

## Evidence of increased circulation of *Bordetella pertussis* in the Italian adult population from seroprevalence data (2012–2013)

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Incidence data on pertussis cases in Italy do not show pertussis resurgence as recently described in other European countries. The aim of this study was to determine the seroprevalence of IgG antibodies to pertussis toxin (PT-IgG) in selected adult age groups, who can serve as a reservoir of *Bordetella pertussis* and be responsible for onward transmission to vulnerable infants. The seroprevalence of PT-IgG was studied in sera collected in 2012–2013 in three age groups: 20–29 years and 30–39 years (reproductive age), and  $\geq 60$  years. These data were compared to those from sera collected in similar age groups in 1996–1997. More than 80% of the adult population analysed in the 2012–2013 group presented detectable levels of PT-IgG ( $>5$  IU ml<sup>-1</sup>). PT-IgG titres of 50–99 IU ml<sup>-1</sup>, considered indicative of infection in the last few years, and PT-IgG titres of  $\geq 100$  IU ml<sup>-1</sup>, considered indicative of recent infection (i.e. within the last year), reached 9.1% [95% confidence interval (CI) 6.9–11.3%; 58/639] and 5% (95% CI 3.3–6.7%; 32/639) seroprevalence, respectively. Notably, the proportion of subjects with a seroprevalence indicative of recent infection increased significantly from 9.3% (95% CI 7.5–11.1%; 96/1037) in 1996–1997 to 14.1% (95% CI 11.4–16.8%; 90/639) in 2012–2013. Overall, our data clearly indicate a significant increase in the circulation of *B. pertussis* in adults in Italy; therefore, it is likely that the statutory notification system underestimates the real incidence of the disease. These findings have implications for preventive strategies.

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Abbreviations: aP, acellular pertussis; CI, confidence interval; ESEN, European Sero-Epidemiology Network; GMT, geometric mean titre; ISS, Istituto Superiore di Sanità; PT-IgG, IgG antibodies to pertussis toxin; WHO, World Health Organization; wP, whole-cell pertussis.

## INTRODUCTION

Pertussis is an infectious disease caused by *Bordetella pertussis*. Close contact enhances the spread of infection and the disease affects all age groups (Mattoo & Cherry, 2005; Melvin *et al.*, 2014). The course of the disease can be particularly severe during the first and second years of life, when incidence, as well as hospitalizations and deaths, are particularly high (case fatality rate 0.2 and 4% in developed and developing countries, respectively) (WHO, 2010).

During recent decades, the incidence of reported cases has increased, especially in adolescents and adults, even in high-vaccine-coverage countries (EUVAC.NET, 2009; Zepp *et al.*, 2011). In immunized children, adolescents and adults,

the disease may have a mild course (Yaari *et al.*, 1999) and may not present typical symptoms of pertussis (Tozzi *et al.*, 2003; Gonfiantini *et al.*, 2014). For this reason, the disease may not be recognized by clinicians and, therefore, these cases can represent a potential source of infection for infants during their first year of life, when they are not yet completely immunized (de Greeff *et al.*, 2010; Wiley *et al.*, 2013). It has been shown that primarily mothers and fathers are the main source of infection for infants, but also grandparents may be responsible for the transmission of infection to newborns (Wendelboe *et al.*, 2007). In a recent review, Wiley *et al.* (2013) reported that in households the main source of infection in newborn cases is the mother [39%; 95% confidence interval (CI) 33–45% ], followed by the father (16%; 95% CI 12–21%) and grandparents (5%; 95% CI 2–10%).

In Italy, after the introduction of the whole-cell pertussis (wP) vaccine in 1961, pertussis incidence started to decrease, even when vaccine coverage was low (10–16% wP vaccine coverage). After 1995, when the acellular pertussis (aP) vaccines were introduced, a sharp decrease in pertussis incidence was observed with <5 cases per 100 000 population after 2000 (Gonfiantini *et al.*, 2014).

Pertussis is a mandatory notifiable disease and, based on routine notification data, Italy is a low-incidence country with outbreaks rarely reported after the achievement of high immunization coverage (Italian Ministry of Health, 1991, 2014). It is an accepted fact that the number of pertussis cases reported to the statutory notification system is greatly underestimated (Ciofi degli Atti *et al.*, 2002; Rota *et al.*, 2005; Gonfiantini *et al.*, 2014). Therefore, the real epidemiological impact of pertussis is not being perceived. To get a better estimate of the circulation of *B. pertussis* in the population, sero-epidemiological studies have proven to be a valuable instrument next to the clinical surveillance programmes based on reporting systems, and can provide a better approximation for the real burden of disease amongst populations (Barkoff *et al.*, 2015).

