Widespread circulation of pertussis and poor protection against diphtheria among middle-aged adults in 18 European countries

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Abstract

Reported incidence of pertussis in member states of the European Union (EU) and the European Economic Area (EEA) varies and may not reflect the real situation. In the EU/EEA, vaccine-induced protection against diphtheria and tetanus seems sufficient as few cases are reported even among the adult population who was vaccinated many years ago. Aim of this study was to determine the seroprevalence of pertussis, diphtheria and tetanus antibodies in EU/EEA countries within the age groups of 40-49 and 50-59 years. Eighteen countries collected around 500 samples between 2015 and 2018 (N=10,302 in total) and they were analysed for IgG-specific antibodies against pertussis toxin (PT), diphtheria toxoid (Dt) and tetanus toxin (TT). The proportion of sera with IgG-PT antibody levels ≥ 100 IU/mL, indicative for recent pertussis infection was comparable for 13/18 countries ranging between 4.0-6.4%. For diphtheria the proportion of sera lacking the protective level (<0.1 IU/mL) varied between 22.8% and 82.0%. For tetanus the protection was sufficient: only very few sera showed unprotective IgG-TT antibody levels. The seroprevalence of pertussis indicates that the circulation of *B. pertussis* is widespread across EU/EEA. The lack of vaccine-induced seroprotection against diphtheria in EU/EEA in these age groups is of concern and deserves further attention.

Introduction

Whooping cough or pertussis is a highly infectious disease that has resurged since the 1990s despite high vaccine coverage. The epidemic of 2012 in several countries resulted in the highest incidence of morbidity and mortality since the large-scale introduction of vaccines in the 1950s. The increase in the number of deaths in neonates, who are too young to be (fully) vaccinated, is particularly alarming. These new-borns represent the highest-risk group for fatal pertussis disease, and often parents and close family members (e.g. grandparents) are the main source of infection.^{1,2}

The incidence numbers of pertussis in European countries vary from 1 up to >100 per 100,000 inhabitants.^{3,4} Moreover, most natural infections among adolescents and adults due to *B. pertussis* result in mild or subclinical disease and are often not reported.⁵ This under-reporting of cases is recognised and the estimated rate of reported infections between European countries ranges from 1/1,000 up to 33/1,000 inhabitants, yearly.^{6,7} To get a better estimate of the circulation of *B. pertussis* in the population, sero-epidemiology is a valuable tool complementary to clinical surveillance programs. Serosurveillance of infections covered by the national vaccination programs is important because it provides relevant information about the pressure of infection and the immunological status of the population, and thus provides a tool to evaluate the risk of infection for not yet vaccinated infants. Furthermore, seroprevalence studies offer an opportunity to study waning immunity in the population. Several European countries have performed such studies for pertussis (Belgium, Denmark, Italy, the Netherlands, Sweden and United Kingdom).⁸⁻¹³ However, comparing serosurveillance studies between countries is not easy due to the wide variety of cohort selection criteria and laboratory tests that were used.⁹

No internationally accepted correlate of protection for pertussis has been established. However, because pertussis toxin (PT) is specific for *B. pertussis*, the level of antibodies against PT (IgG-PT in IU/mL) is used as an etiological marker of pertussis. Based on this marker the proportion of recent infections in the population can be estimated provided that individuals vaccinated at least in the last two years are excluded.^{14,15}

In contrast to pertussis, the incidence of diphtheria and tetanus according to the data reported to ECDC has been very low in the last decade across EU/EEA countries, due to the longstanding vaccination programmes and high coverage.¹⁶ Almost all EU/EEA countries reported a coverage of >90% for the third infant dose of DTP in the last decade.¹⁷ Diphtheria re-emerges when vaccination programmes are compromised and outbreaks have been observed following drops in vaccination coverage to 60% and 80% in the former Soviet Union and former Soviet republics during 1990-1995¹⁸, and more recently in Venezuela.¹⁹

The aim of this study was to determine the seroprevalence levels of IgG-PT, IgG-Dt and IgG-TT within two specific age groups (i.e. 40-49 and 50-59 years of age) in EU/EEA countries to explore the proportion of sera indicative for a recent pertussis infection to determine the circulation of *B. pertussis*, and the persistence of vaccine-induced protection against diphtheria and tetanus in EU/EEA.

Methods

Study design and sample collection

This study was organised by the National Institute of Public Health and the Environment (RIVM) and the University of Turku (UTU) under contract of ECDC/EUPert-LabNet (ECDC/2015/009). All national reference laboratories in EU/EEA countries were invited to participate. The study design was a random sample of retrospective collections of anonymised leftover serum samples or anonymised samples from nationwide serosurveillance studies with formal approval from a medical ethical committee during 2015-2018. No individual donor information was requested except age and date of sampling. Serum samples should not have been collected from individuals suspected for respiratory-infections. The successive age groups of 40-49 years and 50-59 years were chosen, and the recommended number of samples collected per country in each age group was 250 for a prevalence estimation of 20% with 95% confidence intervals (CI) in each age group independently of each other and regardless of country population. An equal distribution of sera from male and female donors was preferred.

