Vaccine 41 (2023) 4057–4063

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Prevalence of poliovirus neutralizing antibodies in Italian population: A systematic review and meta-analysis

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ARTICLE INFO

Article history:
Available online 29 April 2023

Keywords:
Polio eradication
Serosurveys
Polio-free status

ABSTRACT

Introduction: The introduction of anti-poliomyelitis vaccines has driven progress toward the global eradication of wild polioviruses, a millennium goal of the World Health Organization. With the vaccination campaigns carried out since 1964, in 2002 Italy was certified polio-free, considering that no cases had been recorded since 1983. Nevertheless, it is crucial to guarantee high level of immunization coverage also in low-endemicity countries, considering that sporadic polio cases can be recorded. To evaluate the presence of susceptible subjects in the population, seroepidemiological studies are key actions.

Methods: We conducted a systematic review of the relevant literature to evaluate the prevalence of anti-PV neutralizing antibodies in Italian population. Seven studies, selected among scientific articles available in MEDLINE/PubMed, ISI Web of Knowledge and Scopus and published from January 1, 2012, to November 15, 2022, were included.

Results: The pooled prevalence of subjects without PV1 neutralizing antibodies was 6.4% (95%CI = 0.5–16.9), for PV2 it was 5.3% (95%CI = 0.4–14.2), and for PV3 it was 13.0% (95%CI = 4.0–25.7; I2 = 98.5%). Levels of neutralizing antibodies appears to decrease with increasing age; this decline is a proxy for the real risk factor, which is the time since the last vaccine dose.

Conclusions: Public health institutions must be aware of the risk of reintroduction of wild PV in polio-free countries and therefore they must keep high level of immunization in population and reinforce the active surveillance systems.

1. Introduction

Poliomyelitis is a crippling and potentially fatal disease caused by poliovirus (PV) [1]. The three poliovirus serotypes (PV1, PV2, and PV3) show minimal heterotypic immunity between them; therefore, immunity to one serotype does not produce significant immunity to the other serotypes [2].

Two types of polio vaccines are currently available. An inactivated poliovirus vaccine (IPV) was licensed in 1955 and was used extensively until the early 1960s. In 1963, a trivalent oral poliovirus vaccine (OPV) largely substituted IPV, becoming the vaccine of choice in most countries, especially those with a high level of poliovirus endemicity; indeed, it can produce a mucosal immune response in the intestines, the primary site of poliovirus multiplication, a mechanism that has not been demonstrated for IPV [3]. Nevertheless, vaccine-associated paralytic poliomyelitis (VAPP) is a serious but sporadic and rare adverse event following OPV administration [3]. An enhanced-potency IPV was licensed in November 1987 and became available in 1988 [4]. Due to the risk of VAPP, in many countries where advanced control or the elimination of polio has been achieved, IPV has replaced OPV in the routine immunization schedule, even if this formula does not protect against infection [5].

The introduction of these vaccines has driven progress toward the global eradication of wild PV, a millennium goal of the World Health Organization (WHO) [6]. Eradication of wild PV2 was declared on September 20, 2015 (last reported case in October 1999), and the eradication of wild PV3 on October 17, 2019 (last reported case in November 2012) [6]. Cases due to wild poliovirus have decreased by > 99% since 1988, and since 2017 Pakistan and Afghanistan have been the only endemic countries worldwide [7,8], while the last wild PV1 case was reported in Mozambique on May 2022 [9].

Abbreviations: PV, Polio virus; CDC, Center for Disease Control and Prevention; WHO, Word Health Organization; VAPP, Vaccine-associated paralytic poliomyelitis; IPV, inactivated poliovirus vaccine; OPV, oral poliovirus vaccine.