The aim of this study was to determine the seroprevalence of IgG antibodies to pertussis toxin (PT-IgG) in selected adult age groups, which could represent a reservoir of *B. pertussis* and be responsible for sustained transmission to

vulnerable infants. The seroprevalence of PT-IgG was measured in sera collected in the age classes 20–29 years and 30–39 years (representative of the reproductive age), and  $\geq 60$  years (possibly involved in the care of infants). A special emphasis was focused on the proportion of sera with concentrations indicative of recent infection (Hallander *et al.*, 2009a, b).

A further point addressed was the comparison of PT-IgG seroprevalence in sera collected in 2012–2013 to sera collected in 1996–1997 in a similar adult population. This comparison was conducted in order to investigate any increase in circulation of *B. pertussis* in the country. To date, no evidence of pertussis resurgence has been reported by the Italian statutory notification system, in contrast with large outbreaks of pertussis observed in other European countries, the USA and Australia (Chiappini *et al.*, 2013; WHO SAGE Pertussis Working Group, 2014; Fedele *et al.*, 2015).

## METHODS

**Sera collection.** The study is based on anonymous residual samples collected from clinical laboratories within a larger seroprevalence study performed in the framework contract ‘Coordination of activities for laboratory surveillance of whooping cough in member states/EEA countries’ on behalf of the EUpert-Labnet consortium (ECDC/2011/013) comparing sera from 14 European member states. The age groups 20–29 years and 30–39 years are representative of reproductive age, and the  $\geq 60$  years age group are possibly involved in care of infants. We estimated that the sample size needed in each age group, assuming a prevalence of 20% with a 95% CI and a precision of 5%, was 250.

Overall, 639 sera, residual from specimens taken for diagnostic purposes or routine ascertainment, were collected, of which 239, 248 and 152 sera were in the age groups 20–29, 30–39 and  $\geq 60$  years, respectively. Sera were obtained from clinical laboratories in five Italian regions: Lombardy (North Italy), Tuscany and Latium (Central Italy), and Apulia and Sicily (South Italy). Participating regions corresponded to 48% of the national population in 2013.

Table 1 shows the absolute number of sera collected from April 2012 to March 2013 per age group and Italian region. Sera collected in 2012–2013 were compared to similar sera collected in 1996–1997 as described by Edmunds *et al.* (2000). In the 1996–1997 study, a total of 1037 sera were tested for PT-IgG, of which 447, 386 and 204 were in the age groups 20–29, 30–39 and  $\geq 60$  years, respectively.

**Table 1.** Distribution of sera collected in five Italian regions by age group

Age group (years)	Region					Total
	Lombardy	Tuscany	Latium	Apulia	Sicily	
20–29	51	46	48	47	47	239
30–39	49	50	49	50	50	248
$\geq 60$	ND	50	ND	52	50	152
Total	100	146	97	149	147	639

ND, Not determined.

Only age, geographic area and date of sampling were recorded for each sample. Sera from individuals known to be affected by an immunosuppressive condition or by an acute respiratory infection, or to have undergone a blood transfusion within the last 6 months, were excluded.

**Vaccination status.** All individuals in the reproductive age groups, included in both studies, were born before the introduction of aP vaccine in Italy, in 1995, and after the recommendation for immunization with wP vaccine in 1961, with the only exception being individuals aged 30–39 years enrolled in the 1996–1997 study, who were all born prior to the introduction of wP vaccine in Italy (Gonfiantini *et al.*, 2014). With regard to individuals aged  $\geq 60$  years in 2012–2013 and in the 1996–1997 studies, they were all born before the use of wP vaccine in Italy (Gonfiantini *et al.*, 2014).

Considering the lack of a pertussis immunization policy for adults in Italy, it is highly unlikely that recent vaccination occurred in the age classes considered in our study. Furthermore, it is also unlikely that these subjects received the wP vaccine as their primary vaccination, considering that wP vaccine coverage was in general low, with reported figures ranging between 10 and 16% during the years 1974 to 1981 (Gonfiantini *et al.*, 2014). At the national level, until the early 1990s, wP vaccination coverage remained low (>40%; Italian Vaccine Coverage Survey Working Group, 1994).

**Laboratory methods.** The level of PT-IgG, expressed in IU ml<sup>-1</sup>, is a marker of pertussis disease and/or infection. Among the antigens present in the aP vaccine formulation, only PT is specific for *B. pertussis* (Mattoo & Cherry, 2005; Melvin *et al.*, 2014), and PT-IgG dosage is well characterized and has international units. Considering these points, only the PT-IgG response was assessed in the sera (Guiso *et al.*, 2011).

