foxes (41.6 %), with a mean prevalence rate in the Žilina and Prešov Region of 47.7% and 38.5%, respectively (Figure 1).

The annual prevalence varied between 26.0% in 2007 and more than 50.0% in 2001, 2005 and 2010. The mean worm burden reached 2.352 tapeworms per infected fox (Table 1).

Considerable correlation was observed between prevalence of *E. multilocularis* in red foxes and the mean annual air temperature, as well as between prevalence and mean annual precipitation. The prevalence reached the highest values (50.8–58.4%) during the cold and wet years of 2001, 2005 and 2010 (Figure 2). In contrast, prevalence significantly decreased (to 30.2%, 26.0% and 27.3%, respectively) in the much drier years 2003, 2007 and 2012 (Table 1) [17].

Figure 1

Occurrence of alveolar echinococcosis in humans (n=26) and mean prevalence of *Echinococcus multilocularis* in red foxes in the Žilina and Prešov Regions, Slovakia 2000–2012



Locations of human cases are indicated as dots. *Echinococcus multilocularis* prevalence in foxes is indicated in square brackets.
AT: Austria; CZ: Czech Republic; HU: Hungary; PL: Poland; UA: Ukraine.

Echinococcus multilocularis in dogs

Of 138 dog faeces examined, *E. multilocularis* DNA was confirmed in four (2.9 %) samples. The majority, 119 samples, came from the Prešov Region with three infected animals (2.5 %). Among the 19 animals from the Žilina Region, one sample (5.2 %) was found to be positive.

The positive animals were used as guard and shepherd dogs, but neither usage of the dogs nor their age or sex influenced the occurrence of parasites significantly (p>0.05).

Data about free movement in rural areas, feeding and catching rodents were known for 82 cases. Positivity for *E. multilocularis* was closely correlated with the possibility to catch the rodents (p=0.04; OR=9.4; 95% CI: 0.9–95.9) and uncontrolled movement in rural areas (p=0.02; OR=14.5; 95% CI: 1.4–145.5). Feeding with raw viscera was not identified as a risk factor for infection (Table 2).

Human cases of alveolar echinococcosis in Slovakia

The first case of alveolar echinococcosis in Slovakia was confirmed in 2000 in a woman in her 60s from a village in the Žilina Region (north-western Slovakia).

Table 1

Occurrence of *Echinococcus multilocularis* in red foxes, northern Slovakia, 2000–2012

Year	Mean annual air temperature[a] (°C)	Mean annual precipitations[a] (mm)	Number of foxes examined	Number of foxes infected	Prevalence % (range)	Mean worm burden Number per fox (range)
2000	7.7	902.1	175	48	27.4 (19.0 –36.3)	NA
2001	6.7	962.8	185	108	58.4 (48.8–67.5)	1,426 (1–25.000)
2002	7.4	865.7	175	90	51.4 (42.2–60.6)	1,930 (2–40.000)
2003	6.8	629.6	301	91	30.2 (25.6–35.3)	495 (1–10.500)
2004	6.5	895.7	247	108	43.7 (43.7–39.0)	1,497 (1–16.000)
2005	6.0	949.2	333	169	50.8 (45.2–56.3)	5,013 (2–59.900)
2007	6.8	701.0	158	41	25.9 (18.9–34.1)	11,027 (5–245.000)
2010	6.5	1,155.2	122	68	55.7 (48.1–63.1)	1,093 (1–19.900)
2011	NA	NA	58	23	39.7 (29.6–50.3)	1,096 (2–37.920)
2012	NA	NA	121	33	27.3 (20.1–35.0)	3,232 (3–33.120)
Total	-	-	**1,875**	**779**	**41.6**	**2,352 (1–245.000)**

NA: data become available in full-scale form with a delay of two years.

[a] Measured at three weather observatories in northern Slovakia (Oravská Lesná, Poprad, Stropkov).

Although the first clinical symptoms of the disease had appeared already in 1994, the lack of knowledge about the presence of *E. multilocularis* on the territory of Slovakia probably resulted in misdiagnosis. In the patient's anamnesis, a history of keeping a dog and collecting forest fruits were of epidemiological importance [18].

In the same year, a second case of autochthonous alveolar echinococcosis was detected. The patient was a woman in her 70s, from a village in mountain area of north-western Slovakia (Žilina Region), who spent a lot of time at her mountain cottage where she grew vegetables. Except weight loss, the patient had no clinical signs, and the disease was noticed only after observing the high erythrocyte sedimentation rate during a routine medical examination. Subsequent USG and CT examination of the abdomen revealed an irregularly shaped focus 12x11x10 cm in the right liver lobe. Serological (ELISA, Western blot) and histological examinations confirmed the diagnosis of alveolar echinococcosis [19].

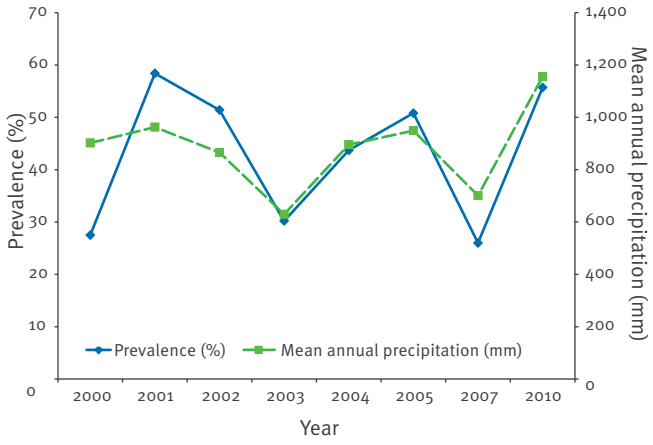
Three years later, in 2004, two further cases were verified. The patients were men from the Prešov and Žilina Region. Since then, new cases of alveolar echinococcosis have been detected every year (Figure 3). The incidence of the disease has followed an increasing trend. As of April 2013, the total number of alveolar echinococcosis in Slovakia has reached 26 cases (Figure 1). Only three of these patients did not come from the highly endemic Prešov or Žilina Regions. One woman came from the Trenčín Region, situated in the west of Slovakia and neighbouring the Žilina Region, and two persons were from the Košice Region neighbouring the Prešov Region (Figure 1). The mean age of the patients was 52.3±20.7 years, ranging between eight and 78 years. Only negligible differences were recorded between the positivity of women (14 cases) and men (12 cases).

In addition to the 26 confirmed cases of alveolar echinococcosis, at least 10 other cases were detected between 2007 and 2012 in whom the aetiological agent of echinococcosis was not determined. In these patients, the diagnosis of echinococcosis was made after serological and USG examination. However, material for histological or PCR examination that could facilitate the confirmation of diagnosis could not be obtained because of the good response to anthelmintic treatment that made surgical intervention unnecessary, or because the patients neglected to appear for their control examination.

Three of the 26 confirmed patients were younger than 20, one of them even younger than 10 years (Table 3). In 2006, alveolar echinococcosis was diagnosed in a teenager from the Prešov Region, living in close vicinity of

Figure 2

Positive correlation between *Echinococcus multilocularis* prevalence in red foxes and mean annual precipitation[a], northern Slovakia, 2000–2010



[a] Measured at three weather observatories (Oravská Lesná, Poprad, Stropkov).

a forest. The patient suffered from headache, fatigue, cough, fever and abdominal enlargement. USG examination confirmed tumour-like infiltrations in the right and left liver lobes and the diagnosis of echinococcosis was confirmed by ELISA, Western blot and histological examination [20]. In 2012, two more cases of alveolar echinococcosis in young patients were detected. One was a teenager from the Žilina Region with multiple cysts in the liver and four cysts in the lungs, found by USG examination; the diagnosis was confirmed by PCR after the resection of suspicious parasitic material from the liver. The other case was younger and came from the Prešov Region, with a primary infection site in the right femur; no other lesions were confirmed using USG and PET-CT examinations. This case was living near the forest at the end of a village, in close vicinity to an area often visited by red foxes. The family also owned a dog. Although the first symptoms, pain in the right leg and knee, were not indicative of alveolar echinococcosis, serological examination confirmed the presence of antibodies to Echinococcus spp. Following histological investigation of material obtained during surgical intervention, Western blot of the serum sample and PCR analyses of DNA from the cyst secretion confirmed the diagnosis of alveolar echinococcosis.

Discussion and conclusion

Our monitoring confirms the presence of highly endemic areas of *E. multilocularis* in the northern part of Slovakia, with a mean prevalence rate of almost 42.0% in its main definitive hosts, red foxes. This 12-year examination of red foxes has unfolded great fluctuation of *E. multilocularis* prevalence between years, probably depending on climatic conditions. Prevalence rates were highest during the cold and wet years of 2001, 2005 and 2010. In those years precipitation totals were considerably above normal. In contrast, prevalence significantly decreased in the warmer years of 2003, 2007 and 2012, which were the first, third and fourth warmest years since 1871 in Slovakia [17].

Given that *E. multilocularis* is considered a parasite adapted to cold climate, environmental conditions of northern Slovakia are very suitable for the spread of echinococcosis. The area is characterised by mountain climate with average annual temperatures between -3 and +4 °C. Annually, rainfall varies between 800 and 2000 mm. Main recreational areas of Slovakia are situated in northern Slovakia, which is of relevance from a public health point of view. Žilina Region is dominated by national parks, and 51.0% of its territory is under some form of protection, accounting for 30.0% of Slovakia's total protected area. In the territory of the Prešov Region are situated the only truly alpine mountains in Eastern Europe, the Tatra National Park [11]. In the past decades, touristic activities have increased, not only in the National Parks but all over the country. The estimated number of tourists visiting Tatra National Park exceeds 3,500,000 per year [21]. In addition, the risk of human infection is also influenced by high population density and urbanisation of red foxes (and other free-living carnivores) in these areas.

Due to close contact with humans and contamination of soil around houses and in gardens, dogs present a considerable risk factor in the spread of echinococcosis to humans. Stehr-Green et al. and Kern et al. identified ownership of a dog as associated with acquisition

Table 2

Impact of usage, feeding and catching rodents on the occurrence of *Echinococcus multilocularis* in dogs, 2000–2005

Variable	Positive/examined (%)	95% CI
Usage		
Hunting dogs	0 / 47 (0.0)	−2.8 to 13.5
Guard dogs	2 / 35 (5.7)	1.1–17.4
Shepherd dogs	2 / 26 (7.7)	1.4–24.6
Pet dogs	0 / 11 (0.0)	0.0–26.4
Unknown	0 / 19 (0.0)	0.0–17.6
Fed with raw viscera		
Yes	3 / 26 (11.5)	3.2–30.2
No	1 / 41 (2.4)	−0.5 to 14.5
I don't know	0 / 15 (0.0)	0.0–22.2
Catching rodents		
Yes	3 / 17 (17.6)	4.9–41.7
No	1 / 54 (1.9)	0.2–7.4
I don't know	1 / 11 (0.1)	0.5–40.4

CI: confidence interval.