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https://doi.org/10.1016/j.vaccine.2023.04.047
0264-410X/

In 1964, the Italian Ministry of Health developed a mass vaccination campaign with monovalent OPVs, offering it free and actively to all children between 6 months and 14 years (vaccination became mandatory in 1966). In 1972, monovalent OPVs were replaced by trivalent OPV. However, between 1964 and 2000, vaccination with OPV resulted in a small number of cases of VAPP; therefore, due to ethical concerns and the favorable epidemiological context, in 2000, a sequential schedule (IPV-IPV-OPV-OPV) was announced. In 2003, the use of OPV was suspended, and IPV was introduced exclusively for immunization in infancy [10]. Three doses are administered to infants of 3, 5, and 11 months of age using a hexavalent formula (IPV-hepatitis B- Haemophilus influenzae type b-tetanus-diphtheria-acellular pertussis), the fourth dose at 5–6 years of age in a tetravalent formula (tetanus-diphtheria-acellular pertussis-IPV), and a fifth dose is recommended during adolescence. In 2017, the Italian government confirmed this immunization routine mandatory for children [11]. The three-dose vaccine coverage achieved in infant cohorts from 2000 to 2019 ranged between 85.4% and 96.8% [12].

With the vaccination campaigns carried out since 1964, in 2002 Italy (together with the entire European region) was certified polio-free by the Regional Commission for the Certification of Poliomyelitis Eradication; indeed, no cases had been recorded since 1983 [13]. Although levels of neutralizing antibodies wane over time, the literature reported that > 99% of the group that received the recommended schedule should be protected for at least 18 years. Therefore immunization may be considered life-long [14,15]. Indeed, it is crucial to guarantee a high level of protection also in low-endemicity countries, pondering that in 2022 in New York City (US) a sporadic polio case (Vaccine-derived poliovirus type 2 was detected in stool specimens obtained on days 11 and 12 after initial symptom onset) and the detection of Sabin-like type 2 polioviruses in wastewater samples have been recorded [16].

From this perspective, we conducted a systematic review and meta-analysis of the relevant literature to evaluate the prevalence of Italian inhabitants without anti-polio neutralizing antibodies. The determinants of seroprevalence and the strategies to maintain a high level of immunity in the population were also analyzed.

2. Material and methods

The systematic review protocol was prepared using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist [17]. The protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO) with reference acknowledgment number CRD42022380761. The systematic review's population, intervention, comparison, and outcome (PICO) framework was used to formulate the review question; the resulting question was "anti-polio neutralizing antibodies seroprevalence in the Italian population".

Search strategy, selection criteria, and data extraction. The Scopus, MEDLINE/PubMed, and ISI web of knowledge were systematically searched. Research articles, brief reports, letters, and editorials published between January 1, 2012 and November 15, 2022 were