PT-IgG levels were measured using an ELISA assay standardized within the European Sero-Epidemiology Network (ESEN) (Giammanco *et al.*, 2003). The 2012–2013 sera were tested in the Istituto Superiore di Sanità (ISS) Rome laboratory using the PT-IgG ELISA, standardized with a Swedish laboratory (Public health agency laboratory, Solna, Sweden). The Swedish method (Reizenstein *et al.*, 1995) was a reference method for a European seroprevalence study of pertussis burden evaluation on behalf of the EUpert-Labnet consortium (ECDC/2011/013) and is carefully monitored for imprecision and drift over time as described elsewhere (Hallander *et al.*, 2009a).

The 1996–1997 sera were tested in the Palermo laboratory, where the ESEN PT-IgG ELISA method was standardized (Giammanco *et al.*, 2008). The Swedish laboratory participated in the ESEN standardization; thus, the Swedish method is traceable and comparable to the Palermo PT-IgG ELISA. In-house reference sera and international standards [06/142, World Health Organization (WHO) International Standard Pertussis Antiserum] were calibrated against the USA Food and Drug Administration standard serum (lot 3-HRP3) (Xing *et al.*, 2009). A total of eight twofold dilutions per sample were used in the ELISA assay to calculate the titre and results were expressed in IU ml<sup>-1</sup> PT-IgG. To ensure comparability between the ELISA assays performed at the ISS Rome and the Swedish laboratory, sets of proficiency panel sera and additional 'monitoring' sera were received by the ISS Rome laboratory for testing. The panel sera were chosen to cover the limit of detection, approximately 40 and >100 IU ml<sup>-1</sup>. The limit of detection of the assay was 1 IU ml<sup>-1</sup>. The monitoring sera were chosen to cover the concentrations of approximately 8 IU ml<sup>-1</sup> and 40 IU ml<sup>-1</sup>.

No internationally accepted correlates of protection have been established yet for pertussis (Plotkin, 2014). In the absence of well-established serological levels of PT-IgG IU ml<sup>-1</sup> for protection against pertussis infection, the following thresholds were selected based on previous studies. In particular, the ELISA assay cut-off for pertussis antibodies was set at 5 IU ml<sup>-1</sup> and a serological detectable response was defined for values of >5 IU ml<sup>-1</sup> PT-IgG level (Edelman *et al.*, 2007; Knuf *et al.*, 2008).

PT-IgG levels  $\geq 20$  IU ml<sup>-1</sup> were considered as potentially protective as in previous studies (Long *et al.*, 1990; Versteegh *et al.*, 2005; Tomovici *et al.*, 2012). In populations not recently vaccinated for pertussis (within the last year), the cut-off value of  $\geq 50$  IU ml<sup>-1</sup> was used to estimate the proportion of subjects infected with *B. pertussis* within the last few years and  $\geq 100$  IU PT-IgG ml<sup>-1</sup> for infection within the last year (Hallander *et al.*, 2009a, b; Guiso *et al.*, 2011; ECDC, 2012).

**Data analysis.** Data were recorded in a computerized database (Microsoft Excel, version 2010) and were analysed using STATA statistical software version 9.2 (STATA), the GraphPad Prism version 4.00 for Windows (Graphpad Software, www.graphpad.com) and IBM SPSS statistics version 21.

To compare differences of continuous variables between groups or within groups, two-sided Mann–Whitney tests or Wilcoxon paired-sample tests were performed, respectively. The Fisher's exact test was used for categorical variables, assessed by the  $\chi^2$  test. To study the association between variables, a linear regression model was applied and the Spearman correlation coefficient was calculated. Cumulative reverse curves were used to show the distribution of antibody titres (Reed *et al.*, 1995). Antibody titres in each study group were presented as geometric mean titre (GMT), with 95% CI.  $P < 0.05$  considered statistically significant.

**Ethics statement.** This study was conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from the Ethical Committee of the ISS, Rome, Italy (PRE293/12; CE12/359). Informed written consent was obtained from individuals providing specimens at the time of sampling.

## RESULTS

### Seroprevalence in 2012–2013

A total of 639 sera were collected within the five Italian regions assessed in the study. Table 2 shows the distribution of sera considering the five levels of PT-IgG titres and the three age classes. On average, only 18% of the population tested demonstrated PT-IgG titres of <5 IU ml<sup>-1</sup>, and the lowest percentage with such titres was in the  $\geq 60$  years age group (6.6%,  $P < 0.0001$  vs total).

The proportion of the population presenting PT-IgG levels compatible with pertussis infection within the last few years (PT-IgG levels in the range of 50–99 IU ml<sup>-1</sup>) was 9.1% (95% CI 6.9–11.3%; 58/639), and within the last year (PT-IgG levels  $\geq 100$  IU ml<sup>-1</sup>) was 5% (95% CI 3.3–6.7%; 32/639). The highest percentage of subjects with PT-IgG levels  $\geq 100$  was in the 20–29 years age group (7.1%, 95% CI 3.8–10.4%; 17/239;  $P < 0.0001$  vs total).

