no PCR products in either reaction, indicating that the target sequences of the *cagA* primers were mutated or deleted. The 28 isolates with ambiguous PCR results were excluded from the evaluation in Table 1. However, if they were included and regarded as *cag* PAI$^+$, in accordance with the interpretations made above, the kappa coefficient was estimated as 0.80 (95% CI 0.64–0.95), compared with 0.57 (95% CI 0.40–0.75) if they were regarded as *cag* PAI$^-$. The former value is essentially identical to that in Table 1, thereby supporting the original interpretations. The occurrence of such PCR ambiguities could be reduced by examining single-colony isolates, but this increases the risk of overlooking strain variants unless numerous colonies are included.

In conclusion, agreement between the results of tests to determine the serological CagA status and the *cag* PAI PCR results of *H. pylori* infections is satisfactory, considering the biological prerequisites. Genotypic approaches are required when the characteristics of individual bacterial clones are being studied. However, in the clinical and epidemiological settings, current and past infections of the whole gastric mucosa are typically of interest. Genotypic methods are then limited by the availability of biological specimens from sometimes asymptomatic individuals, and by the need to assess strain variants dispersed throughout the stomach. Despite previous advice against serology [4], the present data support the application of well-evaluated serological assays in clinical and epidemiological studies.

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RESEARCH NOTE

A seroprevalence study of poliovirus antibody in the population of northern Greece

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ABSTRACT

This study assessed immunity to poliomyelitis in a representative sample of 1064 persons living in

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northern Greece. Antibody prevalences in the individuals tested were 91.1% (95% confidence interval (CI): 89.4–92.8), 92.1% (95% CI: 90.5–93.7) and 83.1% (95% CI: 80.8–85.4) for poliovirus types 1, 2 and 3, respectively. For poliovirus type 3, a gap in immunity was found in individuals aged 10–29 years. Re-vaccination of adolescents living in northern Greece is suggested to ensure herd immunity and to minimise the risk of importation of wild poliovirus from endemic countries.

In the 12 years since the World Health Organization (WHO) launched its initiative to eradicate poliomyelitis by the year 2000, the annual number of cases of poliomyelitis reported to WHO has dropped from >35 000 to 2880 [1]. The eradication of poliomyelitis has been complicated by events such as the importation of wild polioviruses into polio-free countries and the circulation of neurovirulent vaccine-derived polioviruses [2,3]. As a consequence, the WHO target for poliomyelitis eradication has been reset to 2005.

Greece has been free of indigenous cases of poliomyelitis since 1982 [4], but an outbreak of paralytic disease caused by a wild type 1 poliovirus occurred in the neighbouring country of Albania in 1996 [5]. During the same period, five cases of poliomyelitis occurred in Greece in non-vaccinated Gypsy children. The close homology of these Greek isolates with the Albanian genotype suggested direct virus importation [6].

Oral polio vaccine was introduced into Greece in 1964, and was offered to 2 million persons aged ≤18 years [7]. After 1964, a standard vaccination schedule was initiated, and this remains in force today. Four doses are scheduled at the age of 2, 4, 6 and 18 months, with a booster dose at 4–6 years. The estimated vaccination coverage with three or four documented doses, independent of age at immunisation, has been >95% since 1990, but there have been delays in the receipt of three doses by the age of 1 year [8]. Serosurveillance is of value in countries where there are pockets of non-vaccinated individuals [9]. The main objective of the present study was to gain an insight into possible gaps in immunity in the northern Greek population.

The study was carried out in 2001–2002 and involved 1064 apparently healthy individuals (550 males and 514 females), aged from 3 months to ≥70 years, living in 17 prefectures in northern Greece (total population 2.8 million). The prefectures were sampled in proportion to the number of individuals in each age group, based on the 2001 census [10]. An age-stratified sample was selected from each prefecture by single random sampling.

Antibodies against poliovirus types 1, 2 and 3 were determined with a microneutralisation assay with prototype Sabin strains, according to the WHO guidelines [11]. Sera were heat-inactivated, diluted two-fold from 1:8 to 1:1024, and then incubated in duplicate for 3 h at 36°C with $100 \times 50\%$ tissue culture infective dose ($TCID_{50}$) of poliovirus antigen. A cell suspension containing 2×10^4 L20B cells/0.1 mL was added. Cell controls and an in-house reference serum sample of known neutralising activity were included in each batch. After incubation for 5 days, the highest dilution of serum that protected 50% of the cultures was recorded. A serum sample was considered positive if antibodies were present at a dilution ≥1:8. Results were expressed as log_2 reciprocal titres (log_2 titre $1:8 = 3$). The chi-square test was used for group comparisons of the seroprevalence, and Student's *t*-test was used to analyse differences between geometric mean titres (GMTs).