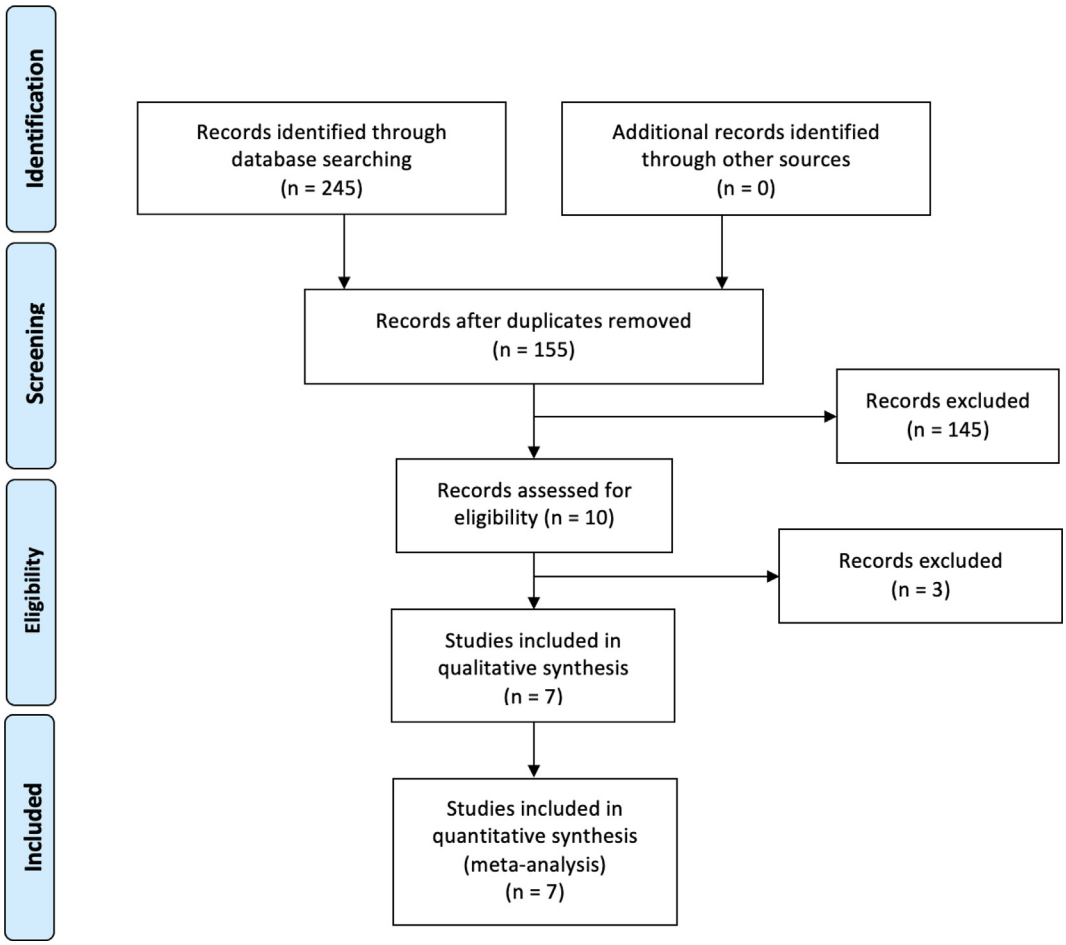

Fig. 1. Flow-chart of the bibliographic research.

included in our search. The following terms were used for the search strategy: (seroprevalence OR prevalence OR immuni* OR antibod*) AND (poliomyelitis OR polio) AND (Ital*). Studies in English or Italian with full text were included. Abstracts without full-text, reviews, meta-analyses, papers not reporting epidemiological data, clinical trials, studies focusing on issues unrelated to the purpose of this review (vaccine knowledge, attitude, etc.), and studies not set in Italy were excluded. When necessary, study authors were contacted to obtain additional information. The list of papers was screened by title and/or abstract independently by two reviewers who applied the predefined inclusion/exclusion criteria. Discrepancies were recorded and resolved by consensus.

Extracted data included year, sample size, susceptible subjects for each virus serotype, Italian region, and determinants of prevalence.

Quality assessment. The methodological quality of included studies was assessed via the Newcastle–Ottawa Scale (NOS), adapted for the evaluation of cross-sectional studies [18]. It is divided into seven categories, checking three quality aspects (selection, comparability, and outcome/exposure), and scores range from 0 to 10. The quality of a study was considered high if the NOS score was between 7 and 10, intermediate if the NOS score was between 4 and 6, and low if it was between 0 and 3.

Two operators independently assessed the risk of bias for each study. Discrepancies were recorded and resolved by agreement.

Pooled analysis. The prevalence of Italian subjects without antipoliovirus neutralizing antibodies was evaluated; a sub-analysis was performed only for those subjects vaccinated or potentially vaccinated, according to the Italian vaccination schedule. The pooled proportion in the meta-analysis was calculated using the Freeman-Tukey double arcsine transformation to stabilize variances, and the DerSimonian-Laird weights for random effects models, with the estimate of heterogeneity obtained from the inverse-variance fixed-effects model. The pooled prevalence and the associated 95% Wald confidence interval were plotted, and a forest plot was drawn. The I^2 statistic was calculated to measure of the proportion of the overall variance attributable to heterogeneity between studies rather than to chance. A p-value < 0.05 was considered to indicate the statistical significance of the heterogeneity.

Four different sensitivity analyses were conducted to evaluate stability, as follows

- Sub-analysis considering only high-quality studies
- Sub-analysis per study sample (500 + vs. < 500 subjects)
- The exclusion of one study at a time, and the conclusion subsequently based on the others was then re-evaluated to avoid severe distortions.

Statistical analysis was conducted using STATA MP17.