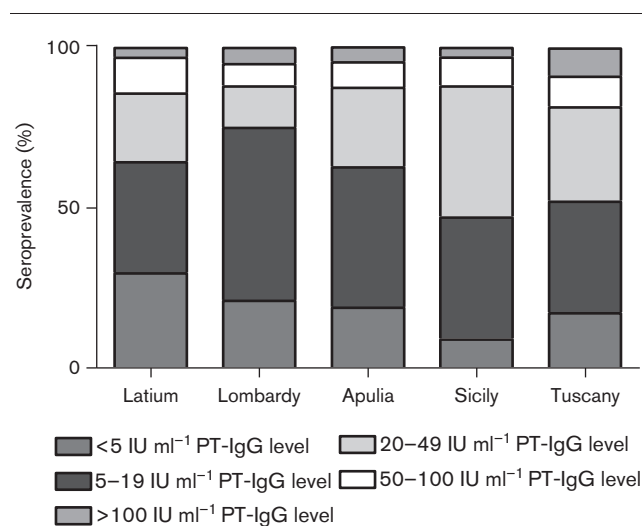
Fig. 1 shows data analysed by region and age group. When the total population was considered, sera collected in Lombardy presented a statistically significant higher percentage of subjects with PT-IgG levels in the range 5–19 IU ml<sup>-1</sup>, compared to Tuscany ( $P = 0.0038$ ), Latium ( $P = 0.0098$ ) and Sicily ( $P = 0.019$ ). In sera collected in Sicily, a statistically significant lower percentage of subjects had values of PT-IgG <5 IU ml<sup>-1</sup>, compared to Lombardy ( $P = 0.008$ ), Latium ( $P < 0.0001$ ) and Apulia ( $P = 0.018$ ). Again in Sicily, the percentage of PT-IgG values in the range 20–49 IU ml<sup>-1</sup> was found to be significantly higher than in Lombardy ( $P < 0.0001$ ), Latium ( $P = 0.0075$ ) and Apulia ( $P = 0.017$ ). A

**Table 2.** Distribution of sera collected in 2012–2013 by age group, showing results of PT-IgG titres

Fisher's exact test results were as follows. PT-IgG  $<5$  IU ml<sup>-1</sup>: 20–29 vs  $\geq 60$   $P<0.0001$ ; 20–29 vs total  $P<0.0001$ ; 30–39 vs  $\geq 60$   $P<0.0001$ ; 30–39 vs total  $P<0.0001$ ;  $\geq 60$  vs total  $P<0.0001$ . PT-IgG 5–19 IU ml<sup>-1</sup>: 20–29 vs  $\geq 60$   $P=0.021$ ; 20–29 vs total  $P<0.0001$ ; 30–39 vs  $\geq 60$   $P=0.0012$ ; 30–39 vs total  $P<0.0001$ ;  $\geq 60$  vs total  $P<0.0001$ . PT-IgG 20–49 IU ml<sup>-1</sup>: 20–29 vs total  $P<0.0001$ ; 30–39 vs total  $P<0.0001$ ;  $\geq 60$  vs total  $P<0.0001$ . PT-IgG 50–99 IU ml<sup>-1</sup>: 20–29 vs total  $P<0.0001$ ; 30–39 vs total  $P<0.0001$ ;  $\geq 60$  vs total  $P<0.0001$ . PT-IgG  $\geq 100$  IU ml<sup>-1</sup>: 20–29 vs total  $P=0.040$ ; 30–39 vs total  $P<0.0001$ ;  $\geq 60$  vs total  $P<0.0001$ .

Age group	Number/total number, seroprevalence (%) and [95 % CI] PT-IgG (IU ml <sup>-1</sup> )				
	<5	5–19	20–49	50–99	$\geq 100$
20–29	53/239 22.2 [16.9–27.5]	92/239 38.5 [32.3–44.7]	54/239 22.6 [17.3–27.9]	23/239 9.6 [5.9–13.3]	17/239 7.1 [3.8–10.4]
30–39	52/248 21.0 [15.9–26.1]	104/248 41.9 [35.8–48.0]	61/248 24.6 [19.2–30.0]	23/248 9.3 [5.7–12.9]	8/248
$\geq 60$	10/152 6.6 [2.7–10.5]	64/152 42.1 [34.3–49.9]	59/152 38.8 [31.1–46.5]	12/152 7.9 [3.6–12.2]	7/152 4.6 [1.3–7.9]
Total	115/639 18.0 [15.0–21.0]	260/639 40.7 [36.2–43.8]	174/639 27.2 [23.7–30.7]	58/639 9.1 [6.9–11.3]	32/639 5.0 [3.3–6.7]