Table 3

Age of first diagnosis of alveolar echinococcosis, Slovakia, 2000–2012 (n=26)

Age category	Number of cases	Incidence/100,000 inhabitants[a]
<10 years	1	0.18
10–19 years	2	0.31
20–29 years	1	0.12
30–39 years	4	0.45
40–49 years	0	0.00
50–59 years	5	0.65
60–69 years	7	1.40
>70 years	6	1.33

[a] According to number of inhabitants in 2010 [36].

of the disease [22,23]. Some of the dogs (2.9%) in our study were infected with *E. multilocularis* and could be a source of human infection. Catching rodents and unattended movement in rural areas were correlated with infection of dogs. Of course, a larger number of samples and further epidemiological analyses would be more valuable, but due to limited staff we did not continue the examination of dogs after 2005. Budke et al. and Ziadinov et al. also recognised a higher probability of infection in dogs that hunt rodents, in hunting dogs and in non-restrained dogs [24,25]. Although *E. multilocularis* is not common in dogs in Slovakia, contact with animals with risk behaviour increases the probability of infection, especially in highly endemic regions of the country.

The list of diagnosed human cases of alveolar echinococcosis confirms northern Slovakia as a risk area. Of 26 infected humans, only three lived outside the Žilina or Prešov Region. However, the prevalence of *E. multilocularis* in red foxes also reaches 18.4 % in the Košice Region and as much as 40.3 % in the Trenčín Region [9]. Thus, the risk of infection in areas in regions other than Žilina and Prešov is not negligible and a rise in the number of new cases is possible in the future. Moreover, as northern Slovakia is a popular tourist destination, we cannot exclude the infection of the three patients in highly endemic localities.

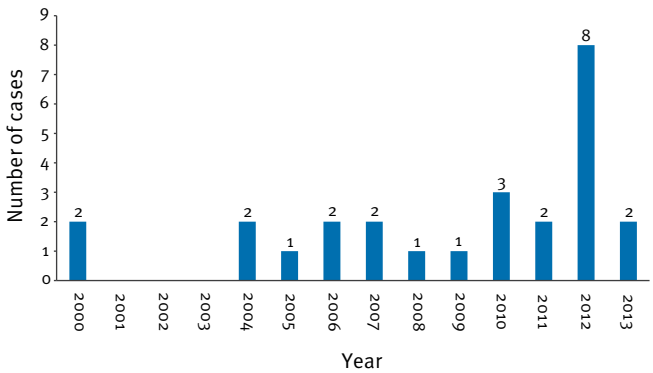
Humans are considered to be unsuitable hosts for *E. multilocularis* [26]. Therefore, it is assumed that repeated or long-term exposure to contaminated environment is needed to establish the infection [27]. In addition, the long incubation period (five to 15 years) contributes to the fact that alveolar echinococcosis is mainly diagnosed in older patients. The mean age of patients with alveolar echinococcosis in Slovakia was 52.3 years, but remarkable is the occurrence of alveolar echinococcosis in three persons younger than 20 years. Two of them came from the Prešov Region and one lived in the Žilina Region. All three patients had severe organ damage indicating their exposure to *E. multilocularis* in early childhood. The mean age of patients reported to the European Echinococcosis Registry between 1982 and 2000 was 52.5 years, but only 2.1% of 559 persons were younger than 20 years [27]. Similarly, 2.1% and 5.2% of patients with affirmed alveolar echinococcosis in the highly endemic Ningxia Hui Region of China were younger than 20 years and 30 years, respectively [28]. On the other hand, relatively high occurrence of the disease in children was recorded in Poland, a country neighbouring Slovakia, where among 121 human cases, four (3.3%) were between the ages of six and 11 years, and the mean age of patients with alveolar echinococcosis was only 47.6 years [29]. In Poland, *E. multilocularis* in red foxes was detected for the first time in 1995 [30] and one of the endemic localities, the Subcarpathian Province, is situated in the south of the country and borders with the highly endemic Žilina and Prešov Regions of Slovakia. Although the reason for increased incidence of alveolar echinococcosis in young people recorded in this study is unknown, we speculated that the environment of northern Slovakia is highly contaminated with infective stages of *E. multilocularis*.

The short interval between the first detection of *E. multilocularis* in foxes and the first human case (with severe organ damage) suggests that the parasite was present in Slovakia before 1999. Since then, increased awareness of public health authorities and physicians the possibility of serological examination of serum samples and new diagnostic methods, together with a constant high prevalence of the parasite in red foxes and increased density of the fox population, has contributed to an increase in the number of human cases detected, especially since 2010.

We can assume that the real number of human alveolar echinococcosis may be several time higher than that shown by official data. A similar situation was recorded

Figure 3

Confirmed human cases of alveolar echinococcosis, Slovakia, 2000–2013 (n=26)



also in other countries; Jorgensen et al., for example, estimate that the incidence of the infection in Germany is three times higher than national surveillance data [31]. In Slovakia, confirmed cases of echinococcosis are reportable to the Regional Authorities of Public Health (RAPH) operated under Ministry of Health, and thereafter, the data are sent to the Epidemiological Information System (EPIS) or reported directly to the EPIS. Such a reporting system has some limitations since reports to EPIS come only from physicians and pathologists registered in the system. On the other hand, reports to the RAPH come from microbiological or parasitological laboratories. Due to the fact that each party relies on the other to report diagnosed cases, many cases can be neglected and the incidence can thus be seriously underestimated. Moreover, in many cases the diagnosis is established only as echinococcosis, and the aetiological agent of the disease, *E. multilocularis* or *E. granulosus*, is not determined. Similarly, the European Food Safety Authority (EFSA) supposes that current data about the occurrence of human echinococcosis in Member States of the European Union do not provide an accurate picture of the epidemiological situation. In 2010, approximately 22% of human cases remained undetermined [32]. Distinction between infections with *E. granulosus* and *E. multilocularis* would be beneficial because the two diseases require different management of prevention and treatment [32].

Despite the fact that alveolar echinococcosis was discovered in Slovakia already 13 years ago and despite (or maybe because of) the popularisation of this medical problem, public knowledge on its epidemiology is still greatly misleading. In Slovakia, as in other European countries, biased information about eating berries and mushrooms as the most important infection risk factor is often promoted in media, while little consideration is given to ownership of dogs and contact with wild carnivores. Nevertheless, several case–control studies aimed at investigating the epidemiology of alveolar echinococcosis have affirmed ownership of dogs and contact with carnivores as the most important risk factors [22,23,33]. Therefore, regular deworming treatment of dogs could be an effective preventive measure. In contrast, two studies from 2004 and 2005 did not find an association of alveolar echinococcosis with picking and eating berries and mushrooms from fields and forests [23,34].

The above facts draw attention to the need to revise the existing reporting system, improve the national surveillance system and engage specialists outside the medical community (public health professionals, parasitologists, veterinarians, zoologists and ecologists) for effective management of prevention and control strategies. Knowledge on how to minimise the risk of *E. multilocularis* transmission is not yet established in new endemic areas. Eradication of the parasite from the environment by means of long-term baiting campaigns in the fox population appears to be an effective tool, but has never been completely successful [35,36]. The baiting programmes depend strongly on several factors, mainly on available financial resources. Therefore the prevention of human alveolar echinococcosis should be based on integrated control measures such as increasing public awareness of hygienic measures, regular anthelmintic treatment of domestic carnivores or vole habitat management [36].

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Conflict of interest

None declared.

Authors' contributions

Daniela Antolová and Martina Miterpáková provided investigation of red foxes and dogs for *E. multilocularis* presence using sedimentation and counting technique, ELISA and PCR methods; and human samples for alveolar echinococcosis by serological and molecular techniques. They were responsible for design and writing of the manuscript. Prof. Radoňák, prof. Szilágyiová, dr. Hudačková and dr. Žáček coordinated human sampling and data collection.

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Incidence and hospitalisation rates of Lyme borreliosis, France, 2004 to 2012

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Lyme borreliosis (LB) has become a major concern recently, as trends in several epidemiological studies indicate that there has been an increase in this disease in Europe and America over the last decade. This work provides estimates of LB incidence and hospitalisation rates in France. LB data was obtained from the *Sentinelles* general practitioner surveillance network (2009–2012) and from the Programme de Médicalisation des Systèmes d'Information (PMSI) data processing centre for hospital discharges (2004–09). The yearly LB incidence rate averaged 42 per 100,000 inhabitants (95% confidence interval (CI): 37–48), ranging from 0 to 184 per 100,000 depending on the region. The annual hospitalisation rate due to LB averaged 1.55 per 100,000 inhabitants (95% CI: 1.42–1.70). Both rates peaked during the summer and fall and had a bimodal age distribution (5–10 years and 50–70 years). Healthcare providers should continue to invest attention to prompt recognition and early therapy for LB, whereas public health strategies should keep promoting use of repellent, daily checks for ticks and their prompt removal.

Introduction

Lyme borreliosis (LB) is the most common vector-borne disease in the northern hemisphere [1-3]. The economic and social costs of managing the disease represent an important burden on both health services and society [4,5]. Several epidemiological studies describe a 2 to 3.6-fold increase in the incidence of this disease over the last decade, in Europe as well as in the United States (US) [6-9].

The American surveillance system, based on notifications of observed cases, has been active since 1991. In Europe, most countries do not have national monitoring data at their disposal. The Czech Republic and Slovenia are among the few exceptions [1]. In France, the only existing nationwide study is a prospective study conducted by the *Sentinelles* network with general practitioners (GPs) from 1999 to 2000. It estimated the incidence of the disease at 9.4 per 100,000, with important inter-regional variations [10]. Several regional studies were also conducted, some on high-risk populations (forest workers), others on the general population in high-risk regions. In eastern France, disease incidence was estimated at 200 per 100,000 inhabitants, and at over 500 per 100,000 in certain areas [11,12].

Estimates of the epidemiological characteristics of LB are useful to orient control and prevention measures, as well as to assess their effectiveness. These data are also necessary to elaborate factual risk communication messages provided to the lay public or the media. Since 1984, the *Sentinelles* network collects, processes, forecasts and dispatches epidemiological data on the activity of GPs in France [13], in real time. In 2009, LB was added to the list of the monitored health indicators.

We analysed the data collected by the *Sentinelles* network over the first four years of LB surveillance in France (2009–2012). We also analysed the national hospitalisation databases from 2004 to 2009 (the latest available data at the time of writing this paper).

Methods

Incidence and characteristics of Lyme borreliosis cases (2009–2012)

The *Sentinelles* network GPs notify weekly the cases of LB they identify during consultations to the network's electronic information system. Notifications are made online throughout the year, in a standardised way. *Sentinelles* GPs make up a representative

sample of the national GPs, both in terms of practice area and age distribution of their patients [14]. To be reported by GPs, cases had to meet the following definitions as stated in the European Concerted Action on Lyme Borreliosis criteria: (i) presence of an erythema migrans (clinical diagnosis); or (ii) appearance of neurological, articular (arthritis only), cutaneous or cardiac symptoms evocative of Lyme disease, in a patient with positive serology [15].