Determinants of seroprevalence and the strategies to maintain a high level of immunity in the population were collected from all available studies. Their respective findings were compared, with particular attention to the evidence presented in several of the included papers.

3. Results

Identification of relevant studies. The flow-chart, constructed following the PRISMA guidance [17] (Fig. 1), shows the article selection process. According to the inclusion criteria mentioned above, 8 articles were identified in ISI Web of Knowledge, 7 in Scopus, and 9 in MEDLINE/PubMed. After the exclusion of duplicate articles in the three databases, there were 10 eligible studies. Of these, three were excluded, one because the results were described in a

Table 1
Characteristics of the selected studies included in the meta-analysis and systematic review.

Author	Year	Quality	Study period	Sample PV1	Not immunized PV1	Sample PV2	Not immunized PV2	Sample PV3	Not immunized PV3	Region	Sample's age	Vaccination status
Larocca AMV	2022	h	2014–2020	123	0	123	1	123	3	Apulia	21.1 ± 2.6	All vaccinated with IPV
Bianchi FP	2021	h	2014–2020	1408	7	967	15	1408	101	Apulia	23.1 ± 4.4	All vaccinated with OPV
Ferlito C	2021	h	2012–2014	52	0	–	–	52	4	All Italy	range: 18–31	All vaccinated
Garonzi C	2020	m	2018	17	1	17	0	17	0	Veneto	<18	All vaccinated
Lupi S	2019	h	2013–2014	1073	76	1073	41	1073	178	All Italy	range: 12–50	Unknown
Giammanco GM	2018	h	2009	418	104	418	123	418	216	Tuscany	range: 0–88	Unknown
Baldo V	2012	h	2010	219	67	219	19	219	47	Veneto	27.1 ± 4.3	All vaccinated with OPV

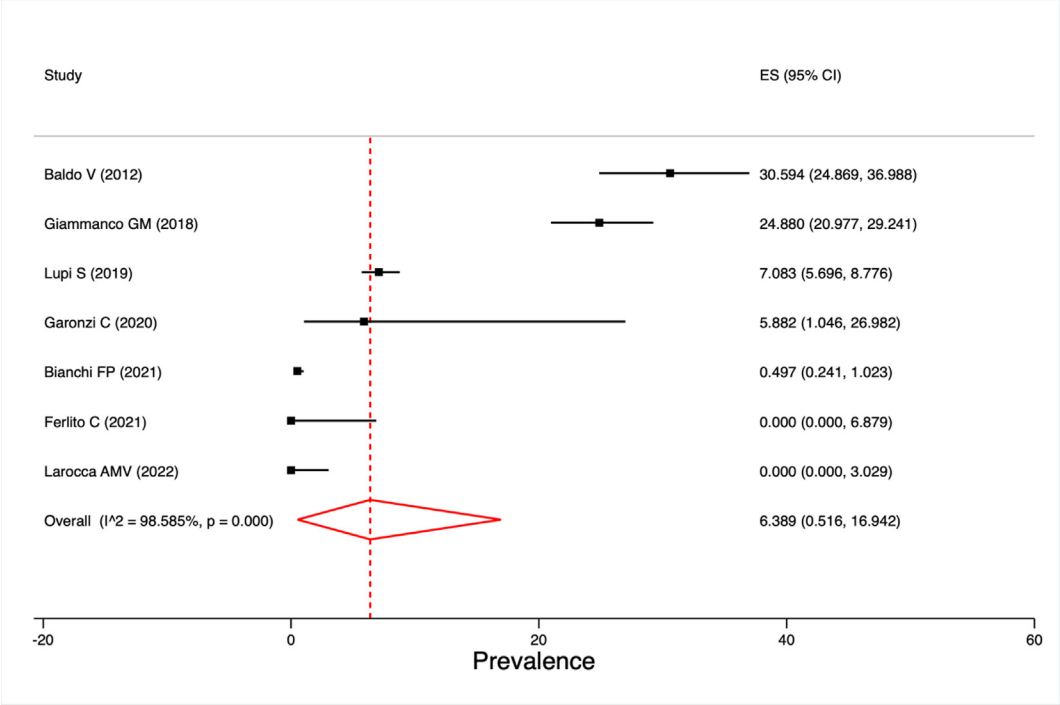

Fig. 2. Forest plot of the pooled prevalence of subjects without PV1 neutralizing antibodies. Black dots and lines: Prevalence from individual studies with 95%CI; dashed red line: x-axis reference value; red rhombus: pooled prevalence with 95%CI. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