smaller, non-statistically significant, percentage of subjects



**Fig. 1.** Seroprevalence data obtained in 2012–2013 in five Italian regions showing relative distributions of seroprevalence per PT-IgG cut-off, per region. Statistically significant differences are indicated. PT-IgG level  $<5$  IU ml<sup>-1</sup>: Latium/Apulia  $P=0.046$ , Latium/Sicily  $P<0.0001$ , Latium/Tuscany  $P=0.027$ , Lombardy/Sicily  $P=0.008$ , Apulia/Sicily  $P=0.01$ . PT-IgG level 5–19 IU ml<sup>-1</sup>: Latium/Lombardy  $P=0.0098$ , Lombardy/Sicily  $P=0.019$ , Lombardy/Tuscany  $P=0.0038$ . PT-IgG level 20–49 IU ml<sup>-1</sup>: Latium/Sicily  $P=0.0075$ , Lombardy/Apulia  $P=0.024$ , Lombardy/Sicily  $P<0.000$ , Lombardy/Tuscany  $P=0.003$ ; Apulia/Sicily  $P=0.017$ .

with values compatible with a recent infection ( $\geq 100$  IU ml<sup>-1</sup>) was found in Sicily.

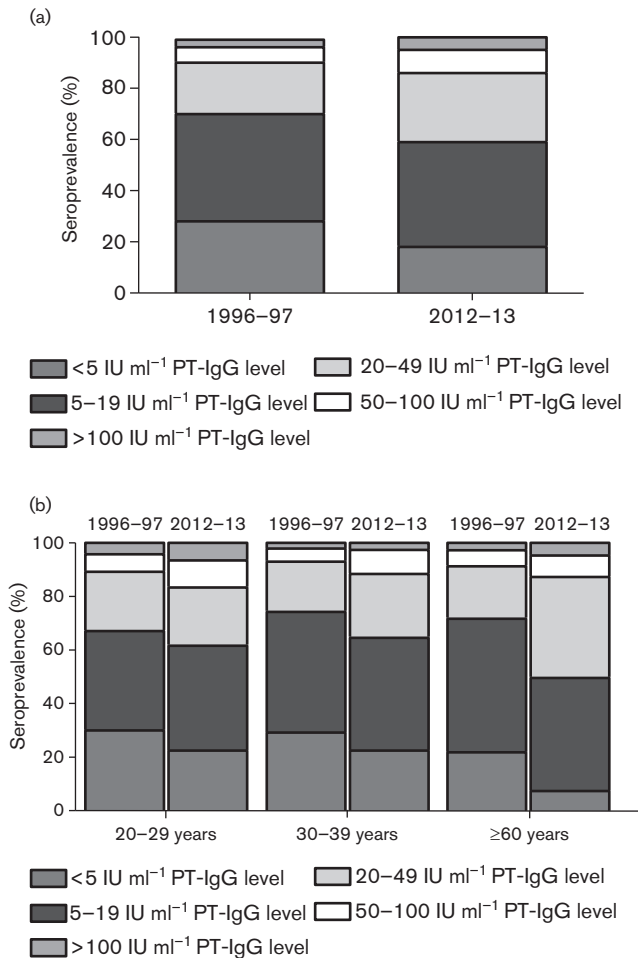
A significantly greater number of subjects with antibody titres compatible with recent infection was found in sera collected in Latium (50–100 IU ml<sup>-1</sup>) and Tuscany ( $\geq 100$  IU ml<sup>-1</sup>), with a statistical significance only in the  $\geq 60$  years age group in Tuscany versus Apulia (50–99 IU ml<sup>-1</sup>,  $P=0.030$ ).

### Comparison of seroprevalence in Italy in a 16-year interval: 1996–1997 versus 2012–2013

To evaluate whether pertussis seroprevalence changed in Italy over 16 years, we compared serological data obtained in 2012–2013 to data obtained in 1996–1997 in the same age groups (Fig. 2a).

The proportion of individuals with  $<5$  IU ml<sup>-1</sup> PT-IgG level significantly decreased (28.1 % in 1996–1997 vs 18.0 % in 2012–2013;  $P<0.0001$ ), whilst the proportion of seropositive subjects increased. In particular, the percentage of individuals with PT-IgG titres 20–49 IU ml<sup>-1</sup> rose from 20.2 % in 1996–1997 to 27.2 % in 2012–2013 ( $P=0.0012$ ), and the proportion of subjects with PT-IgG titres 50–99 IU ml<sup>-1</sup> and  $\geq 100$  IU ml<sup>-1</sup> increased from 6.2 to 9.1 % ( $P=0.03$ ) and from 3.1 to 5.0 % ( $P=0.049$ ) in 2012–2013, respectively.