Procedures

In this study, centralised testing for all collected samples was performed with the multiplex immunoassay (MIA). The use of the validated DTP-MIA showing 1:1 correlation with the original FDA-ELISA for pertussis and 1:1 correlation with the neutralisation assay for diphtheria²⁰⁻²² offered the advantage of measuring PT, Dt and TT antibodies simultaneously in one assay run^{23,24}. In short, purified antigens were coupled

covalently to distinct color-coded activated carboxylated beads (Luminex, Austin, Texas, USA). Serum samples were measured in two dilutions (1/200 and 1/4,000), with in-house references calibrated against international standards, control sera and blanks included on each plate and MFI was converted to IU/mL by interpolation from a five-parameter logistic standard curve. As control for possible drift of the assay in time and different bead batches used, 5% of randomly selected samples per country were assessed in an extra duplicate measurement.

Outcomes

The first primary outcome was the proportion of samples from the middle-aged cohorts with a suspected recent pertussis infection. In absence of an internationally accepted correlate of protection for pertussis, the cut-off value of IgG-PT ≥ 100 IU/mL was defined as indicative for a recent infection and a level of ≥ 50 IU/mL for the prevalence of infection two years prior the sample being taken.^{11,25} In addition, the proportion of sera below the level of 5 IU/mL was used as indication of subjects potentially susceptible to pertussis. The second primary study outcome was to estimate the level of vaccine-induced protection against diphtheria and tetanus based on the WHO cut-off level of 0.01 IU/mL for basic immunity and 0.1 IU/mL for protection for anti-diphtheria (IgG-Dt) and anti-tetanus (IgG-TT) antibodies^{26,27}. The lower limit of quantification (LLOQ) was 0.85 IU/mL for PT and 0.001 IU/mL for Dt and TT.

Statistical analysis

The following information was collected: country, laboratory and location of sample collection, date of collection, sample size, age group, gender and type of collection. The final database included approximately 31,000 IgG results. Antibody levels below the LLOQ were replaced by LLOQ/2. The outcomes were grouped by country, age category (40-49 years, 50-59 years, total) and gender. For each group, seroprevalences were calculated with their corresponding 95% CI using the exact method²⁸ and geometric mean concentrations (GMCs) with 95% CI were calculated based on the t-distribution. *P*-values for differences in seroprevalences between groups were calculated using Fisher's exact 2x2 test and differences in GMCs between groups using the two-sample t-test. All reported *p*-values are 2-sided, *p*-values smaller than 0.05 are considered significant. Overall differences in seroprevalences between age groups, gender and countries were assessed using the likelihood ratio test on a binomial generalised linear regression model. All statistical analyses were carried out in R (version 3.6.1).

Role of the funding source

The funder of the study had a role in the study design, data interpretation, and writing of the manuscript, but no role in data collection or data analysis. The corresponding authors had full access to all data from the study, and final responsibility for the decision to submit for publication was by consensus of all co-authors.

Results

From the 28 invited countries, 18 countries agreed to participate and shipped around 250 samples for both targeted age cohorts of 40-49 and 50-59 years to the RIVM. The characteristics of the sample collections like collection period (2015-2018) and locations are summarised in Table 1. The gender distribution of the samples was obtained from 16 countries. To avoid a selection bias, all samples (N=1,644) from the two age groups collected in a national serosurveillance study in the Netherlands were included. UK data comprised samples from England only. Altogether, the number of subjects included was 10,302.

The percentages of sera per country with a level for IgG-PT ≥ 100 IU/mL varied between 1.8% (Finland) and 9.4% (Norway) with 13/18 countries showing a level between 4.0% and 6.4 % (Figure 1; Table 2). The levels between 50-100 IU/mL for IgG-PT (Table 2), ranged between 4.8% (United Kingdom) and 9.9% (France) for all countries, excluding Norway with a level of 12.5%. The proportion of subjects potentially susceptible to infection, IgG-PT < 5 IU/mL, varied between 22.1% (Norway) and 41.5% (United Kingdom) (Table 2). The seroprevalence (IgG-PT ≥ 100 IU/mL) of the two age cohorts separately and in total per country is illustrated in Figure. 2A with a subdivision per gender. We found no influence of age and gender on the seroprevalence overall ($p=0.138$ and $p=0.06$ resp.), but the country effect was highly significant ($p<0.001$). For overall seroprevalence of IgG-PT levels 50-100 IU/mL or >50 IU/mL, no effect was found for age ($p=0.305$, $p=0.071$ resp.), the country effect remained (both $p<0.001$), but the gender effect was now significant ($p=0.001$, $p<0.001$) (Suppl. Table 1). The GMC values for IgG-PT antibodies ranged from 7.2 to 14.8 IU/mL within the 18 countries (Table 3). Significant GMC differences between the age cohorts were found for Austria, Lithuania, Latvia, the Netherlands and Norway with elevated IgG-PT concentrations in the 50-59 year cohort, except for Austria (Table 3). Between gender, significant GMC differences could be observed in the total cohort for Finland, Hungary, the Netherlands and Portugal with elevated IgG-PT concentrations in males (Suppl. Figure. 1).