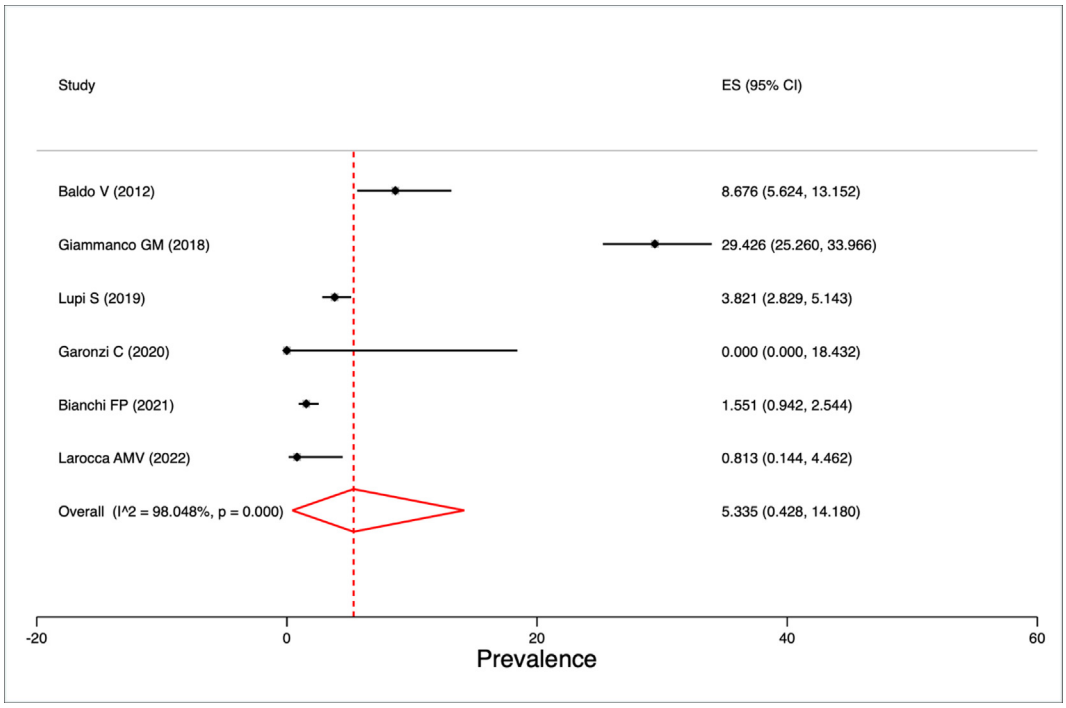

Fig. 3. Forest plot of the pooled prevalence of subjects without PV2 neutralizing antibodies. Black dots and lines: Prevalence from individual studies with 95%CI; dashed red line: x-axis reference value; red rhombus: pooled prevalence with 95%CI. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

more recent and comprehensive article already included in the systematic review, and two because the full text was unavailable. Thus, 7 studies were eligible [19–25] (Table 1). Overall, 148 studies did not meet the inclusion criteria, and therefore they were excluded.

Quality assessment. The NOS has applied appropriately to the included studies, and 85.7% were evaluated as high quality (Table 1).