Statistically significant differences were found when the data were stratified by age group (Fig. 2b). In all age groups, a significant reduction of subjects with PT-IgG titres  $<5$  IU ml<sup>-1</sup> was observed in 2012–2013 with respect to 1996–1997, from 29.8 to 22.2 % ( $P=0.038$ ), from 29.3 to



**Fig. 2.** (a) Seroprevalence data measured in 1996–1997 versus 2012–2013 showing relative distribution of seroprevalence per PT-IgG cut-off. Statistically significant differences between the two sera collections are indicated. Fisher’s exact test for PT-IgG titres in 1996–1997 versus 2012–2013: <5 IU ml<sup>-1</sup>, from 28.1 to 18.0, *P*<0.0001; 20–49 IU ml<sup>-1</sup>, from 20.2 to 27.2, *P*=0.0012; 50–100 IU ml<sup>-1</sup>, from 6.3 to 9.1, *P*=0.034; ≥100 IU ml<sup>-1</sup>, from 3.0 to 5.0, *P*=0.047. (b) Comparison of the seroprevalence data measured in 1996–1997 versus 2012–2013. Relative distributions of seroprevalence data per age class and PT-IgG cut-off. Statistically significant differences between the two sera collections are indicated. Fisher’s exact test 1996–1997 versus 2012–2013: age class 20–29 years, PT-IgG level <5 IU ml<sup>-1</sup> from 29.8 to 22.2%, *P*=0.038; age class 30–39 years, PT-IgG level <5 IU ml<sup>-1</sup> from 29.3 to 21.0%, *P*=0.021; age class ≥60 years, PT-IgG level <5 IU ml<sup>-1</sup> from 22.5 to 7.2%, *P*<0.001; PT-IgG level 20–49 IU ml<sup>-1</sup> from 18.6–38.8%, *P*<0.001

21.0% (*P*=0.021) and from 22.5 to 7.2% (*P*<0.001), in the 20–29, 30–39 and ≥60 year age groups, respectively.

A significant increase in the percentage of potentially protected subjects, with PT-IgG titres in the range 20–49 IU ml<sup>-1</sup>, was observed in the age group ≥60 years in 2012–2013 compared to the 1996–1997 cohort (38.8 vs

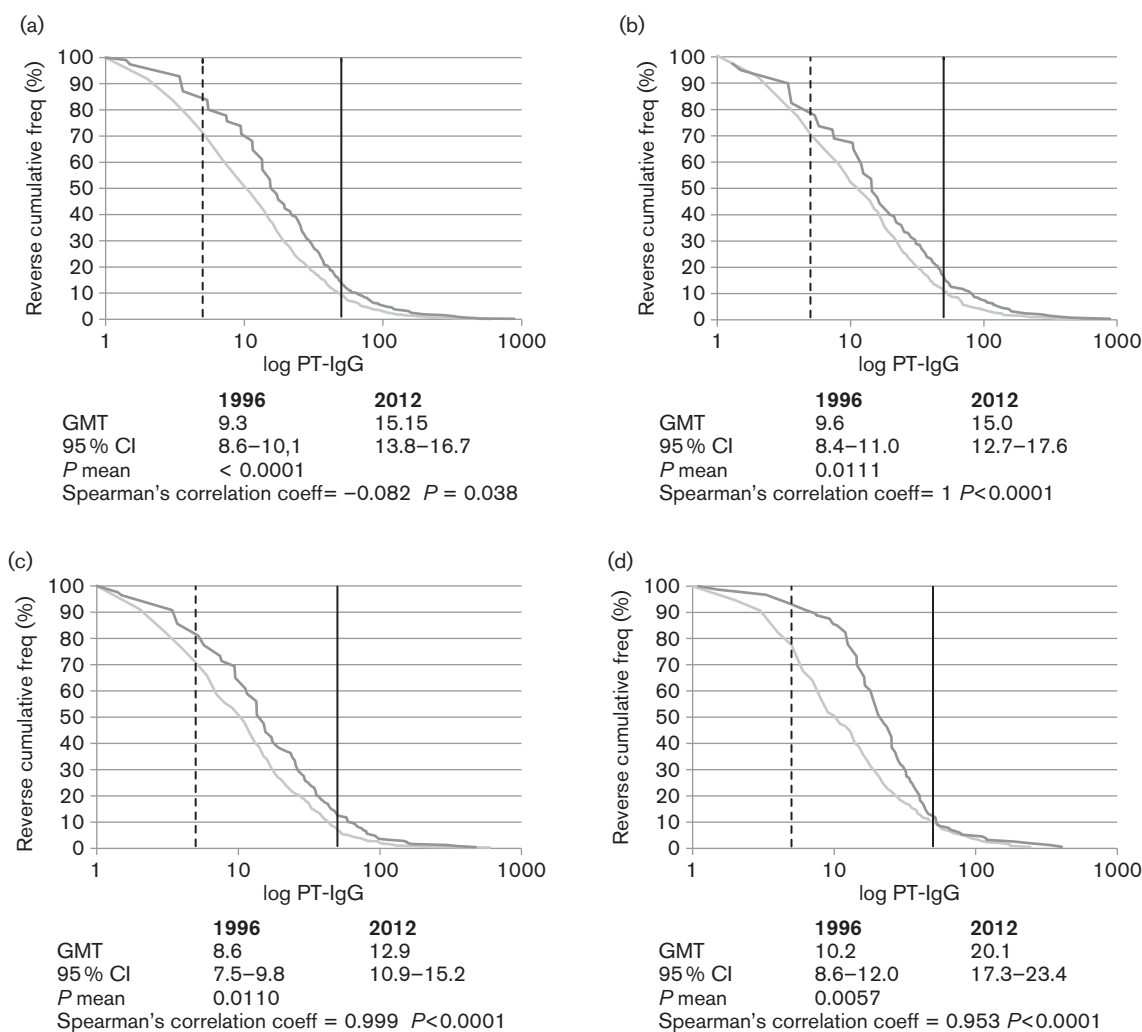
18.6%; *P*<0.0001) (Fig. 2b). An increase of the percentage of individuals with PT-IgG titres 50–99 and ≥100 IU ml<sup>-1</sup> was found in all age classes in 2012–2013 versus 1996–1997 cohorts, but the numbers were too small to reach statistical significance (Fig. 2a).