For each notified case, a standardised questionnaire provided information on the patient's age and sex, the date of diagnosis, a history of tick bite before the consultation (date of bite), presence of asthenia, myalgia, cutaneous manifestations (erythema migrans with a single or numerous lesion(s), lymphocytoma, acrodermatitis (ACA)), neurological manifestations (meningoradiculitis, clinical signs of meningitis, meningoencephalitis, radiculitis, facial paralysis, events related to another cranial nerve), the presence of arthritis (articulation(s) concerned) or cardiac events (atrioventricular block, pericarditis, myocarditis, other) and, when available, the results of cerebrospinal fluid (CSF) analysis and serological tests. Three of the authors (AV, CA, TH) validated the reported cases when they met the adopted case definition. Validation of cases is an ongoing regular activity of the surveillance procedure and involves checking the consistency of data reported in the standardised questionnaire for each reported cases. When needed, GPs are contacted for more information. Cases of meningoradiculitis or unilateral facial paralysis were validated even if CSF fluid analysis had not been done, when they were clinically very suggestive (consensus agreement between three authors, AV, CA and TH) in patients who reported a history of erythema migrans less than two months before the onset of neurological manifestations [16].

Cases reported from 1 January 2009 through 31 December 2012 were analysed. The annual incidence rate was calculated as follows: the average number of cases notified by *Sentinelles* GPs (adjusted for participation and geographic distribution) was multiplied by the total number of private GPs practicing in France [17]. This product was then divided by the French population [18]. Confidence intervals were estimated under the assumption that the number of reported cases followed a Poisson distribution.

Lyme borreliosis hospitalisations (2004–2009)

Hospitalisation data were collected by reviewing all hospital discharge reports containing an LB code from 1 January 2004 through 31 December 2009 (the latest available data at the time of writing this paper), all obtained through the Programme de Médicalisation des Systèmes d'Information (PMSI) data processing centre [19]. This database is a national register of all discharges from all short-stay/acute-care hospitals. It collects data described by the physicians who took care of the patients during their hospitalisation, using the International Classification of Diseases, 10th revision (ICD-10) [20]. We identified all the hospitalisation reports for which a LB code was reported, i.e. M01.2* (Lyme arthritis), L90.4 (acrodermatitis) or A69.2 (Lyme disease), either for primary or secondary diagnoses. A hospitalisation was considered compatible with LB and retained in the analyses when the data in the hospital discharge report satisfied one of the following criteria (Table): (i) a hospital discharge report with a code specific for LB diagnosis (ICD-10 codes M01.2* or L90.4), (ii) a hospital discharge report with ICD-10 Code A69.2 in the absence of any other diagnosis, or (iii) a hospital discharge report with ICD-10 Code A69.2 together with one or more associated diagnosis code(s) compatible with LB symptoms (neurological, cardiac, articular and ocular disorders). Hospitalisations were described by age, sex, region of residence of the patient, and types of disorders. Also, seasonality and the mean duration of hospitalisation were presented. To estimate the average annual incidence rate of hospitalisations (national and regional), the number of hospital stays in a given geographical area was divided by the average population in the study period, and then multiplied by 100,000 [18].

Results

Incidence and characteristics of Lyme borreliosis cases (2009–2012)

From 2009 through 2012, GPs of the *Sentinelles* network reported 441 cases of Lyme borreliosis, 110 of which were not chosen for validation because they did not meet the criteria. These were mostly late manifestations without serological confirmation (n=42), tick bites without any clinical event (n=25), insufficiently completed questionnaires (n=19), positive serology without any associated clinical event (n=8), diagnosis errors later confirmed by the GP (n=7), data capture errors (n=4), prevalent but non incident cases (n=5). The remaining 331 cases were validated and analysed.

The estimated yearly LB incidence averaged 26,584 cases (95% confidence interval (CI): 23,053–30,115), representing an estimated average incidence rate of 42 per 100,000 national population (95% CI: 37–48). This result was stable over the four years of monitoring: 42 per 100,000 inhabitants (95% CI: 30–54) in 2009, 42 per 100,000 (95% CI: 32–52) in 2010, 41 per 100,000 (95% CI: 31–51) in 2011 and 44 per 100 000 (95% CI: 32–56) in 2012. Regionally, the average incidence rate ranged from 184 (95% CI: 31–356) per 100,000 population in the Limousin region and 157 (95% CI: 34–279) in Alsace to 0 in Nord-Pas-de-Calais and Bourgogne (Figure 1A). The incidence peaked during the months of June to October (Figure 2A).

Women represented 52% of these 331 cases (p=0.37). The age distribution revealed two peaks, between 5 and 10 years and between 50 and 70 years (Figure 3). A tick bite was reported by 74% of patients. The average delay between the bite and the date of diagnosis was 28 days, with a median of 10 days. Most reported cases

Table 1

ICD-10 codes of clinical disorders that may be related to Lyme borreliosis

ICD-10 chapters and group of conditions concerned[a]	ICD-10 code concerned
Chapter VI: Diseases of the nervous system	
Inflammatory diseases of the central nervous system	G00-9
Disorders of the trigeminal nerve	G50.8, G50.9
Facial nerve disorders	G51, G51.0, G51.8, G51.9
Disorders of other cranial nerves	G52, G52.0-3, G52.7-9
Cranial nerve disorders in diseases classified elsewhere	G53, G531, G538
Nerve root and plexus disorders	G54, G54.0-5, G54.8-9
Other polyneuropathies	G62, G62.8-9
Polyneuropathy in diseases classified elsewhere	G63, G63.0
Other disorders of the peripheral nervous system	G64
Chapter VII: Diseases of the eye and adnexa	
Iridocyclitis	H20, H20.0-1, H20.8-9
Other disorders of iris and ciliary body	H21, H21.8-9
Disorders of iris and ciliary body in diseases classified elsewhere	H22, H22.0-1, H022.8
Chorioretinal inflammation	H30, H30.0-9
Other disorders of the choroid	H31, H31.8-9
Chorioretinal disorders in diseases classified elsewhere	H32, H32.0-8
Chapter IX: Diseases of the circulatory system	
Acute pericarditis	I30, I30.0-9
Pericarditis in diseases classified elsewhere	I32, I32.0-8
Acute myocarditis	I40, I40.0-9
Myocarditis in diseases classified elsewhere	I41, I41.0, I41.2, I41.8
Cardiomyopathy	I42, I42.9
Cardiomyopathy in diseases classified elsewhere	I43, I43.0
Atrioventricular and left bundle branch block	I44, I44.0-7
Other conduction disorders	I45, I45.0-9
Other heart disorders in diseases classified elsewhere	I52, I52.0-8
Chapter XIII: Diseases of the musculoskeletal system and connective tissue	
Arthritis and polyarthritis due to other specified bacterial agents	M00.8, M00.80-9
Direct infections of joint in infectious and parasitic diseases classified elsewhere	M01, M01.2, M01.20-9, M01.30-9, M01.80-9
Other arthritis	M130-9
Arthropathies in other diseases classified elsewhere	M14, M14.8

[a] International Classification of Diseases, 10th Revision [20].

had erythema migrans (93.6%), with a single lesion in 91% of cases and multiple lesions in 9% of cases. Other clinical forms of the disease were reported in a limited number of cases: cutaneous lymphocytoma (n=4), ACA (n=3), arthritis (n=9), neurological disorders (n=5), and one patient presented both arthritis and neurological disorders. For one patient only presenting a neurological form of LB, a lumbar puncture was performed to confirm the diagnosis.

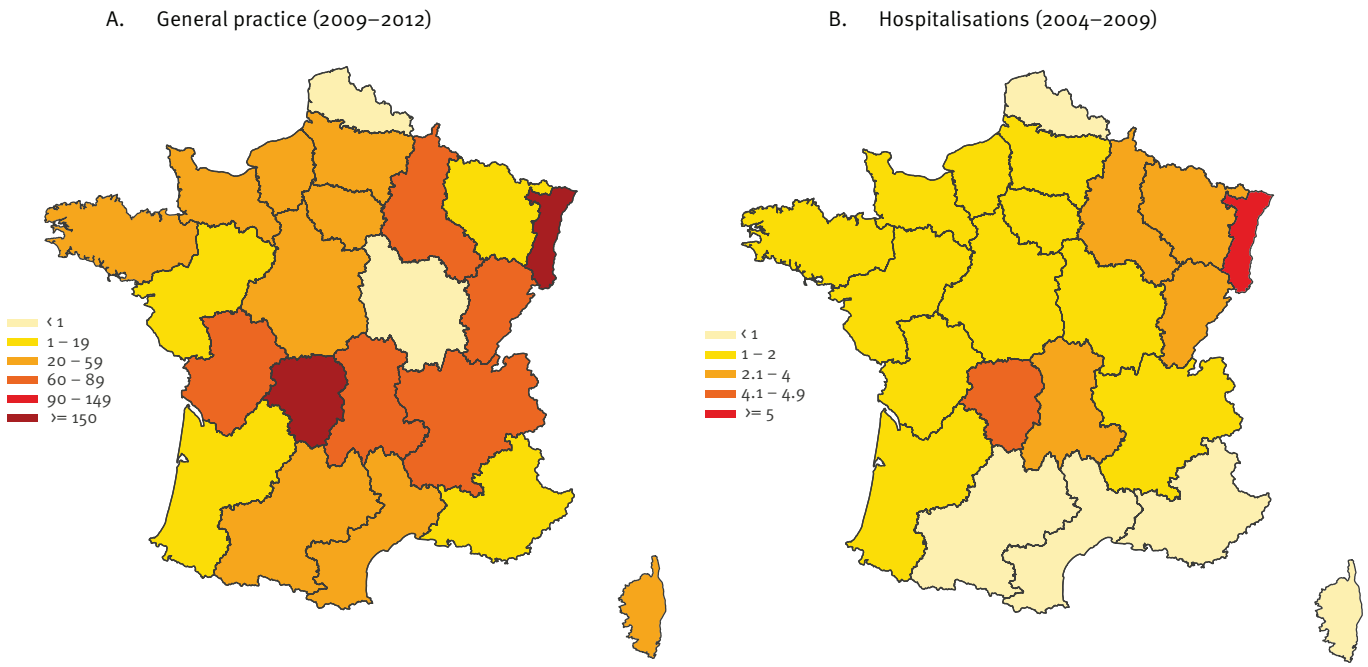
Lyme borreliosis hospitalisations (2004–2009)

Among the 14,348 files extracted in France from the PMSI from 2004 through 2009, 5,727 were considered compatible with a diagnosis of Lyme disease, i.e. 40% of all hospitalisation records with an LB code. Therefore, the estimated average incidence of hospitalisations was 954 per year (Figure 4), which represents an estimated annual average hospitalisation rate over the period of 1.55 per 100,000 inhabitants (95% CI: 1.42–1.70), ranging from 1.38 to 1.87. Regional variations of hospitalisation rates were remarkable, ranging from 6.72 per 100,000 inhabitants per year in Alsace, to 0.30 in Corsica (Figure 1B).