The proportion of sera with Dt antibody levels below the basic immunity level of 0.01 IU/mL varied between 4% (Finland) and 43% (Greece) and for the protective level of 0.1 IU/mL from 23% for Finland up to around 80% for Greece, Ireland, Romania and United Kingdom (Figure.1 and Table 2). Age, gender and country had a significant influence on the seroprotection for both cut-offs ($p<0.001$, Suppl. Table 1). Significant differences for gender within the countries were found for the levels <0.01 IU/mL in the 40-49 years groups for Denmark, the Netherlands and Sweden, in the 50-59 year olds for Hungary, the Netherlands and United Kingdom and in the total cohorts for Denmark, the Netherlands and United Kingdom (Figure 2B). For the levels <0.1 IU/mL differences in gender were found in the 40-49 year olds for Denmark, Hungary, Ireland and the Netherlands, in the 50-59 year olds for Denmark and the Netherlands, and in the total cohorts for Denmark, Ireland and the Netherlands. The GMC's of IgG-Dt levels were low for all participating countries, not exceeding 0.1 IU/mL in the total cohorts for 11/18 countries (Table 3). The GMC's in the 50-59 year olds were always lower than those of the 40-49 year olds, except for the Netherlands and Romania equally low and a significant difference in GMC between the age groups was found in 12/18 countries (Table 3).

In contrast with diphtheria, the seroprotection levels for tetanus were sufficient with only very few sera lacking basic immunity (Figure. 1; Table 2). The proportion of sera with levels below 0.01 IU/mL ranged from 0-1.2%, apart from Greece (2.8%). For the total cohort, seven countries were considered as fully protected (Austria, Finland, France, Hungary, Latvia, Portugal and Slovenia). The protective level of 0.1 IU/mL was reached in more than 90% of the sera in all countries, apart from Greece (79%) and Ireland (83%). In the other 16 countries the proportion of sera with unprotected levels (<0.1 IU/mL) ranged from 0.4% to 8.2%. Whereas age, gender and country had significant impact on the seroprotection level (<0.1 IU/mL, $p<0.001$), the unprotected level (<0.01 IU/mL) depended only on age and country ($p=0.003$, $p<0.001$ resp.), but not gender ($p=0.061$) (Suppl. Table 1). Between countries no significant gender differences were found for the unprotected level in both age groups and the total cohort (Figure 2C). However, for the seroprotection level gender differences were found in the age group 40-49 years for Sweden, in the 50-59 years for Denmark, Hungary, Ireland and Latvia, and in the total cohorts for Belgium, Denmark, Hungary, Latvia and Sweden. The GMC's of IgG-TT were above 1.0 IU/mL in 14 countries ranging from 1.15 IU/mL to 3.16 IU/mL, and below 1.0 IU/mL in 4 countries ranging from 0.36 IU/mL to 0.83 IU/mL. In 12 countries the GMC's in the 50-59 years olds were lower than in the 40-49 year olds. Significant differences in GMC between the age groups were found in France, Greece, Latvia and Romania (Table 3).

Table 1. Summary of sample collections: Period of sample collection, number of samples collected by age group, type of samples and geographical origin by country. The participating countries are listed in alphabetical order of the complete name and not according to the abbreviation.

Country	Collection time	Number of samples			Type of samples	Geographical origin
		40–49 y	50–59 y	Total		
Austria (AT)	2015-2016	250	250	500	Left over samples	Vienna, Lower Austria, Carinthia, Salzburg, Burgenland
Belgium (BE)	2017	252	252	504	left over samples	Liège, Charleroi, Brussels, Bruges, Ghent
Denmark (DK)	2015-2016	249	242	491	Left over samples (diagnostic laboratories)	Whole country
Finland (FI)	2015-2016	250	250	500	Left over samples (patients with suspected coeliac disease)	Varsinais-Suomi, Pohjanmaa
France (FR)	2015-2016	299	298	597	Left over samples	Institut Pasteur, Paris
Greece (GR)	2015	250	250	501	Left over samples	Attikon University General Hospital, Athens
Hungary (HU)	2017	260	273	533	Left over samples (health care workers)	Békés, Budapest, Csongrád, Hajdú, Szabolcs
Ireland (IE)	2016-2017	250	249	499	Left over samples (patients)	Whole country
Latvia (LV)	2015-2016	250	250	500	Left over samples	Riga
Lithuania (LT)	2016	250	250	500	Left over samples (people admitted to outpatient clinics)	Kaunas
Netherlands (NL)	2016-2017	830	814	1644	Population-based seroprevalence study	48 municipalities of the Netherlands
Norway (NO)	2015-2016	251	251	502	Left over samples (Immunol. and clin. biochemistry dept.)	Akershus
Portugal (PT)	2015-2016	250	250	500	Population-based seroprevalence study	Alentejo, Algarve, Centro, Lisboa, Norte, Açores, Madeira
Romania (RO)	2018	252	252	504	Left over samples	41 counties of Romania + Bucuresti Municipality