Pooled analysis. The pooled prevalence of subjects without PV1 neutralizing antibodies, estimated on 3,310 subjects, was 6.4%

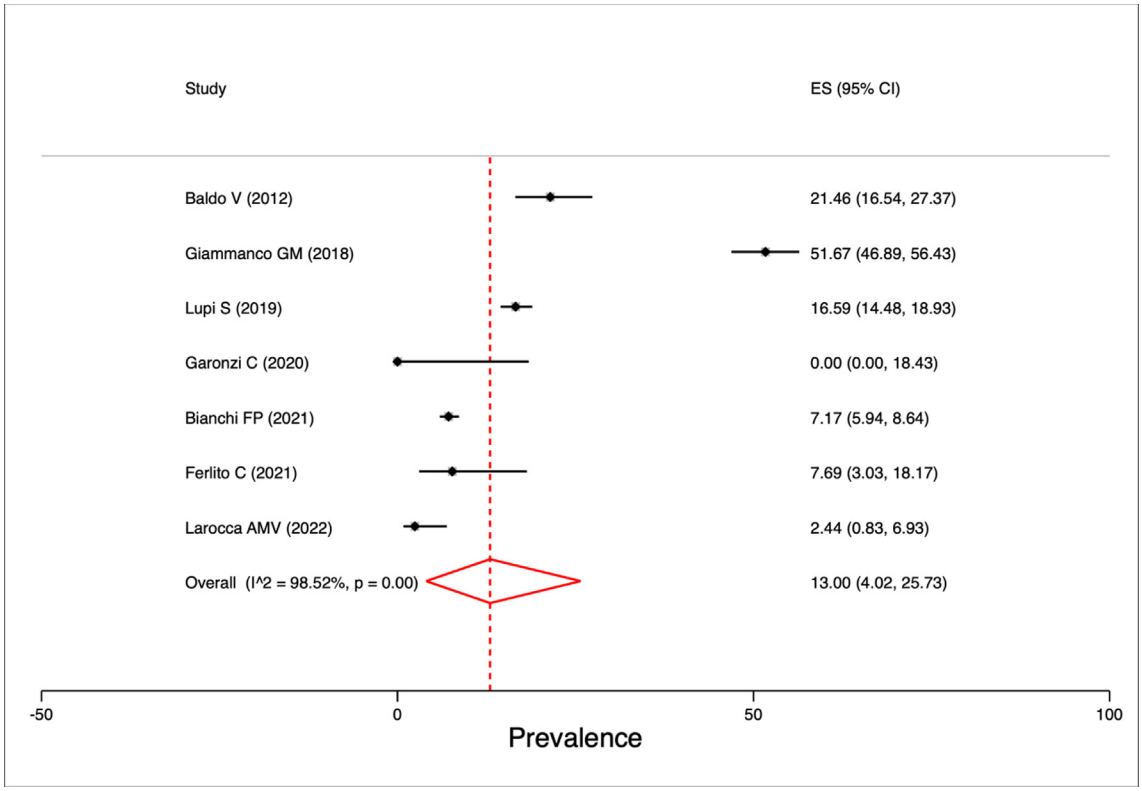

Fig. 4. Forest plot of the pooled prevalence of subjects without PV3 neutralizing antibodies. Black dots and lines: Prevalence from individual studies with 95%CI; dashed red line: x-axis reference value; red rhombus: pooled prevalence with 95%CI. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(95%CI = 0.5–16.9; I^2 = 98.6%; p-value for the heterogeneity test < 0.0001; Fig. 2), the pooled prevalence of subjects without PV2 neutralizing antibodies, estimated on 2,817 subjects, was 5.3% (95%CI = 0.4–14.2; I^2 = 98.0%; p-value < 0.0001; Fig. 3), and the pooled prevalence of subjects without PV3 neutralizing antibodies, estimated on 3,310 subjects, was 13.0% (95%CI = 4.0–25.7 ; I^2 = 98.5%; p-value < 0.0001; Fig. 4).

Considering only vaccinated or potentially vaccinated subjects, the pooled prevalence for PV1 was 6.3% (95%CI = 0.6–16.2; I^2 = 98.4%; p-value < 0.0001), for PV2 it was 5.3% (95%CI = 0.8–12 .9; I^2 = 97.3%; p-value < 0.0001), and for PV3 it was 12.9% (95%C I = 4.8–24.0; I^2 = 98.0%; p-value < 0.0001).

The sensitive analyses showed no severe distortion of the estimated pooled prevalence.

Determinants of seroprevalence and the strategies to maintain a high level of immunity in the population.