Fig. 3 shows the reverse cumulative curves drawn for the entire population sampled and by age group in both studies. GMTs of PT-IgG in the overall population and in the three age groups considered were statistically higher in 2012–2013 compared to 1996–1997. The GMT and statistical significance values are shown in Fig. 3.

## DISCUSSION

Our data on PT-IgG seroprevalence in adult age groups clearly support the view that *B. pertussis* is circulating widely in the Italian population. Overall, more than 80% of the adult population analysed in the 2012–2013 study showed a detectable serological response with values of PT-IgG >5 IU ml<sup>-1</sup>. The PT-IgG levels indicative of a recent infection were 9.1% (95% CI 6.9–11.3%) in the whole population, with the highest value (9.6%, 95% CI 5.9–13.3%) found in the 20–29 years age group (*P*<0.0001 vs total). Seroprevalence with a PT-IgG level ≥100 IU ml<sup>-1</sup> was 5% (95% CI 3.3–6.7%) in 2012–2013, and the 20–29 years age group appears to have had a greater exposure to pertussis, with a seroprevalence of 7.1% (95% CI 3.8–10.4%) (*P*<0.0001 vs total) in accordance with studies from Denmark (Rønn *et al.*, 2014) and in Italy in 2004 (Gabutti *et al.*, 2008). In particular, Gabutti and colleagues performed a sero-epidemiological study in six Italian regions. They found that in the age groups 25–44 and ≥65 years the seroprevalence for a PT-IgG level ≥100 IU ml<sup>-1</sup> was 5.7 and 6.0%, respectively. The data reported recently on the pertussis prevalence in adult patients with acute cough (Teepe *et al.*, 2015), which used sera collected from October 2007 to April 2010, were not in line with our data and those of Gabutti *et al.* (2008). The study by Teepe *et al.* (2015) was carried out in 12 European countries, including Italy, and found the overall prevalence of recent infections (cut-off PT-IgG ≥125 IU ml<sup>-1</sup>) was 3%, whilst in sera collected in Italy (Lombardy region) the prevalence was equal to 0. When our data were analysed in the same region and using the same cut-off, we found a seroprevalence of 5%. Several differences in the study design may have contributed to this discrepancy. In the study by Teepe *et al.* (2015) the size of the Italian sample was extremely small, only 80 patients having been analysed. Furthermore, the recruited population was different, being adult patients with acute cough.

When our data were analysed by region, we found that in Sicily a smaller percentage of subjects had titres of PT-IgG <5 IU ml<sup>-1</sup>, suggestive of an increased circulation of *B. pertussis* in Sicily compared to the other Italian regions. A greater number of subjects with antibody titres compatible with a recent infection was found in sera collected in Latium and Tuscany. In Italy, pertussis immunization of infants has been recommended since the 1960s, but



**Fig. 3.** Reverse cumulative distribution curves for log PT-IgG: in all age classes (a), the in 20–30 years age class (b), in the 30–39 years age class (c) and in ≥60 years age class (d). The black and grey reverse cumulative distribution curves represent data obtained using the 2012–2013 and 1996–1997 sera series, respectively. The vertical lines mark the PT-IgG cut-off levels 5 IU ml<sup>-1</sup> (---) and 50 IU ml<sup>-1</sup> (—).

vaccination policies were established at the regional level. In some regions, pertussis vaccine was immediately provided free of charge, while in others, parents were required to pay for the vaccine (Binkin *et al.*, 1992). This situation lasted for many years and led to differences in vaccination coverage across the country. In fact, at the national level, vaccination coverage remained low (less than 40%) until the early 1990s with wide variations between regions (15–71%), the regions of southern Italy being in the lower range of vaccination coverage (Italian Vaccine Coverage Survey Working Group, 1994). However, since 2002, after the inclusion of pertussis immunization in the national vaccination calendar in 1999, the aP vaccines have been offered free of charge to children <1 year of age in all Italian regions, allowing

achievement of an immunization coverage of approximately 96% after 2006 (Italian Ministry of Health, 2014).