					(emergency hospital laboratories)	
Slovak Republic (SK)	2016- 2018	250	250	500	Left over samples (patients inspected for borreliosis)	Banská Bystrica
Slovenia (SI)	2015- 2016	263	261	524	Left over samples (diagnostic borreliosis and measles)	Kranj, Maribor, Nova Gorica, Ljubljana
Sweden (SE)	2016	253	251	504	Left over samples (clinical chemistry labs)	Halland, Jämtland/Härjedalen, Jönköping, Kalmar, Skåne, Stockholm, Västerbotten, Västra, Götaland, Örebro, Östergötland
United Kingdom (UK)	2015- 2016	250	249	499	Left over samples (genitourinary medicine)	Manchester, Exeter, Newcastle, Leicester, Leeds, London, Cambridge

Table 2. Percentages of pertussis seroprevalence and Dt and TT seroprotection per country and by age group.

Country	Age group	N total	IgG-PT (IU/mL)					IgG-Dt (IU/mL)				IgG-TT (IU/mL)			
			<5	50 to <100		≥100		<0.01		<0.1		<0.01		<0.1	
			%	%	N	%	N	%	N	%	N	%	N	%	N
AT	40-49	250		11.2	28	5.2	13	10.4	26	53.6	134	0.0	0	1.6	4
	50-59	250		5.2	13	3.6	9	20.0	50	59.2	148	0.0	0	1.6	4
	Total	500	31.0	8.2	41	4.4	22	15.2	76	56.4	282	0.0	0	1.6	8
BE	40-49	252		7.1	18	7.5	19	13.5	34	54.8	138	0.0	0	6.0	15
	50-59	252		11.5	29	7.1	18	27.8	70	63.1	159	1.2	3	8.7	22
	Total	504	27.8	9.3	47	7.3	37	20.6	104	58.9	297	0.6	3	7.3	37
DK	40-49	249		7.6	19	5.6	14	12.9	32	52.6	131	0.4	1	4.0	10
	50-59	242		9.5	23	11.2	27	15.3	37	52.9	128	0.0	0	5.8	14
	Total	491	28.9	8.6	42	8.4	41	14.1	69	52.8	259	0.2	1	4.9	24
FI	40-49	250		4.0	10	2.0	5	3.2	8	20.4	51	0.0	0	0.4	1
	50-59	250		6.8	17	1.6	4	4.4	11	25.2	63	0.0	0	0.4	1
	Total	500	34.4	5.4	27	1.8	9	3.8	19	22.8	114	0.0	0	0.4	2
FR	40-49	299		9.4	28	4.4	13	2.0	6	23.1	69	0.0	0	2.7	8
	50-59	298		10.4	31	8.1	24	10.7	32	48.0	143	0.0	0	3.4	10
	Total	597	22.6	9.9	59	6.2	37	6.4	38	35.5	212	0.0	0	3.0	18
GR	40-49	250		6.0	15	4.8	12	26.8	67	72.4	181	0.0	0	11.6	29
	50-59	250		5.2	13	3.2	8	59.6	149	91.6	229	4.8	12	31.2	78
	Total	501	35.1	5.6	28	4.0	20	43.3	217	82.0	411	2.4	12	21.4	107
HU	40-49	260		5.0	13	1.5	4	3.5	9	60.4	157	0.0	0	2.7	7
	50-59	273		4.8	13	3.7	10	12.5	34	68.1	186	0.0	0	2.2	6
	Total	533	38.3	4.9	26	2.6	14	8.1	43	64.4	343	0.0	0	2.4	13
IE	40-49	250		8.4	21	5.6	14	33.2	83	76.4	191	0.4	1	12.8	32
	50-59	249		10.4	26	5.6	14	41.8	104	77.1	192	2.0	5	21.3	53
	Total	499	28.9	9.4	47	5.6	28	37.5	187	76.8	383	1.2	6	17.0	85
LV	40-49	250		4.8	12	4.0	10	1.2	3	22.0	55	0.0	0	0.8	2
	50-59	250		9.6	24	5.2	13	9.2	23	44.0	110	0.0	0	3.6	9
	Total	500	34.6	7.2	36	4.6	23	5.2	26	33.0	165	0.0	0	2.2	11
LT	40-49	250		7.2	18	4.8	12	2.8	7	27.6	69	0.4	1	3.6	9
	50-59	250		10.8	27	8.0	20	10.8	27	48.4	121	0.8	2	8.4	21
	Total	500	23.4	9.0	45	6.4	32	6.8	34	38.0	190	0.6	3	6.0	30
NL	40-49	830		8.6	71	4.9	41	12.3	102	56.5	469	0.5	4	4.6	38
	50-59	814		7.5	61	5.9	48	13.3	108	58.6	477	0.1	1	5.8	47
	Total	1644	29.9	8.0	132	5.4	89	12.8	210	57.5	946	0.3	5	5.2	85
NO	40-49	251		12.0	30	7.6	19	5.6	14	31.9	80	0.0	0	3.6	9
	50-59	251		13.1	33	11.2	28	8.8	22	32.7	82	0.4	1	6.8	17
	Total	502	22.1	12.5	63	9.4	47	7.2	36	32.3	162	0.2	1	5.2	26
PT	40-49	250		6.0	15	4.0	10	7.6	19	42.4	106	0.0	0	0.4	1
	50-59	250		6.4	16	4.4	11	10.0	25	41.6	104	0.0	0	0.4	1
	Total	500	37.6	6.2	31	4.2	21	8.8	44	42.0	210	0.0	0	0.4	2
RO	40-49	252		7.1	18	4.0	10	25.8	65	77.4	195	0.0	0	4.0	10
	50-59	252		11.1	28	4.0	10	25.0	63	74.2	187	0.4	1	12.3	31
	Total	504	28.0	9.1	46	4.0	20	25.4	128	75.8	382	0.2	1	8.1	41
SK	40-49	250		6.0	15	7.2	18	5.2	13	64.4	161	0.0	0	2.0	5
	50-59	250		5.6	14	4.8	12	6.8	17	71.2	178	0.4	1	1.2	3
	Total	500	34.8	5.8	29	6.0	30	6.0	30	67.8	339	0.2	1	1.6	8
SI	40-49	263		7.2	19	4.6	12	1.9	5	26.6	70	0.0	0	0.4	1
	50-59	261		5.0	13	3.8	10	10.3	27	37.9	99	0.0	0	1.5	4
	Total	524	33.0	6.1	32	4.2	22	6.1	32	32.3	169	0.0	0	1.0	5