These hospitalisations concerned women in 42.2% of cases ($p<0.001$). The age distribution revealed two peaks: one between 5 and 10 years and another around the age of 55 years (Figure 5). Reasons for hospitalisation were neurological disorders (n=2,820), LB with no associated diagnosis (n=1,860), arthritis (n=660), cardiac events (n=304), ocular disorders (n=87) and ACA (n=85). The monthly distribution of hospitalisations (month of entry) peaked between June and November (Figure 2B). The average duration of stay was 5.3 days

Figure 1

Estimated annual regional incidence rates of Lyme borreliosis in general practice (2009–2012) and hospitalisations (2004–2012), France



Incidence rates per 100,000 inhabitants.

and varied, according to the LB form, from an average of 3.0 days for hospitalisations coded with an isolated LB code, to 8.3 days for LB associated to cardiac disorders.

Discussion

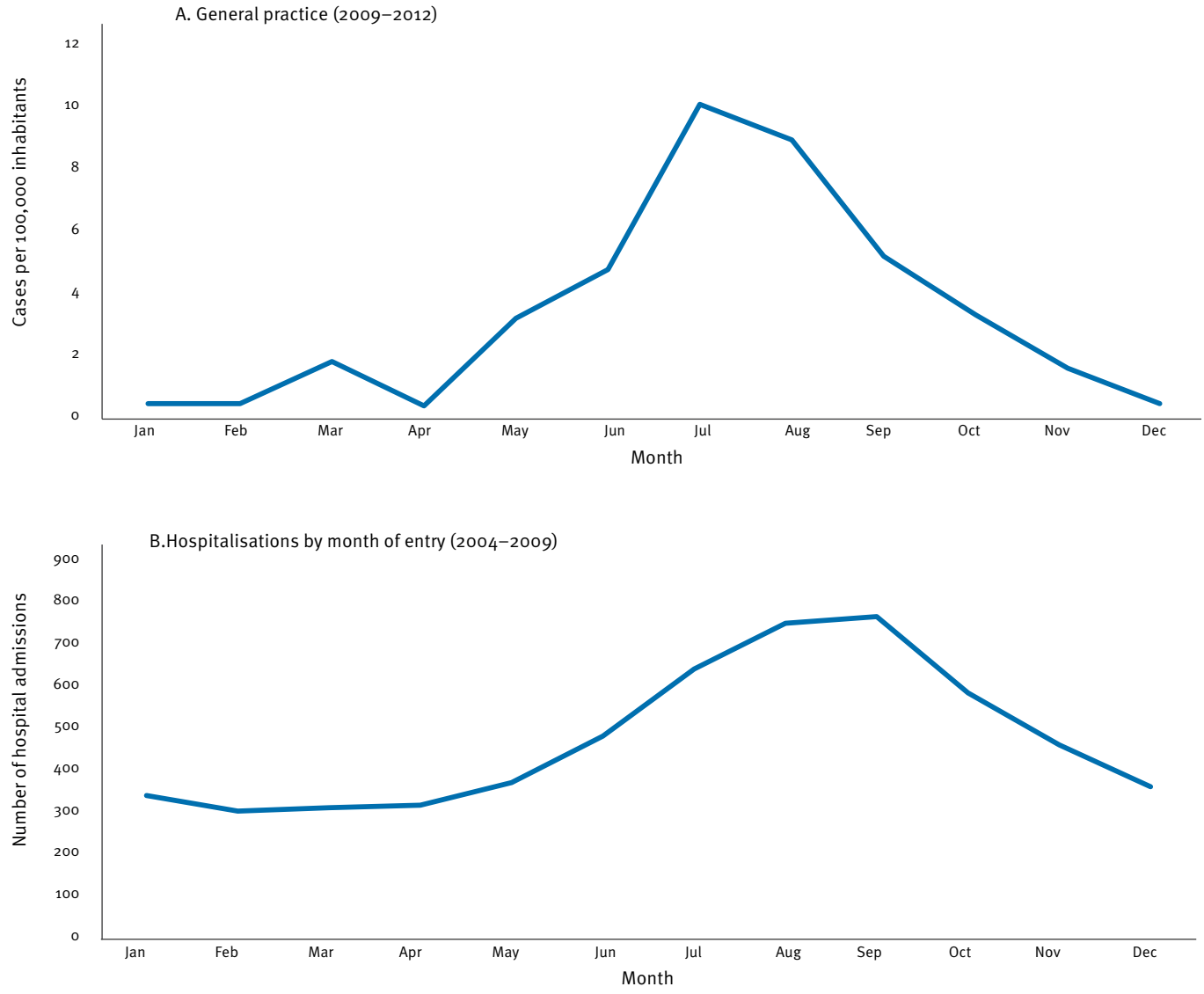
Data from the *Sentinelles* GP network and administrative hospitalisation database allowed for an updated and ongoing description of the epidemiology of LB in France. Although they covered different time periods, there was consistency between the data sources in regard to age distribution, seasonality and spatial distribution. The study confirmed that the incidence was greater in the eastern and central regions of France, increased in summer, and was higher among young children and older adults. It also confirmed that GPs play a major role in diagnosing and treating the early forms of the infection. Even though they were unusual, severe forms made hospitalisation necessary.

On the basis of the 331 cases collected and validated by the *Sentinelles* network, the LB incidence was estimated at 42 (95% CI: 37–48) for 100,000 inhabitants. The actual LB incidence could in fact be higher than this estimate, since patients who did not consult a GP were not covered by this study. There is no published or unpublished information that could help assess the magnitude of this effect. However, nowadays in France, people cannot go directly to a specialist without first consulting a GP. It is therefore likely that the vast majority of patients had been seen by a GP. The incidence rates estimated here can be compared with incidence rates in other European countries, although such comparisons must be interpreted with caution. Indeed, there are differences between countries in the process of data collection. Only some countries use a continuous surveillance system, and case definitions and laboratory confirmation methods vary [1,9]. In 2005, the highest incidence rates of LB in Europe were reported in Slovenia with 206 cases per 100,000 inhabitants, Austria with 135 cases per 100,000 inhabitants and the Netherlands with 103 cases per 100,000 inhabitants [9]. A high yearly incidence rate of LB (131 per 100,000 inhabitants) was also reported in Switzerland between 2008 and 2011 [21]. Incidence measured in Germany in 2006 amounted to 37.3 per 100,000 inhabitants in six of the 16 federal states [8]. In the Czech Republic in 2006, it was 42.6 per 100,000, close to the national incidence in France. In Belgium, it was 16 per 100,000 in 2005 [9], whereas the Belgian network of sentinel GP estimated the incidence rate of erythema migrans at 90.2 per 100,000 in 2009 [22]. The incidence rate was below 1 per 100,000 in Italy and Portugal in 2005 [9].

The only earlier available data on French national incidence of LB were provided by a study conducted by the *Sentinelles* network in 1999 and 2000. It estimated the national incidence rate at 9.4 per 100,000 inhabitants [10]. The higher incidence reported in the present study could suggest that the LB incidence rate has increased in France. However, these incidences must be compared with caution, since different artifacts may have contributed to the higher numbers. Firstly, it has been shown

Figure 2

Mean monthly incidence rate of Lyme borreliosis in general practice (2009–2012) and number of hospital admissions (2004–2009), France)



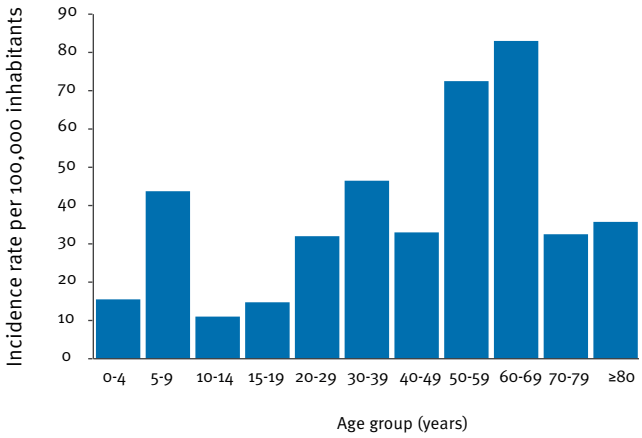
Incidence rates per 100,000 inhabitants.

that the incidence of LB is highly sensitive to changes in surveillance methods [23]. Indeed, the methodology used by Letrilliart et al. in 1999–2000 was different from the one we applied, and probably led to an underestimation of the incidence rate in their study. At the time, data were not collected through a routine systematic and standardised surveillance, as they have been since 2009. The routine surveillance of the *Sentinelles* network is based on periodic reports from sentinel physicians (usually weekly reports). Thus, if a physician does not provide report for several weeks, they will not be counted as a participating during those weeks. In the 1999 survey, it was assumed that physicians reported cases actively during the entire study period. Thus, compared to routine surveillance, the number of cases declared in the 1999 study was attributed to an overestimated number of participating GPs, resulting in lower incidence estimates. Secondly, LB today is better known to the public and the GPs than it was in 1999, which may have contributed to the strong increase in estimated LB incidence we report here [6]. Finally, the number of hospitalised cases was stable over the period 2004–2009 and seems incompatible with a four-fold increase in incidence suggested by a comparison of incidence estimates derived from the 1999–2000 study and the 2009–12 surveillance data. The hospital discharge database being a stable system, surveillance bias is unlikely, and the database can therefore provide useful data for trend analyses.

Nevertheless, numerous studies and surveillance data from other European countries and the US showed

Figure 3

Annual incidence rates of Lyme borreliosis by age group as estimated by the general practitioners' *Sentinelles* network, France, 2009–2012



that the LB incidence may indeed have increased over the past few decades. In the US, a continued surveillance system was created in 1991, and the incidence has increased by 101% since then [6]. In Europe, an increase in LB was observed in several countries [9]. Between 1990 and 2001, the incidence rate in the Czech Republic doubled [1]). In Germany, Fulop et al. describe a 110% increase between 2001 and 2006 [8]. In the United Kingdom, case numbers have increased 3.6-fold between 2001 and 2011 [24]. In addition, some factors not related to surveillance artifacts may have contributed to an actual incidence increase, such as climate changes [25,26], increases in the population of wild animals hosting ticks, modifications in agricultural and forest landscapes that can lead to a higher density of tick populations [1], or reduction in biodiversity that can increase the prevalence of ticks carrying Borellia [27].

Regional incidence rates varied considerably in France, from 0 per 100,000 inhabitants to 184 per 100,000. These variations could be explained by participation biases. However, they were the same as those observed in the earlier *Sentinelles* network study [10] and, more importantly, the regional hospitalisation incidences corroborated the GP surveillance data, as shown by the similar distribution of GP and hospitalisation data in Figure 1. Other published studies confirmed the regional distribution reported here [11]. In the US, incidence also varied among states, from less than 0.01 per 100,000 inhabitants in Montana or Colorado, to 73 per 100,000 in Connecticut [6]. The confirmation of interregional variations is an asset to guide future studies and develop more efficient public health actions.