Bianchi FP and Larocca AMV [19,20] described the results of a biological screening protocol offered to students and residents of the Medical School of the University of Bari. Bianchi FP et al. [20] evaluated 1,408 students and residents, vaccinated with four doses of OPV. There were no significant differences in the seroprotection rate between the different age groups for each serotype. However, the titer of neutralizing antibodies significantly decreases with age, especially for PV1 and PV2. On the same population sample, Larocca AMV et al. [19] compared the long-term immunogenicity of IPV with that of OPV. The prevalence in the study population of non-protective neutralizing anti-PV1 antibodies was 0.20% (95% CI: 0.01–1.12), and that of anti-PV2 was 1.45% (95% CI: 0.47–3.35), with no statistically significant differences between OPV and IPV (p > 0.05); regarding the PV3 serotype, the prevalence in the study sample was 6.50% (95% CI: 4.49–9.06; n = 32/492), with a statistically significant difference between the OPV and IPV groups (7.9 % vs. 2.4%; p = 0.035). A more significant absence of not protective neutralizing antibodies for PV3 was evidenced by many authors [23–25]. Neutralizing antibodies decreased with age but without substantial differences between the OPV and IPV groups [19]. This decline is a proxy for the real risk factor, the time since the last vaccine dose, as also assessed by many studies [20,23,25]. The protective antibodies against all three serotypes persist at least 18 years after administering the last dose of OPV or IPV; a longer duration of immunity against PV3 was demonstrated for IPV compared to OPV [19,20].

In summary, the time between the last vaccination dose and the antibody titer assessment is a determinant of persistent neutralizing antibody levels. The authors suggested that while the antibody titer decreases over time, immunity against PV1 and PV2 can be considered life-long. Conversely, a fifth additional dose of IPV can strengthen the persistence of protective immunity in the long term, particularly against PV3 [19,20]. Indeed, the introduction of a fifth IPV dose in adolescence is intended to raise protective antibody titers, and to extend protective immunity and it is supported by many authors [19,20,23,24]; furthermore, a single IPV dose has been demonstrated to boost and maintain at high levels mucosal IgA response in previously OPV-vaccinated persons [24]. Therefore, since Immunization Plan 2017–2019 the Italian Ministry of Health officially recommended administering a fifth IPV dose in adolescents, and most regional health authorities have already implemented the new schedule.

Garonzi C et al. [22] evaluated the rates of protective antibodies after chemotherapy in a group of pediatric oncological patients; the authors reported that 21–26% of patients did not show protective antibody titer after therapy, and 33% of them lost protective antibodies titer comparing blood test before and after chemotherapy. The authors described a protocol to revaccinate the patients

starting from 6 months from the end of treatments; 46% of patients were compliant with this policy, and it was associated with a 100% restoration of protective antibody level for polio.

Ferlito C et al. [21] evaluated the immunity status of 31 vaccinated militaries at baseline and after administering an IPV booster dose, introduced by the Italian military health authorities in the compulsory vaccination schedule for the military in 1998. Anti-polio antibody types 1 and 3 were present in all tested individuals (PV2 was not evaluated), except for only four individuals who lacked protection against PV3. After the IPV booster, all seronegative subjects seroconverted (at least a fourfold titer increase). The authors concluded that despite the continuous decline of wild PV in circulation globally, maintaining a high level of immunity is pivotal, especially for the military. This can be considered as such not only to avoid possible reintroduction of the wild PV in Italy due to infections taken during international missions abroad but also to prevent possible vulnerabilities, which may be exploited in case of malicious use of PV as biological weapons [21].

The results of the investigations described above must be considered in light of the absence of natural boosters in Italy. In the last 30 years, no cases of poliomyelitis (or use of supplementary immunization activities) have been reported. Anyway, polio-free regions must maintain high levels of immunity in the population and reinforce surveillance systems, since it may be possible to import polioviruses at any time as long as they will not be eradicated [19–21,25].