SE	40-49	253		9.5	24	6.3	16	11.5	29	58.5	148	0.0	0	5.5	14
	50-59	251		6.0	15	4.0	10	21.1	53	62.2	156	0.4	1	6.4	16
	Total	504	29.2	7.7	39	5.2	26	16.3	82	60.3	304	0.2	1	6.0	30
UK	40-49	250		4.0	10	5.2	13	24.8	62	71.6	179	1.2	3	7.6	19
	50-59	249		5.6	14	4.4	11	35.7	89	79.1	197	0.4	1	8.8	22
	Total	499	41.5	4.8	24	4.8	24	30.3	151	75.4	376	0.8	4	8.2	41

Table 3. GMCs for IgG-PT, IgG-Dt and IgG-TT in IU/mL per country and by age group. P values are given for the difference between the two age groups.

Country	Age group	IgG-PT (IU/mL)		IgG-Dt (IU/mL)		IgG-TT (IU/mL)	
		GMC (95%CI)	p-value	GMC (95%CI)	p-value	GMC (95%CI)	p-value
AT	40-49	11.8 (9.8-14.1)		0.08 (0.06-0.10)		1.84 (1.64-2.07)	
	50-59	7.8 (6.5-9.4)		0.05 (0.04-0.07)		1.85 (1.63-2.10)	
	Total	9.6 (8.4-10.9)	0.002	0.06 (0.05-0.08)	0.014	1.85 (1.70-2.01)	0.952
BE	40-49	12.1 (9.9-14.7)		0.07 (0.06-0.09)		1.64 (1.38-1.95)	
	50-59	12.4 (10.3-14.9)		0.04 (0.03-0.05)		1.34 (1.09-1.64)	
	Total	12.2 (10.7-14.0)	0.838	0.05 (0.04-0.06)	<0.001	1.48 (1.30-1.69)	0.136
DK	40-49	10.8 (9.0-13.0)		0.08 (0.07-0.10)		1.80 (1.52-2.13)	
	50-59	13.3 (10.9-16.2)		0.08 (0.06-0.10)		2.02 (1.70-2.41)	
	Total	12.0 (10.5-13.7)	0.142	0.08 (0.07-0.09)	0.754	1.91 (1.69-2.15)	0.346
FI	40-49	7.4 (6.3-8.8)		0.29 (0.24-0.35)		3.23 (2.93-3.57)	
	50-59	8.0 (6.8-9.5)		0.22 (0.18-0.26)		3.10 (2.77-3.47)	
	Total	7.7 (6.8-8.7)	0.518	0.25 (0.22-0.29)	0.030	3.16 (2.94-3.41)	0.582
FR	40-49	12.9 (11-15.1)		0.27 (0.23-0.33)		1.94 (1.72-2.20)	
	50-59	14.0 (11.9-16.5)		0.09 (0.08-0.11)		1.28 (1.12-1.45)	
	Total	13.4 (12.0-15.0)	0.488	0.16 (0.14-0.18)	<0.001	1.58 (1.44-1.73)	<0.001
GR	40-49	8.0 (6.6-9.8)		0.03 (0.02-0.04)		0.62 (0.51-0.75)	
	50-59	7.6 (6.3-9.2)		0.01 (0.01-0.01)		0.21 (0.17-0.26)	
	Total	7.8 (6.8-8.9)	0.696	0.01 (0.01-0.02)	<0.001	0.36 (0.31-0.42)	<0.001
HU	40-49	7.5 (6.4-8.7)		0.08 (0.07-0.09)		2.38 (2.02-2.79)	
	50-59	8.2 (6.9-9.7)		0.05 (0.05-0.06)		2.24 (1.92-2.62)	
	Total	7.8 (7.0-8.8)	0.464	0.07 (0.06-0.07)	0.002	2.31 (2.06-2.58)	0.614
IE	40-49	10.7 (8.9-12.8)		0.02 (0.02-0.03)		0.79 (0.64-0.97)	
	50-59	12.2 (10.1-14.7)		0.02 (0.01-0.02)		0.64 (0.50-0.81)	
	Total	11.4 (10.0-13.0)	0.322	0.02 (0.02-0.02)	0.026	0.71 (0.60-0.83)	0.184
LV	40-49	7.0 (5.8-8.4)		0.26 (0.22-0.30)		1.94 (1.70-2.20)	
	50-59	11.1 (9.3-13.1)		0.11 (0.09-0.14)		1.42 (1.21-1.65)	
	Total	8.8 (7.8-10.0)	<0.001	0.17 (0.15-0.20)	<0.001	1.66 (1.50-1.83)	0.002
LT	40-49	10.6 (8.9-12.6)		0.17 (0.14-0.20)		1.29 (1.10-1.51)	
	50-59	15.0 (12.8-17.6)		0.08 (0.07-0.10)		1.03 (0.85-1.24)	
	Total	12.6 (11.2-14.2)	0.004	0.12 (0.10-0.13)	<0.001	1.15 (1.02-1.30)	0.076
NL	40-49	9.7 (8.7-10.7)		0.06 (0.06-0.07)		1.24 (1.13-1.36)	
	50-59	11.2 (10.2-12.4)		0.06 (0.06-0.07)		1.20 (1.10-1.32)	