Clear evidence that the data from the national routine surveillance are not representative of the true Italian situation comes from the comparison of seroprevalence data obtained in 2012–2013 to the data obtained in 1996–1997. In the 2012–2013 sera, an increased percentage of positive PT-IgG subjects was found in general, and specifically was compatible with recent and very recent pertussis infections. Reverse cumulative distribution curves (Fig. 3) showed that the overall range of values in the two studies was the same, but within the range, the individual values in 2012–2013 were higher than those in 1996–1997, with higher median and mean and lower variance (steeper mid-section). The 20–29 years age group appears to have been more exposed

to pertussis, with a higher proportion of subjects with PT-IgG levels compatible with recent infection (Figs 2b and 3).

Even though our data show interesting results, it should be taken into account that the sample size of the two seroprevalence studies compared is different. The 2012–2013 sera were collected from five Italian regions, while the 1996–1997 sera were collected from 19 out of 21 regions. However, the age groups and the inclusion/exclusion criteria are the same, and both samples are representative of the Italian population (Edmunds *et al.*, 2000). Moreover, despite a reduced sample size in the age group  $\geq 60$  years, the study allowed the detection of statistically significant differences in the two PT-IgG seroprevalence surveys conducted.

Our 2012–2013 cohort was part of a larger seroprevalence study performed in the framework contract ‘Coordination of activities for laboratory surveillance of whooping cough in member states/EEA countries’ on behalf of the EUpert-Labnet consortium (ECDC/2011/013) comparing sera from 14 European member states (Huygen *et al.*, 2014; L. Wehlin and others, unpublished data). The outcome of the study indicated that Italian samples, together with sera from Portugal, showed the highest proportion of elevated PT-IgG titres, indicating an infection within the last few years (L. Wehlin and others, unpublished data). The experts within the WHO SAGE pertussis working group (WHO SAGE Pertussis Working Group, 2014), in order to identify a true pertussis resurgence in 2012, analysed several countries in the world, but not Italy, and found, at the European level, that the UK and Portugal were the only countries demonstrating true resurgence.

Overall, our results suggest that pertussis is resurgent in Italy also, even though the data from the routine surveillance system do not, due to limitations of the system itself (e.g. under-diagnosis, under-notification and delay of notification). Pertussis incidence is underestimated as many cases in adolescents, young adults and adults are not identified because of their atypical clinical characteristics and the lack of laboratory assessment (Tozzi *et al.*, 2003; Gonfiantini *et al.*, 2014). Indeed, a study conducted in 2012 showed that Italian physicians seldom suspect pertussis in old patients with chronic cough and do not ask for laboratory confirmation (Gonfiantini *et al.*, 2013). Furthermore, in Italy, laboratory confirmation is not yet required for pertussis disease notification, in contrast with European Union recommendations (He *et al.*, 2012; Ausiello *et al.*, 2014).

In the sera collected in 2012–2013, the younger age group considered in the study was born in 1983–1992, before the introduction of aP vaccines in Italy and its inclusion in the national vaccination calendar in 1999. Thus, all sera were collected from subjects who could have been vaccinated with the wP vaccine. However, vaccination coverage was quite low, until 1991, reaching around 40% in children aged 12–24 months (ICONA Working Group, 2009; Italian Ministry of Health, 1991). Thus, a minority of our examined subjects could have had a primary vaccination course. However, the majority of the PT-IgG responses are likely to be due to

natural exposure and undiagnosed pertussis infection rather than pertussis vaccination (Fedele *et al.*, 2015).

Our data clearly demonstrate that *B. pertussis* continues to circulate in the Italian adult population. This finding has several implications for future preventive strategies, including the need to raise clinicians’ awareness on pertussis and its possible different manifestations, the importance of a primary vaccination course, including a booster in adolescence, and the need to recommend a booster vaccination in adults. To better estimate the pertussis burden in Italy, clinical diagnosis is not sufficient, particularly in vaccinated and adult subjects with atypical manifestations. The laboratory confirmation of pertussis, strongly recommended by the European Centre for Disease Prevention and Control, should be implemented.

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