In 94% of the cases in France, the infection was diagnosed at the erythema migrans stage. In the US, the proportion of erythema migrans was lower, estimated at 69% [6]. However, the data collection process in the US included cases reported through laboratory-based surveillance that are more likely to have late manifestations of LB [23]. An earlier study conducted in France with GPs, specialists and hospital practitioners reported a proportion of erythema migrans of 68% [11]. In Europe, published data varied from 65% to 95% [11,28,29]. These observations suggest that either the incidence of late Lyme disease forms in general practice in France is underestimated or the incidence of erythema migrans is overdiagnosed. Indeed, the lesion can be confused with other dermatological disorders, and the positive predictive value of an erythema migrans diagnosis made by GPs in France has been estimated at 72% [30].

There was a marked difference in the sex ratio between outpatients (predominantly female) and inpatients (predominantly male), for which we have no explanation. Sex differences in the risk of contracting tick bites, incidence rates, and clinical picture of erythema migrans have been reported, although the biological, immunological, and sociological mechanisms causing these differences have not been determined [31].

There are limits to the use of data coming from the national hospitalisation statistics, including diagnosis and coding mistakes, unintentional omissions, partial reporting of pre-existing conditions, lack of information about treatments (whether or not patients received an LB-effective antibiotic could help validate the diagnosis), the frequent unavailability of medical history forms to the certifying practitioner or difficulties in determining the initial cause of hospitalisation when several associated pathologies are involved. Indeed, it is possible that the recorded diagnoses were given by physicians who did not base their decision on accepted case definitions. Also, the diseases defined by the ICD-10 codes that we used are not specific for LB, their aetiologies may be numerous and in many cases idiopathic. It has been previously shown that the positive

Figure 4

Lyme borreliosis hospitalisations per year, France, 2004–2009

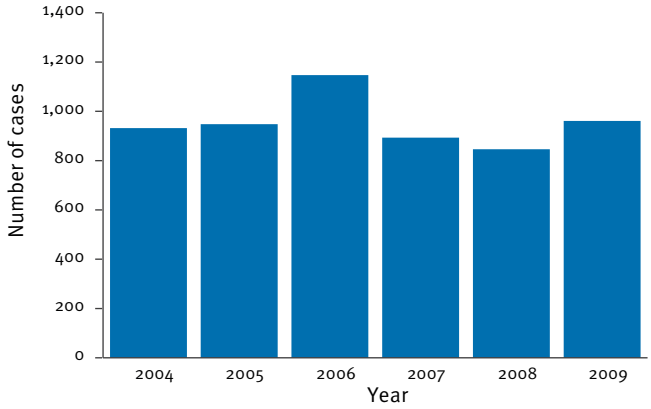
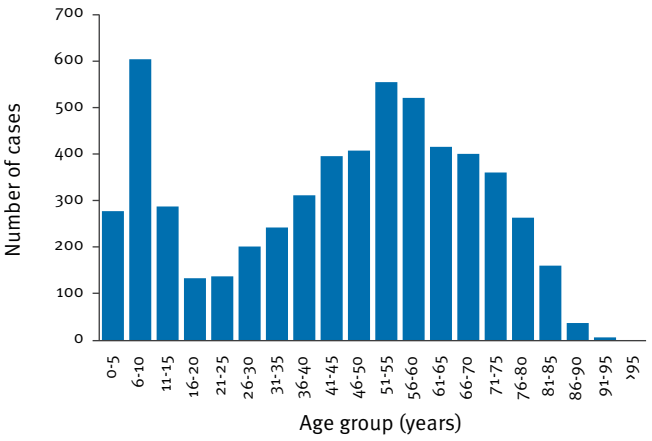


Figure 5

Age-specific distribution of Lyme borreliosis hospitalisations, France, 2004–2009



predictive value of the LB code in the PMSI database was 65% [32]. We therefore considered it necessary to set up a selection process for the reported hospitalisations. In the end, we only kept 40% of the registered reports. Nevertheless, the predictive value of our case definition could remain low due to the lack of specificity of the retained hospitalisation definitions (related to the complexity of databases such as the PMSI hospitalisation database). Therefore, our study could also overestimate the hospitalisation rates for LB in France. However, the PMSI database proved a useful tool to monitor trends over time, determine the seasonality, determine high-risk regions, and provide details on characteristics of hospitalised patients.

Conclusions

In conclusion, a countrywide sentinel network and hospitalisation statistics produced epidemiological data that were sustainable and consistent over time and space. They were suitable for following trends and estimating the burden of this disease for which there is significant public concern. Determination of the healthcare burden associated with LB can inform public health policy and enable valid analysis of the efficiency of LB control measures. Furthermore, we believe that this study could help elaborate factual risk communication messages provided to the lay public or the media. Healthcare providers should continue to invest attention into prompt recognition and early, appropriate therapy for LB. Also, public health strategies should keep promoting the use of repellent, daily checks for ticks and their prompt removal, mainly in the age groups, in the regions and during the months with the highest incidences.

Acknowledgements

The authors wish to thank all the *Sentinelles* network GPs. This work was funded by the French Institute for Health and Medical Research (INSERM) and the French Institute for Public Health Surveillance (InVS).

Conflict of interest

None declared

Authors' contributions

All authors participated in the conceptualisation and writing of this manuscript. AV and TH designed the research, analysed data and drafted the article; CT, AA, VC and CA analysed data; EC, BJ, EF, CS, EC, VV and TB interpreted the data and revised it critically for important intellectual content

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Effectiveness of seasonal trivalent inactivated influenza vaccine in preventing influenza hospitalisations and primary care visits in Auckland, New Zealand, in 2013

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This study reports the first vaccine effectiveness (VE) estimates for the prevention of general practice visits and hospitalisations for laboratory-confirmed influenza from an urban population in Auckland, New Zealand, in the same influenza season (2013). A case test-negative design was used to estimate propensity-adjusted VE in both hospital and community settings. Patients with a severe acute respiratory infection (SARI) or influenza-like illness (ILI) were defined as requiring hospitalisation (SARI) or attending a general practice (ILI) with a history of fever or measured temperature ≥38 °C, cough and onset within the past 10 days. Those who tested positive for influenza virus were cases while those who tested negative were controls. Results were analysed to 7 days post symptom onset and adjusted for the propensity to be vaccinated and the timing during the influenza season. Influenza vaccination provided 52% (95% CI: 32 to 66) protection against laboratory-confirmed influenza hospitalisation and 56% (95% CI: 34 to 70) against presenting to general practice with influenza. VE estimates were similar for all types and subtypes. This study found moderate effectiveness of influenza vaccine against medically attended and hospitalised influenza in New Zealand, a temperate, southern hemisphere country during the 2013 winter season.

Introduction

Influenza infection causes a major burden of illness in adults and children [1,2]. Seasonal trivalent influenza vaccines (TIVs) are effective in preventing a range of laboratory-confirmed outcomes [3], but effectiveness varies by severity and season, the presence of comorbidities and age [4,5].

The SHIVERS (Southern Hemisphere Influenza Vaccine Effectiveness, Research and Surveillance) study has allowed estimation of vaccine effectiveness (VE) against influenza illness requiring hospitalisation since 2012 and against influenza illness requiring primary care (general practice) since 2013.

In New Zealand, seasonal non-adjuvanted inactivated trivalent influenza vaccine is available annually free of charge to all adults aged 65 years and over, pregnant women and all those over six months of age with chronic medical conditions that are likely to increase the severity of the infection. Influenza vaccines are also available on the private market for all others over six months of age. Two commercial vaccine products were available in the New Zealand market in 2013: Fluarix (GlaxoSmithKline) and Fluvax (bioCSL). Both vaccines contained A/California/7/2009 (H1N1)-like virus, A/Victoria/36/2011 (H3N2)-like virus and B/Wisconsin/1/2010-like virus (belonging to B/Yamagata/16/88 lineage).

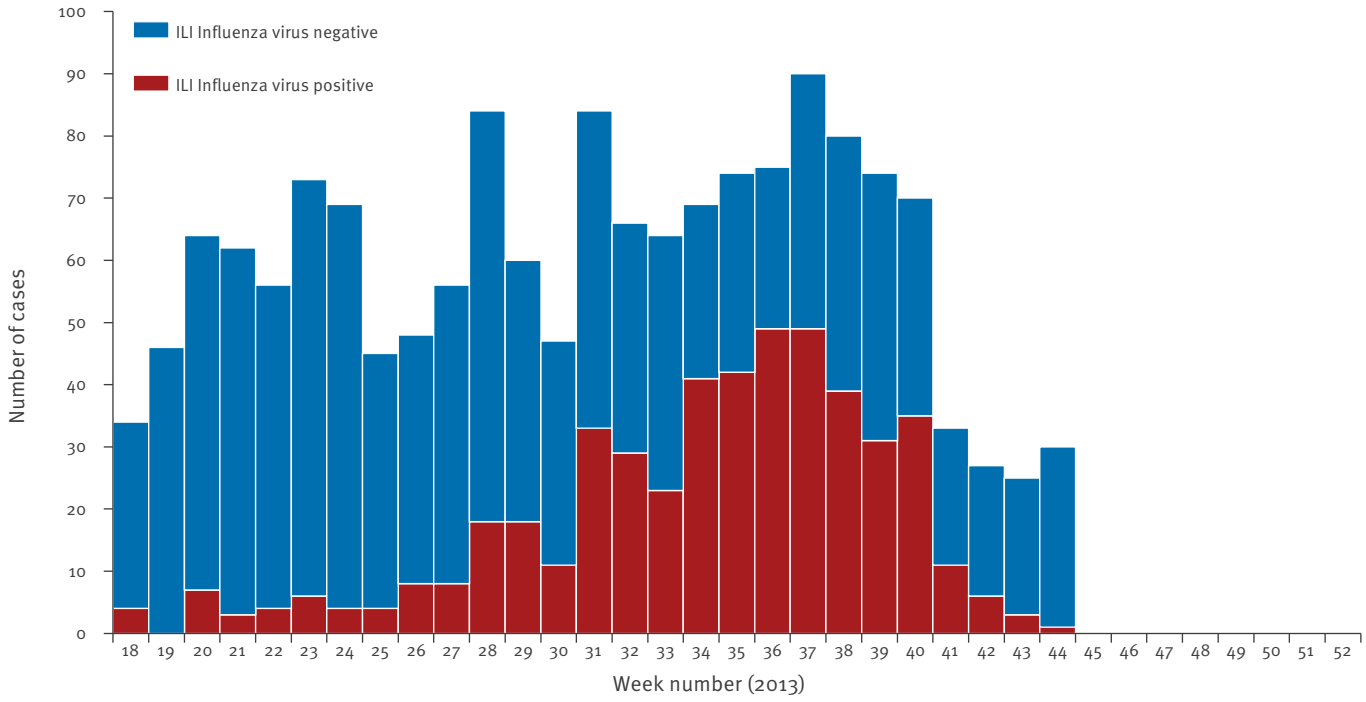
Using the case test-negative design, we estimated the effectiveness of seasonal trivalent inactivated influenza vaccine in preventing laboratory-confirmed influenza in patients hospitalised with severe acute respiratory infections (SARI) and in patients presenting to general practice with an influenza-like illness (ILI) during the 2013 influenza season, which is from March to September in New Zealand.