	Total	10.4 (9.7-11.2)	0.038	0.06 (0.06-0.07)	0.692	1.22 (1.14-1.30)	0.690
NO	40-49	13.0 (10.9-15.6)		0.17 (0.14-0.21)		1.45 (1.24-1.68)	
	50-59	16.9 (14.0-20.3)		0.16 (0.13-0.19)		1.18 (0.98-1.41)	
	Total	14.8 (13.0-16.9)	0.048	0.16 (0.14-0.19)	0.538	1.31 (1.16-1.47)	0.084
PT	40-49	6.9 (5.6-8.4)		0.12 (0.10-0.15)		2.63 (2.36-2.92)	
	50-59	8.8 (7.3-10.6)		0.11 (0.09-0.14)		2.63 (2.38-2.91)	
	Total	7.8 (6.8-8.9)	0.070	0.12 (0.10-0.14)	0.708	2.63 (2.44-2.83)	0.974
RO	40-49	9.9 (8.3-11.9)		0.03 (0.02-0.04)		0.80 (0.69-0.93)	
	50-59	10.9 (9.2-12.9)		0.03 (0.02-0.04)		0.56 (0.47-0.66)	
	Total	10.4 (9.2-11.8)	0.470	0.03 (0.03-0.03)	0.756	0.67 (0.60-0.75)	0.002
SK	40-49	9.5 (7.8-11.6)		0.06 (0.05-0.07)		1.56 (1.36-1.80)	
	50-59	8.2 (6.8-9.9)		0.05 (0.04-0.06)		1.64 (1.42-1.89)	
	Total	8.8 (7.7-10.1)	0.274	0.06 (0.05-0.06)	0.052	1.60 (1.45-1.77)	0.632
SI	40-49	10.4 (8.8-12.2)		0.18 (0.15-0.21)		2.03 (1.79-2.30)	
	50-59	8.6 (7.3-10.2)		0.12 (0.09-0.14)		2.01 (1.76-2.30)	
	Total	9.4 (8.4-10.6)	0.132	0.14 (0.13-0.16)	0.002	2.02 (1.85-2.21)	0.916
SE	40-49	11.6 (9.7-13.9)		0.07 (0.06-0.09)		1.14 (0.97-1.36)	

	50-59	9.4 (7.9-11.1)		0.05 (0.04-0.06)		1.22 (1.01-1.46)	
	Total	10.4 (9.2-11.8)	0.092	0.06 (0.05-0.07)	0.016	1.18 (1.04-1.34)	0.626
UK	40-49	7.3 (6.1-8.8)		0.03 (0.03-0.04)		0.86 (0.72-1.02)	
	50-59	7.2 (5.9-8.7)		0.02 (0.02-0.03)		0.80 (0.67-0.95)	
	Total	7.2 (6.3-8.3)	0.904	0.03 (0.02-0.03)	0.006	0.83 (0.73-0.93)	0.578

Discussion

The proportion of sera with an IgG-PT antibody level ≥ 100 IU/mL indicative for a recent pertussis infection was comparable for 13 out of 18 EU/EEA countries ranging between 4.0-6.4% with outliers up to 1.8% and 9.3% as illustrated by RCDCs (Figure. 3). In addition, the GMC's of IgG-PT antibodies in all countries varied between 7-15 IU/mL, suggesting that the epidemiological situation for pertussis across EU/EEA is broadly similar. In contrast, for diphtheria the proportion of sera with no basic immunity showed a broad range between 3.8-43.3%. For the protective level these proportions ranged from 22.8% to 82.0%, suggesting that the protection against diphtheria is insufficient in older age cohorts in most EU/EEA countries. For tetanus the protection seems sufficient with only very few sera lacking basic immunity. More than 90% of the sera from all countries possessed protective levels except one country with 83%. To our best knowledge, this is the largest seroprevalence study of pertussis, diphtheria and tetanus conducted in EU/EEA since DTP vaccines were introduced, using centralised testing of specific antibody levels to minimise the variation of methods used, and which enables direct comparison between all participating countries.