4. Conclusion

Our study showed that among almost 3,000 Italian subjects, neutralizing antibodies against all three poliovirus types were present in > 85%, with rates of > 93% for PV1, >94% for PV2, and 87% for PV3. These values are similar to the ones reported in other serosurveys set in several European polio-free countries. A 2021 study [26] estimated the seroprevalence of neutralizing antibodies in a sample of 333 Portuguese healthcare workers, showing that 92.8% were immune to PV1, 86.5% to PV2, and 63.3% to PV3. Koivisto K et al. [27] assessed the immunity against polio in 157 pediatric HCWs in Helsinki Children's Hospital, finding that over 95% of them resulted seroprotected. A 2014 paper [28] described the seroprotection against poliomyelitis in the Dutch population using banked serum samples; 6,386 samples from a nationwide group were analyzed, showing a seroprevalence of 94.6% (type 1), 91.8% (type 2) and 84.0% (type 3).

Our systematic review reported that many authors described a decrease in the levels of neutralizing antibodies with increasing age; this decline is a proxy for the real risk factor, which is the time elapsed since the last dose of the vaccine. Over time, polio vaccines seem to trigger a longer-lasting immune response that leads to higher neutralizing antibodies for PV1 and PV2 and lower levels for PSV3. A role for age (or time elapsed since the last dose) in the long-term immunogenicity of other non-polio vaccines has been demonstrated in many surveys [29–34]. Nevertheless, two Italian studies reported that protective antibodies against all three PVs persist for at least 18 years after administering the last dose of OPV or IPV [19,20]. Indeed, International Public Health institutions reported solid scientific evidence for the long-term (>5–10 years) persistence of protective antibodies in $\geq$ 80% of the population vaccinated with $\geq$ 3–4 doses of OPV and low scientific evidence for the long-term (>5–10 years) persistence of protective antibodies in $\geq$ 80% following $\geq$ 3–4 doses of IPV [14,15]. As proposed by many authors, the fifth IPV dose recommended in adolescence could guarantee life-long immunity [19,20,23,24].

The principal limitation of this study was the significant heterogeneity between papers, as suggested by the I^2 values; nevertheless, the use of random-effects analysis diminished the bias. However, a strength of our paper was the considerable sample size after the assortment of selected studies, which enhanced the statistical analysis, providing an improved interpretation of polio immunity status among Italians. Moreover, we investigated the scientific literature published in the last 10 years, and therefore we considered a long time-span period, even if only seven studies were included. Another limit is that we could not perform any sub-analysis per type of vaccine (IPV vs. OPV), age, sex, etc.. In the future, similar meta-analyses should embrace more papers to conduct sub-analyses by age, vaccination status, and geographic area.

Geographically, Italy is a border region that, in recent years, has taken in many migrants, such that there is a risk of poliovirus importation from endemic areas, even if several papers [35–37] reported a good level of immunization among refugees. However, as long as there are areas where polio is still present, its reappearance in polio-free countries is still possible. Many experiences are reported in the literature, such as the 2010 WPV1 outbreak in Tajikistan (polio-free country) and the 1992–1993 outbreak in the Netherlands [38,39]. Even imported cases of vaccine-associated paralytic poliomyelitis may be a critical concern; in 2013, a VAPP case was described in Italy in an immunodeficient Albanian pediatric patient [40].

Consequently, until the eradication of PV1 and to deal with variants of Sabin-like polioviruses, public health institutions must be alert to its possible manifestation (indeed, stocks of OPV vaccine are prepared in case of reintroduction of the wild PV in European countries [41]). In this light, the active surveillance for cases of acute flaccid paralysis seems to be crucial to maintain the polio-free status in Italy. All cases of acute flaccid paralysis in patients under the age of 15 and all cases of suspected poliomyelitis, regardless of patient age, must be reported [42]. Furthermore, high polio vaccine coverage is still an aim of the most recent Italian immunization plan [43]; thus far, the effectiveness of the immunization campaign executed since 1960s has freed the world from a disease that has plagued humanity for centuries.

5. Data statement

No original data are reported on this review.

Funding

This research was founded by Puglia Region.

CRediT authorship contribution statement

Silvio Tafuri: Conceptualization, Supervision, Project administration. **Eustachio Cuscianna:** Data curation, Writing – original draft. **Francesco Paolo Bianchi:** Investigation, Formal analysis, Methodology.

Data availability

No data was used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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