Methods

Ethics approval for the study was obtained from the Northern A Health and Disability Ethics Committee (NTX/11/11/102 AM02).

Figure 1

Study participants with influenza-like illness who were influenza positive or negative by week, New Zealand, 2013 influenza season



ILI: influenza-like illness.
Weeks 18 to 52 are shown (29 April to 31 December 2013).

Study design

In both hospital and community settings, we conducted a study using a standard case test-negative design [6], drawing on an urban population of approximately 838,000 people in Central, South and East Auckland [7].

For community cases, we recruited 18 sentinel general practices with 103,884 enrolled patients. Patients in these sentinel practices were broadly representative of the ethnically diverse urban population of Auckland by age and sex distribution, but with more Pacific people (27% in the practices compared with 15% in the source population) and slightly fewer people of Asian descent (14% versus 19%, respectively)[7].

The practices recruited individuals aged six months and older who presented to a general practitioner or practice nurse with ILI, defined as a history of fever or measured fever of ≥38 °C and cough, with onset during the preceding 10 days [8,9].

All patients presenting to one of the sentinel general practices with suspected respiratory infections were screened by the general practitioner or nurse for ILI. All identified ILI cases were entered on an electronic form in the practice management system and a nasopharyngeal or throat swab was collected for influenza virus testing from all consenting patients.

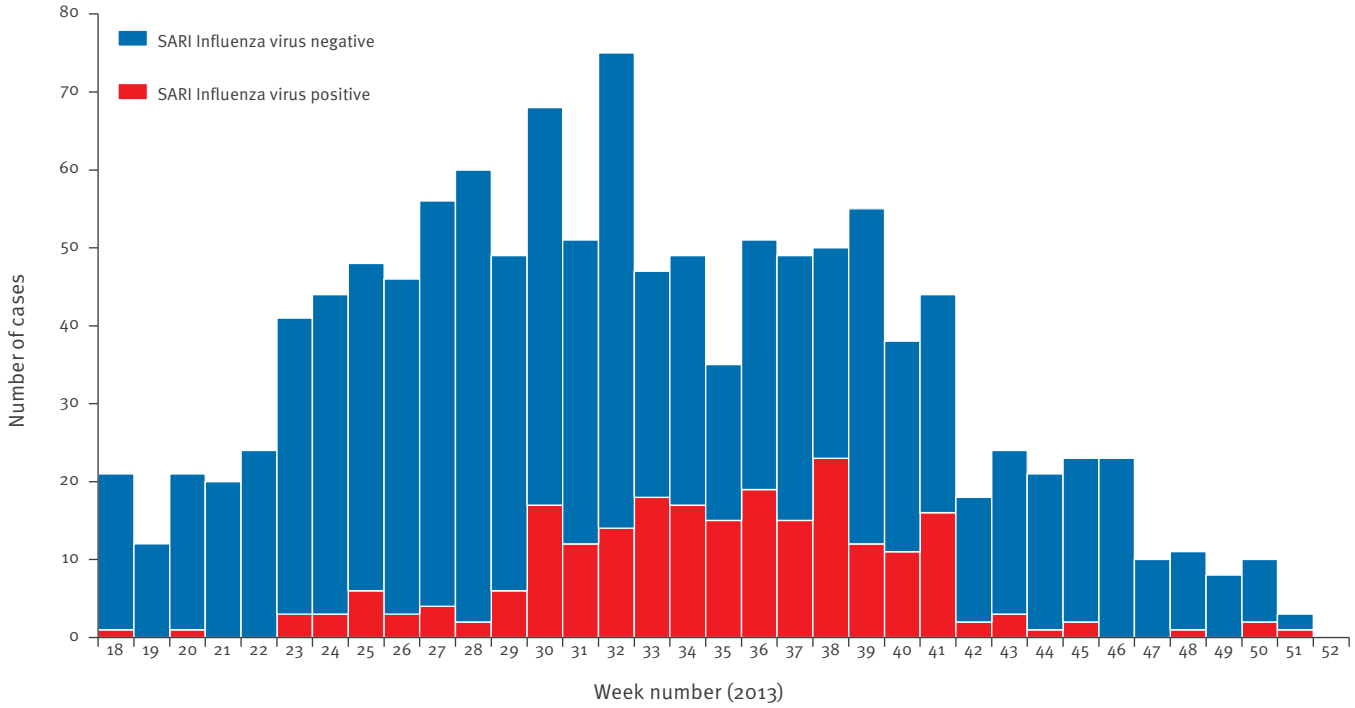
For hospitalised patients, we enrolled individuals aged six months and older who were admitted with SARI to one of the four public hospitals covering the whole population in this catchment area. On the basis of the World Health Organization (WHO) definition, SARI was defined as hospitalisation with a patient-reported history of a fever or a measured temperature of ≥38 °C and cough with onset within the past 10 days [10]. A confirmed case of influenza was defined as a SARI or ILI patient with a positive laboratory result for any influenza virus detected by real-time reverse transcription polymerase chain reaction (RT-PCR), while non-cases (controls) were patients with SARI or ILI who tested negative for all influenza viruses.

SARI patients were identified following screening of patients admitted with respiratory disease by dedicated research nurses. Overnight admissions of patients with respiratory symptoms were screened by the nurses on the following day. All patients satisfying the SARI case definition were invited to participate in the study. Patients who gave verbal consent completed a case report form and provided a nasopharyngeal swab or aspirate for influenza virus testing.

Patients who were identified at seven days post onset of symptoms were excluded from the ILI and SARI analysis, based on the pattern of shedding, which peaks in

Figure 2

Study participants with severe acute respiratory infections who were influenza virus positive and negative by week, New Zealand, 2013 influenza season



SARI: severe acute respiratory infection.

Weeks 18 to 52 are shown (20 April to 31 December 2014).

the first three days, then declines steadily until days 7–9 [9]. We also excluded patients with incomplete data for vaccination status or age, all infants under 6 months of age, children aged under 9 years who were only given one dose of trivalent inactivated influenza vaccine, patients who were vaccinated less than 14 days before admission to hospital or presentation to general practice. For patients with multiple episodes, the first influenza virus-positive episode was used for the analysis, or the first illness episode if there was no influenza virus-positive episode.

The influenza season was defined as starting when there were two consecutive weeks with two or more cases and ending when there were no consecutive weeks with two or more cases. Analysis was thus undertaken from 3 June to 10 November 2013 for SARI cases and from 13 May to 27 October 2013 for ILI cases (Figures 1 and 2).

Participant information

For all ILI patients, variables extracted from the electronic form and patient management system included age, sex, ethnicity, chronic medical conditions and current smoking status, socio-economic status as identified by the New Zealand deprivation status (a meshblock measure reflecting eight dimensions of deprivation distributed into deciles) [11] and a subjective assessment of obesity by the clinician as body mass index measurements were not consistently available.

Similar information was collected for all SARI patients, but for this group we also collected the following: a patient- or caregiver-reported measure of dependence (which assessed requirement for assistance with normal activity or full dependency on nursing care); a measure based on long-term use of oxygen that we labelled 'frailty'; a history of chronic medical conditions; and a self-defined, standardised functional well-being health status score from a national survey [12], combining fair or poor well-being versus all other more positive well-being scores.

In New Zealand, almost all influenza vaccinations are administered in general practices. For ILI cases, vaccination status was taken from the general practice record. SARI vaccination status for the 12 months before hospitalisation was determined by self-report.

Laboratory methods

Nasopharyngeal swabs, aspirates and other respiratory samples were collected according to hospital or general practice standard procedures. Samples were tested using the United States Centers for Disease Control and Prevention (CDC) real time RT-PCR protocol [13] or the AusDiagnostic PCR protocol [14]. The AusDiagnostic assay had a sensitivity of 100% and

Table 1

Characteristics of study participants with influenza-like illness and severe acute respiratory infection, New Zealand, 2013 influenza season*

Characteristic	Hospitalised with severe acute respiratory infection		General practice visit for influenza-like illness	
	Cases n (%)[a]	Controls n (%)[a]	Cases n (%)[a]	Controls n (%)[a]
Vaccinated	82 (36.6)	372 (45.4)	44 (9.1)	177 (17.4)
Median age in years	49 (21.9)	41 (5.0)	25 (5.2)	21 (2.1)
Male	105 (46.9)	410 (50.1)	224 (46.5)	415 (41.0)
Age group				
6 months–5 years	40 (17.9)	271 (33.1)	74 (15.4)	255 (25.2)
6–17 years	11 (4.9)	35 (4.3)	141 (29.3)	221 (21.8)
18–45 years	51 (22.8)	129 (15.8)	173 (35.9)	330 (32.6)
46–64 years	51 (22.8)	156 (19.1)	75 (15.6)	159 (15.7)
65–79 years	47 (21.0)	134 (16.4)	16 (3.3)	41 (4.0)
≥80 years	24 (10.7)	93 (11.4)	3 (0.6)	7 (0.7)
Ethnicity				
Māori	29 (12.9)	169 (20.7)	18 (3.7)	57 (5.6)
Pacific	77 (34.4)	238 (29.1)	97 (20.1)	203 (20.0)
Other characteristics				
Mean New Zealand deprivation score [b]	5.3	5.9	4.95	4.9
Pregnant	5 (2.2)	5 (0.6)	Not collected	Not collected
Current smoker	24 (10.7)	86 (10.5)	30 (6.2)	56 (5.5)
Chronic disease	138 (61.6)	528 (64.5)	Not collected	Not collected
Obese[c]	44 (19.6)	134 (16.4)	17 (3.5)	42 (4.2)
Self-defined well-being health status of poor or fair[d]	28 (12.5)	120 (14.7)	Not collected	Not collected
Frailty[e]	5 (2.2)	24 (2.9)	Not collected	Not collected
Dependence[f]	10 (4.5)	50 (6.1)	Not collected	Not collected
Total	**224 (100)**	**818 (100)**	**482 (100)**	**1,013 (100)**

[a] Unless otherwise indicated.

[b] A meshblock measure reflecting eight dimensions of deprivation distributed into deciles.

[c] Subjective assessment of obesity by the clinician.

[d] A self-defined, standardised functional well-being health status score.

[e] Defined as currently on long-term oxygen use.

[f] Requirement for assistance with normal activity or full dependency on nursing care, as reported by the patient or caregiver.

specificity of 96.6% when the United States CDC method was used as the comparator [15]. RT-PCR assays detected influenza virus types A and B and subtyping was performed for type A. All influenza virus PCR-positive samples were forwarded to the National Influenza Centre and characterised antigenically using established methods [14].