In Europe, vaccination programs including whole cell pertussis (wP) vaccines were implemented during the 1950s, so the majority of participants of this study would have received a wP vaccine. However, it may be expected that due to waning immunity the vaccinated participants are susceptible to infection just like the non-vaccinated and have been re-infected potentially with milder symptoms. From the late 1990s up until 2006 all European countries (except Poland) switched to acellular pertussis vaccines (aP). However, despite continuous high pertussis infant vaccination coverage in most countries ($\geq 95\%$) the pathogen is still circulating. Based on the ECDC and WHO websites^{3,4} an increase in the incidence of pertussis has been reported during the last decade in Austria, Belgium, Denmark, Ireland, Latvia, the Netherlands, Norway, Slovak Republic, Slovenia, Sweden and United Kingdom, although increased awareness and improved laboratory diagnostics by serology and PCR also could have contributed. After the initial increase, the incidence has remained high in most countries, while in the other countries (Finland, France, Greece, Hungary, Lithuania, Portugal and Romania) low incidence was reported.

In 11 countries, the serum collection period coincided with the increase and subsequent higher incidence. In the other seven countries, low incidence numbers were reported during that period. Distribution of the collection period into three groups (2015-2016, 2016-2017 and ≥ 2017) revealed no statistically significant influence on the pertussis seroprevalence data. Also, the geographical origin of the samples did not affect the pertussis serosurveillance outcome when the countries were divided into three groups of

one location, 2-7 locations or whole country. The source of the serum samples was very diverse: from patients (three countries), from healthy people (three countries), but mostly of unknown origin consisting of leftover samples for diagnostics (12 countries). Therefore, the possibility that the different sources might have affected the results seemed minimal considering the non-matching different outcomes per country and source. Whereas no age and gender effect on the seroprevalence results for IgG-PT $\geq$ 100 IU/mL for the whole study was observed, the country effect was very clear because the whole range of proportions of recently infected participants in the EU/EEA was still quite large. This country effect might be explained by the differences in pertussis vaccination schedules, including adult boosters, and vaccines used in the EU/EEA countries throughout the years. Geography and density of the population did not seem to play a role, as Finland and Norway (extremes) are both low-density Nordic countries. A trend towards higher GMC's in males was observed in 14 countries reaching significance in four countries, while in the two other countries GMC's were almost identical between females and males. This might be due to booster vaccinations for the military service and/or a slightly better immune response upon natural infection in males. Gender-specific susceptibility for pertussis might be a relevant factor.

This cross-sectional seroprevalence study shows widespread circulation of pertussis among these middle-aged adults in EU/EEA despite well implemented childhood vaccination programs and underscores the need for vigilant surveillance of pertussis. This study also emphasizes that a large proportion of middle-aged adults in most EU/EEA countries is still susceptible for pertussis infection as the percentages of subjects with IgG-PT $<$ 5 IU/mL are high. This situation is of concern for young infants at high risk for serious disease, because recent studies have shown that parents, siblings and close family (grandparents) are the main source of whooping cough in infants.^{1,2} The national vaccination programs for pertussis are designed to protect vulnerable infants, but despite the high vaccine coverage, an increasing incidence of pertussis among children has been reported. This has led to a renewed focus on how to protect infants, such as maternal immunisation, which might be needed to prevent pertussis but must be considered nationally and should take into account the local epidemiology.²⁹ In EU/EEA, ten countries have now implemented maternal immunization which has been found highly effective at preventing pertussis in young infants from birth until they receive primary vaccinations.^{30,31} Also, 11 countries have implemented more aP vaccinations, like adolescent boosters, boosters for military service, adult boosters and/or cocooning. However, only a high country-wide coverage of these boosters might have a significant influence on the pertussis incidence. Moreover, there is evidence that the immunogenicity of repeated aP boosters seems to diminish, although the persistence of aP vaccine-induced antibodies after a first booster in wP-primed adults appeared to be longer compared to children.³²

The reported pertussis cases of the study countries varied enormously ranging from below 1 per 100,000 citizens up to 500/100,000, annually.^{3,4} In this study we show a heavy burden of pertussis infection in the adult population consistent with other serosurveillance studies⁸⁻¹³, suggesting a large underreporting of adult pertussis cases. Many of these cases will occur as mild infections with subclinical symptoms and are therefore not reported and not captured by routine surveillance systems.³³ Although the real ratio of pertussis infections to reported cases, and thus disease incidence, is unknown, it has been shown that

between 13% to 25% of adults with prolonged cough have high levels of pertussis antibodies³⁴, demonstrating that the current monitoring system based on case reporting is under-ascertaining the true burden of disease. To get a better estimate of the circulation of *B. pertussis* in the population, (regular) sero-epidemiology is a valuable tool complementary to surveillance programs based on case reporting.

The high proportion of sera with unprotected levels for diphtheria is of concern, leaving at least a quarter up to over three-quarters of the middle-aged adult population sampled not well protected against diphtheria. Presumably the vaccine-induced antibody levels against diphtheria have waned in these middle-aged adult cohorts, while the original responses to the primary series in the first year of life would be expected to have been good and in many countries (several) boosters have been administered during childhood. In some countries DT boosters are administered when people travel to endemic countries, but this is too sporadic to influence the outcome of this study. From an epidemiological perspective the protection against diphtheria seems sufficient because no increase in cases has been noticed in EU/EEA during the last decades but import from locations with diphtheria outbreaks remains a real threat. Age, gender and country affected the seroprotection levels in this study. The age effect can be explained by waning immunity due to ageing. The country effect can be attributed to the different vaccination schedules, but not to the used vaccines because the Dt component is similar in most combination vaccines. The gender effect is rather surprising, and might be interpreted as a gender-specific difference in immunity. Moreover, a trend towards higher IgG-Dt antibody levels in females compared to males in 12/14 countries was found reaching significance in five countries. Waning of diphtheria immunity over the years appears to proceed faster in males than in females. Overall, the protection against diphtheria in EU/EEA in the older age groups is suboptimal and certainly not sufficient, and might indicate a need for boosting immunity. It emphasizes the potential risk of suboptimal protection against diphtheria in a time of high population mobility, outbreaks in certain parts of the world and the global shortage of DAT.

In contrast with diphtheria, the seroprotection levels for tetanus were very reassuring leaving only 38 (3.7‰) sera without protective levels and in seven countries seroprotection was complete. The immunogenicity of the tetanus vaccine is superior to the diphtheria vaccine as reflected by higher vaccine-induced antibody responses to tetanus in numerous vaccine studies in all age groups.^{26,27} The age and country effect on the seroprotection levels were similar to diphtheria. For gender the effect reached significance only at the protective cut-off in the whole study and within the countries there was also a trend towards higher levels of tetanus antibodies in females compared to males in 12/14 countries reaching significance in six countries, pointing to a gender-specific difference in immunity.⁹ Overall, the protection against tetanus in EU/EEA needs no extra further action.

In conclusion, this cross-sectional retrospective seroprevalence study among middle-aged adults in 18 EU/EEA countries showed that the circulation of *B. pertussis* is widespread despite highly implemented childhood vaccination programs. Furthermore, it indicates a large underreporting of pertussis cases, also in the middle-aged population. Clearly the current monitoring system of pertussis based on case reporting is under-ascertaining disease, emphasizing that the current monitoring system of pertussis based on case reporting is not accurate enough and that sero-epidemiology is a valuable tool to monitor disease

complementary to the current surveillance programs. For diphtheria, the proportion of sera with non-protective IgG levels is of concern, leaving between 23% and 82% of the middle-aged population unprotected. Therefore, the protection against diphtheria in EU/EEA in these older age groups is not sufficient and deserves proper attention. In contrast to diphtheria, the seroprotection levels for tetanus were much higher, leaving only 3.7% of the sera with non-protective IgG levels. The seroprotection against tetanus is reassuring warranting no extra action but still requires ongoing monitoring to ensure this situation remains unchanged.

Declarations

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The authors declare that they have no conflict of interest.

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Figures

Figure 1

Relative distribution of samples by IgG-PT (A), IgG-Dt (B) and IgG-TT (C) intervals and by country. Proportion of pertussis seroprevalence divided in samples <5 IU/mL (grey), 5 to <50 IU/mL (white), 50 to <100 IU/mL (light grey) and ≥ 100 IU/mL (dark grey) and seroprotection against diphtheria and tetanus in <0.01 IU/mL, 0.01 to <0.1 IU/mL and ≥ 0.1 IU/mL within the 18 countries. The bars sum up to 100%.

A

B

C

Figure 2

A. Percentage of pertussis infected sera in the two age groups (40-49 and 50-59) separately and in the total cohort, and subdivided by gender per country (Y-axis). On the X-axis the percentage of seroprevalence for PT ≥ 100 IU/mL is displayed. B. Percentage of sera protected against Diphtheria in the two age groups (40-49 and 50-59) separately and in the total cohort, and subdivided by gender per country (Y-axis). On the X-axis the percentage of lack of seroprotection against diphtheria for IgG-Dt < 0.01

IU/mL and <0.1 IU/mL is displayed. C. Percentage of sera protected against Tetanus in the two age groups (40-49 and 50-59) separately and in the total cohort, and subdivided by gender per country (Y-axis). On the X-axis the percentage of lack of seroprotection against tetanus for IgG-TT <0.01 IU/mL and <0.1 IU/mL is displayed.

Figure 3

Reverse cumulative distribution curves for IgG-PT (IU/mL) illustrated by increasing values at 50% per country. Values below the LLOQ are not included.

Supplementary Files

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