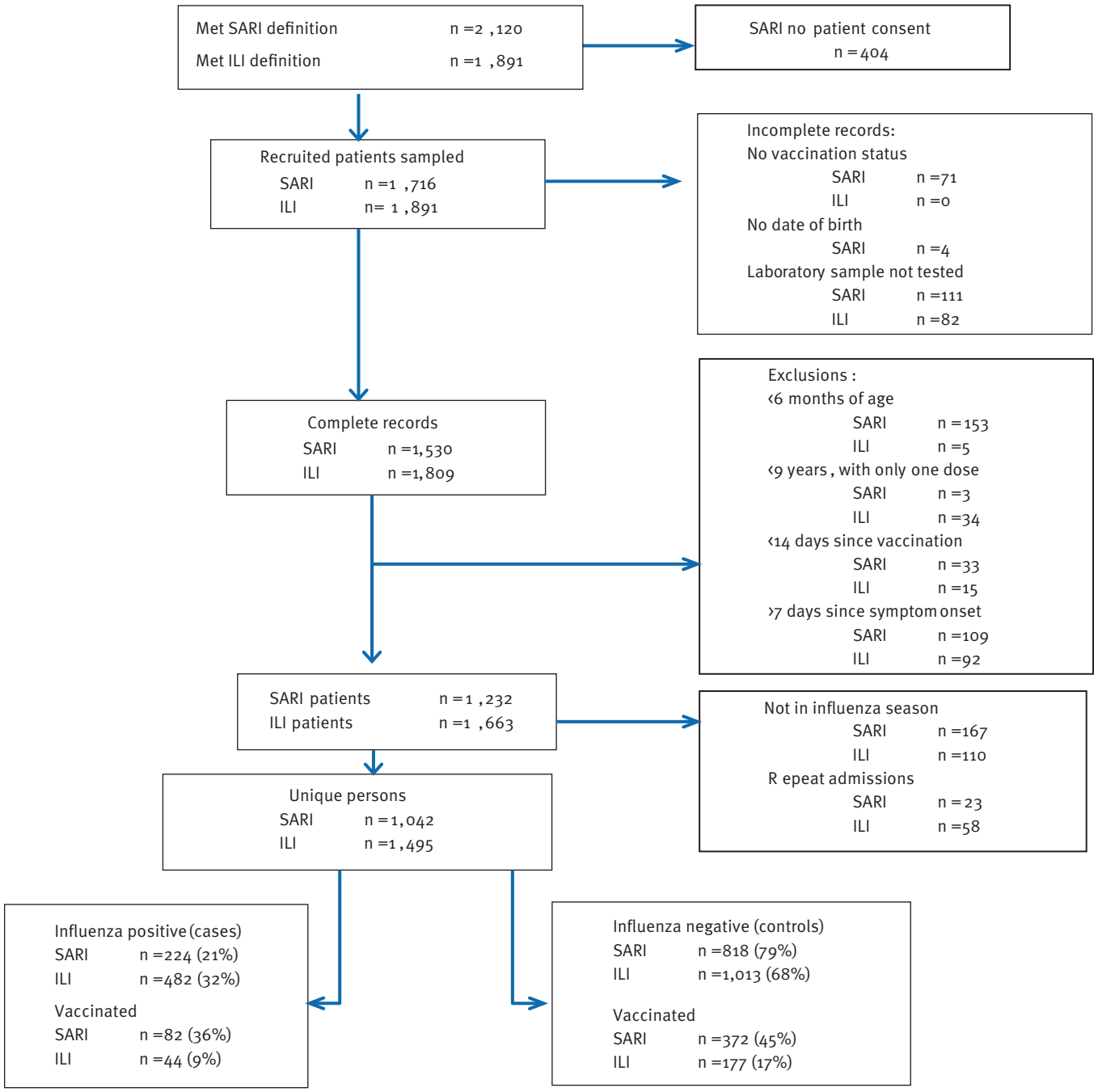
Statistical analysis

Univariate chi-squared tests were used to compare characteristics of patients who were influenza virus positive (cases) and negative (controls). A multivariate logistic model restricted to the test-negative controls was used to calculate an adjusted odds ratio for the propensity to be vaccinated for a range of patient characteristics possibly associated with vaccination (Table 1) and used in a previous study [16]. The results from this propensity model are presented as odds ratios. This propensity model was then applied to the complete dataset to generate individual propensity scores for vaccination. In order to ensure these propensity scores were linear with respect to influenza virus positive status, we used the cubic spline for these scores as an adjustment variable for estimating VE. For both SARI and ILI, we calculated the crude VE, adjusting only for the timing of presentation relative to the influenza season (defined as weeks from the peak), and the adjusted VE, which included the timing of presentation and the cubic spline of the fitted values of the propensity model. VE estimates were calculated against both SARI and ILI, by influenza virus type and subtype and by age group (6 months–17 years, 18–64 years and ≥65 years).

For all patient characteristics, other than age and vaccination status, each missing data point was imputed as the baseline (referent) value for the corresponding variable. The baseline values were: non-Māori, non-Pacific ethnicity, female, not in New Zealand deprivation groups 8,9 or 10 (the three lowest deprivation deciles), not pregnant, current non-smoker, without chronic disease, not obese, with self-rated health average or better, not using long-term oxygen and living without assistance). All male patients and all female

Figure 3

Flowchart of all selected, recruited and tested patients with influenza-like illness and severe acute respiratory infection for influenza vaccine effectiveness analysis, New Zealand, 2013 influenza season



ILI: influenza-like illness; SARI: severe acute respiratory infection.

patients not aged 13–55 years were assumed to be not pregnant. Overall, 53 SARI (5%) cases and 1 ILI (0.1%) case had any imputed covariates. Sensitivity analyses were performed using only those individuals with complete data.

As a further sensitivity analysis, we also compared the overall VE estimate from the propensity-adjusted model with an epidemiological model that included covariates that were assessed as potential confounders and/or effect modifiers (Table 1) and a statistical model, derived from the epidemiological model, where only covariates that were significant at the 0.05 level were included in the final model.

Results

Characteristics and vaccination status of participants ILI and SARI patients in this study by influenza virus status are shown in Figures 1 and 2.

Table 2

Vaccinated and unvaccinated influenza cases by virus type and subtype in hospitalised and community study participants, New Zealand, 2013 influenza season*

Influenza virus type	Hospitalised with severe acute respiratory infection		General practice visit for influenza-like illness	
	Number vaccinated (%)	Number unvaccinated (%)	Number vaccinated (%)	Number unvaccinated (%)
All	82 (100)	142 (100)	44 (100)	438 (100)
A(H1N1)	5 (6)	8 (6)	3 (7)	27 (6)
A(H3N2)	51 (62)	68 (48)	20 (45)	196 (45)
All A[a]	68 (83)	95 (67)	28 (64)	262 (60)
All B	14 (17)	48 (34)	16 (36)	180 (41)

ILI: influenza-like illness; SARI: severe acute respiratory infection.

[a] One SARI case and four ILI cases tested positive for both influenza A and B viruses. Not all cases of influenza A were subtyped. The number of subtypes does not add up to the number of all influenza A viruses identified.

A total of 1,042 SARI admissions and 1,495 ILI patients were included in the analysis, of whom 224 (21%) and 482 (32%) were influenza virus positive, respectively. Of the 224 SARI admissions who tested influenza virus positive, 82 (37%) were vaccinated, compared with 372/818 (45%) who tested negative. Despite our attempts, we were unable to verify self-reported vaccination status of SARI patients with the vaccine providers.

Of the 482 ILI patients who tested influenza virus positive, 44 (9%) were vaccinated, compared with 177/1,013 (17%) who tested negative (Figure 3). The proportion vaccinated did not change throughout the season. In those excluded because of incomplete laboratory tests, self-reported vaccination in the previous 12 months among SARI cases was 49/135 (36%), slightly less than the proportion of included SARI cases, 454/1,042 (44%). In the ILI cases, the proportion vaccinated was much higher, at 8/21 (38%), in those excluded because of incomplete laboratory tests compared with the ILI cases included, of whom 9% (44/482) were vaccinated.

Influenza-positive cases and influenza-negative controls were compared across a range of patient characteristics. SARI and ILI patients who were aged 6 months to 5 years or over 80 years, and those presenting outside the influenza season were less likely to test positive. In comparison with the community patients, the hospitalised patients were more likely to be vaccinated, to be older, to live in a deprived area, to be of Māori or Pacific ethnicity, to be a current smoker and to be obese (Table 1). Details on pregnancy were poorly recorded but less than 3% (30/1,042) of other data fields were missing for both SARI and ILI patients.

Of the 706 influenza cases detected in both SARI and ILI patients, 453 (64%) were type A, 335 (47%) A(H3N2), 43 (6%) A(H1N1) and 75 (11%) A not subtyped) and 258 (37%) type B (107 B/Wisconsin/1/2010-like of the B/Yamagata lineage, 2 (0.3%) B/Brisbane/60/2008-like of the B/Victoria lineage and 149 (21%) where the B lineage was not determined). Five cases (0.7%) tested positive for both influenza A and B (Table 2).

Although vaccination was more common in SARI patients, the same factors affected the propensity to be vaccinated in persons with ILI or SARI. The adjusted odds ratios for the association of various patient characteristics with the likelihood of vaccination showed that older age groups and those with chronic diseases were most likely to be vaccinated (Table 3). In contrast, there was no statistically significant difference in the likelihood of vaccination by ethnicity, sex, deprivation score, pregnancy, obesity, self-rated health, smoking, assisted living or the timing of the admission relative to the influenza season (Table 3).

Vaccine effectiveness

The VE against all circulating influenza virus strains, adjusted only for the number of weeks from the peak of the influenza season, was 32% (95% confidence interval (CI): 7 to 50) for influenza-confirmed SARI and 56% (95% CI: 37 to 70) for influenza-confirmed ILI (Table 4). After also adjusting for the propensity to be vaccinated, the estimated VE was 52% (95% CI: 32 to 66) for SARI and 56% (95% CI: 34 to 70) for ILI. Thus, adjusting for the propensity to be vaccinated had more effect on the VE estimate for SARI than for ILI. For ILI, the crude and adjusted VE point estimates were the same.

There was no significant change to these estimates when excluding patients with missing values (for SARI, unadjusted VE was 29% (95% CI: 3 to 48) and adjusted VE 50% (95% CI: 29 to 66); for ILI, unadjusted VE was 56% (95% CI: 37 to 70) and adjusted VE 56% (95% CI: 34 to 70)). When a logistic regression model was constructed and directly adjusted for all the covariates in Table 1, the VE was 54% (95% CI: 33 to 69) for SARI admissions and 58% (95% CI: 37 to 72) for ILI patients. Adjusting for only the variables that were significant in the model ($p<0.05$) resulted in a VE estimate of 54% (95% CI: 33 to 69) for SARI and 59% (95% CI: 41 to 72) for ILI. When restricting the analysis to within four days of onset of symptoms, the adjusted VE for ILI was

Table 3

Characteristics of non-influenza virus-positive study patients with severe acute respiratory infection and influenza-like illness (controls) and their association with influenza vaccination status, New Zealand, 2013 influenza season

Characteristic	Hospitalised with severe acute respiratory infection		General practice visit for influenza-like illness	
	OR (95% CI)	P value	OR (95% CI)	P value
Age group				
6 months–5 years	0.15 (0.08 to 0.27)	<0.01	0.14 (0.07 to 0.25)	<0.01
6–17 years	0.26 (0.11 to 0.63)	<0.01	0.19 (0.11 to 0.34)	<0.01
18–45 years	0.42 (0.25 to 0.70)	<0.01	0.31 (0.20 to 0.50)	<0.01
65–79 years	2.28 (1.30 to 4.00)	<0.01	7.9 (3.35 to 18.64)	<0.01
≥80 years	2.81 (1.38 to 5.73)	<0.01	8.87 (1.01 to 77.66)	0.05
Ethnicity				
Māori	0.72 (0.44 to 1.17)	0.19	0.60 (0.25 to 1.47)	0.26
Pacific	0.94 (0.59 to 1.49)	0.79	0.68 (0.38 to 1.22)	0.19
Additional characteristics				
Male	1.02 (0.71 to 1.45)	0.92	0.64 (0.43 to 0.96)	0.03
Mean New Zealand deprivation score[a]	0.98 (0.91 to 1.05)	0.53	0.98 (0.91 to 1.06)	0.61
Pregnant	1.28 (0.19 to 8.50)	0.80	Not collected	–
Current smoker	0.92 (0.54 to 1.56)	0.74	0.57 (0.26 to 1.26)	0.16
Chronic disease	2.65 (1.64 to 4.26)	<0.01	2.07 (1.38 to 3.09)	<0.01
Obese[b]	1.18 (0.73 to 1.92)	0.50	1.08 (0.45 to 2.56)	0.87
Self-defined well-being health status of poor or fair[c]	1.03 (0.62 to 1.71)	0.92	Not collected	–
Frailty[d]	3.25 (0.96 to 11.07)	0.06	Not collected	–
Dependence[e]	1.25 (0.55 to 2.83)	0.6	Not collected	–
Early in influenza season[f]	Not applicable	–	1.07 (0.68 to 1.67)	0.78

OR: adjusted odds ratio compared with referent group: female, aged 46–64 years, non-Māori, non-Pacific ethnicity, not in the New Zealand deprivation measure of the three lowest deciles (8,9 or 10), not pregnant, current non-smoker, without chronic disease, not obese, with self-rated health average or better, not on long-term oxygen use, living without assistance and admitted to hospital for severe acute respiratory infection during the peak influenza season.

[a] A meshblock measure reflecting eight dimensions of deprivation distributed into deciles.

[b] Subjective assessment of obesity by the clinician.

[c] A self-defined, standardised functional well-being health status score.

[d] Defined as currently on long-term oxygen use.

[e] Requirement for assistance with normal activity or full dependency on nursing care, as reported by the patient or caregiver.

[f] Admission or presentation before 1 June 2013.

48% (95% CI: 3 to 68) and for SARI 57% (95% CI: 36 to 71). When analysed by restricting to a shorter period around the peak (weeks 28–40), the VE for ILI was 46% (95% CI: 16 to 65) and for SARI 53% (95% CI: 27 to 50). For both SARI and ILI influenza-positive cases, the vaccination rate was constant over time.

The vaccine was significantly protective among patients aged 18–64 years. Specifically, VE was 61% (95% CI: 34 to 77) against SARI and 55% (95% CI: 24 to 73) against ILI. Although with wider CIs, the vaccine was also signficantly protective for those aged 0–17 years, with an estimated VE of 78% for SARI (95% CI: 2 to 95) and 56% for ILI (95% CI: 6 to 79). For those aged 65 years and older, VE point estimates were lower for SARI at 34%, although CIs crossed zero (95% CI: –28 to 66), and higher for ILI at 76% although with wide CIs (95% CI: 15 to 93) (Table 4).

For SARI patients, VE against influenza A was 39% (95% CI: 10 to 58) and against influenza B was 76% (95% CI: 54 to 87). For ILI patients, VE against influenza A was 58% (95% CI: 32 to 74) and against influenza B 54% (95% CI: 19 to 75) (Table 4).

The influenza viruses isolated from all of New Zealand during February to September 2013 were forwarded to the WHO Collaborating Centre for Research and Surveillance of Influenza in Melbourne, Australia, for further antigenic characterisation. Most of the New Zealand influenza A(H1N1) viruses reacted well with ferret antisera raised against A/California/7/2009 virus. Almost all of the A(H3N2) viruses reacted well with ferret antisera raised against cell-propagated A/Victoria/361/2011 or A/Texas/50/2012 viruses. B/Yamagata lineage viruses were the predominant B viruses in New Zealand in 2013. Although this lineage was included in the 2013 southern hemisphere vaccine formulation, antigenic drift was observed in these viruses, as they reacted better with ferret sera raised against B/Massachusetts/2/2012-like virus (selected for the southern hemisphere 2014 vaccine) than B/Wisconsin/1/2010 virus (included in the southern hemisphere 2013 vaccine).

Table 4

Estimated influenza vaccine effectiveness, by participant age group and by influenza virus type and subtype: crude and propensity adjusted models, New Zealand, 2013 influenza season

Influenza virus and age group	Hospitalised with severe acute respiratory infection		General practice visit for influenza-like illness	
	Crude model[a]	Propensity adjusted model[a]	Crude model[a]	Propensity adjusted model[a]
	VE (95% CI)	VE (95% CI)	VE (95% CI)	VE (95% CI)
Influenza virus type or subtype				
Overall	32 (7 to 50)	52 (32 to 66)	56 (37 to 70)	56 (34 to 70)
A(H1N1)	25 (−132 to 76)	48 (−74 to 85)	50 (−68 to 85)	49 (−90 to 86)
A(H3N2)	11 (−33 to 40)	34 (−2 to 57)	56 (27 to 74)	61 (32 to 77)
All A	15 (−21 to 40)	39 (10 to 58)	55 (29 to 71)	58 (32 to 74)
All B	65 (36 to 81)	76 (54 to 87)	60 (32 to 77)	54 (19 to 75)
Age group				
6 months–17 years	72 (−22 to 93)	78 (2 to 95)	56 (6 to 79)	56 (6 to 79)
18–64 years	66 (43 to −79)	61 (34 to 77)	59 (32 to 75)	55 (24 to 73)
≥65 years	35 (−25 to 66)	34 (−28 to 66)	74 (12 to 92)	76 (15 to 93)

VE: vaccine effectiveness, as a percentage.

[a] All models were adjusted for the number of weeks from the influenza peak.

Discussion

The 2013 New Zealand influenza season was characterised by low incidence and a late peak, with influenza A(H3N2) and influenza B most commonly detected. The circulating influenza A subtypes were antigenically similar to the H1 and H3 components of the 2013 vaccine, while the predominant circulating B viruses were lineage matched, although antigenic drift was observed.

This is the first study comparing VE against medically attended ILI and hospitalised SARI due to laboratory-confirmed influenza from the same population in the same season in New Zealand. The ILI surveillance season started and finished two weeks earlier than SARI surveillance. This may reflect a delay between onset of cases in the community and their referral to hospital.

After adjustment for the propensity to be vaccinated, we found moderate VE, around 50%, against both ILI and SARI, suggesting there was unlikely to be a substantial difference in VE by severity of influenza illness. However, the study was not powered to test for this difference. In addition, the patient groups differed by a number of important factors including age and ethnicity. The propensity score among patients with SARI varied much more than among those with ILI, due to the increased likelihood of vaccination among SARI patients and more available data on covariates. In particular, influenza was more often detected in the older age groups in the SARI patients and the adjustment for the propensity score therefore had a bigger effect for SARI patients. There was no significant change to VE estimates when the analysis was restricted to four days post onset of symptoms, rather than seven days.

The vaccine showed significant effectiveness against influenza A for ILI and SARI patients, with protection estimated to be lower for SARI patients. On the other hand, protection against influenza B appeared higher for the SARI patients, but the CIs overlapped for all comparisons. The vaccine prevented about 55–60% of ILI presentations and SARI hospitalisations in the 18–64-year age group. In the younger age group, the vaccine appeared to be more effective against SARI presentations compared with ILI, but the CIs were wide. The sample size was too small to make definitive VE estimates for the older age group.

Our point estimate for VE against medically attended influenza-confirmed ILI was very similar to northern hemisphere estimates for the 2012/13 influenza season, with interim adjusted estimates of 56% (95% CI: 47 to 63) from the United States [17], a United Kingdom mid-season estimate of 51% (95% CI: 27 to 68) [18] and a Canadian estimate of 50% (95% CI: 33 to 63) [19]. Similarly, VE results for ILI in the 2012/13 season from seven study sites in Europe reported the same virus circulation and adjusted estimates for the three circulating strains of 49% (95% CI: 32.4 to 62) for influenza B, 50% (95% CI: 28.4 to 65.6) for A (H1N1) and 42% (95% CI: 14.9 to 60.7) for A(H3N2) [20].

While we collected information on most suspected potential confounding variables, we could not control for residual confounders. In future years, we will collect data on previous presentations with respiratory illnesses and previous vaccination. New Zealand has added influenza vaccination to its national

immunisation register from 2014. This will provide more accurate vaccination history for SARI cases than patient self-report.

In conclusion, this study shows a moderate protective effectiveness of influenza vaccine against medically-attended and hospitalised influenza, generally supporting the current national immunisation strategy in New Zealand. Pooled data from future years of the SHIVERS study will allow more precise VE estimates for high-risk subgroups and will also allow more extensive comparisons between VE estimates in primary care (general practice) and hospital settings.

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Conflict of interest

None declared.

Authors' contributions

Nikki Turner: principal investigator, involved in study design, implementation, analysis, manuscript development. Nevil Pierse: involved in study design, methodological design, data analysis, interpretation and manuscript development. Ange Bissielo: involved in study design, data collection and analysis, and manuscript development. Q Sue Huang: principal investigator for the larger SHIVERS study, involved in study design, implementation, and manuscript development. Sarah Radke: involved in data collection, analysis, interpretation and manuscript development. Michael Baker: involved in study design, data interpretation and manuscript development. Marc-Alain Widdowson: involved in study design, analysis, interpretation and manuscript development. Heath Kelly: involved in study design, methodological analysis, data analysis and interpretation, manuscript development and editing.

* Erratum:

In Table 2, the column headings for number and percentage unvaccinated were incorrectly labelled. This was corrected on 29 August 2014. In addition, in Table 1, the column headings for cases and controls were incorrectly labelled. This was corrected on 22 June 2015.

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