Tables 1 and 2 show that the population in northern Greece is generally well-protected against poliovirus types 1 and 2, but less so against type 3, the seroprevalences being 91.1%, 92.1% and 83.1%, respectively, according to WHO criteria. While the seroprevalence of types 1 and 2 was found to be close to or >90% in almost all age groups, unsatisfactory levels of immunity were observed for type 3 in the group aged 10–29 years (seroprevalence range 75–76.2%, p 0.013–0.003).

Although individuals with a neutralising antibody titre of ≥1:8 are regarded as immune, it is unclear whether all individuals with undetectable antibodies are susceptible to infection, particularly the elderly, who may be protected by memory immunity. Immunity to poliovirus is

Table 1. Age-specific prevalence of antibodies (95% confidence interval (CI)) against poliovirus types 1, 2 and 3 in 1064 individuals living in northern Greece, 2001–2002

Age group	No. tested	Poliovirus type 1 protective antibodies		Poliovirus type 2 protective antibodies		Poliovirus type 3 protective antibodies	
		%	95% CI	%	95% CI	%	95% CI
3–11 months	66	84.8	76.1–93.5	89.4	82.0–96.8	78.8	69.0–88.6
1–4 years	91	96.7	93.0–100	96.7	93.0–100	87.9	81.2–94.6
5–9 years	107	97.2	94.1–100	98.1	95.5–100	89.7	83.9–95.5
10–14 years	104	96.2	92.5–99.9	96.2	92.5–99.9	75.0[a]	66.8–83.2
15–19 years	80	95.0	90.2–99.8	96.2	91.9–100	76.2[a]	66.9–85.5
20–29 years	117	89.7[a]	84.2–95.2	93.2	88.6–97.8	76.1[a]	68.3–83.9
30–39 years	103	89.3[a]	83.3–95.3	93.2	88.3–98.1	82.6	75.3–89.9
40–49 years	104	90.4[a]	84.7–96.1	88.5[a]	82.4–94.6	88.5	82.4–94.6
50–59 years	90	87.8[a]	81.0–94.6	86.7[a]	79.7–93.7	85.6	78.3–92.9
60–69 years	107	86.0[a]	79.4–92.6	86.0[a]	79.4–92.6	86.9	80.5–93.3
≥ 70 years	95	87.4[a]	80.7–94.1	88.4[a]	82.0–94.8	85.3	78.2–92.4
Total	1064	91.1	89.4–92.8	92.1	90.5–93.7	83.1	80.8–85.4

[a]Significant differences between this age group and the age group with the highest prevalence (5–9 years).

Table 2. Age-specific geometric mean titre (GMT) of antibodies (95% confidence interval (CI)) for poliovirus types 1, 2 and 3 in 1064 individuals living in northern Greece, 2001–2002

Age group	No. tested	Poliovirus type 1 GMT		Poliovirus type 2 GMT		Poliovirus type 3 GMT	
		Value	95% CI	Value	95% CI	Value	95% CI
3–11 months	66	7.0	6.4–7.6	7.1	6.4–7.6	6.5	5.8–7.1
1–4 years	91	6.6	6.2–7.0	6.7	6.3–7.0	5.7	5.3–6.1
5–9 years	107	6.2	5.8–6.5	6.2	5.8–6.5	5.2[a]	4.8–5.6
10–14 years	104	5.7	5.3–6.1	5.6[a]	5.3–6.0	4.9[a]	4.5–5.4
15–19 years	80	5.2[a]	4.8–5.6	5.1[a]	4.7–5.6	4.5[a]	4.2–4.9
20–29 years	117	5.3[a]	5.0–5.6	5.2[a]	4.8–5.5	4.8[a]	4.4–5.2
30–39 years	103	5.1[a]	4.8–5.4	5.3[a]	5.0–5.6	4.9[a]	4.6–5.3
40–49 years	104	5.3[a]	5.0–5.6	5.4[a]	5.0–5.7	5.1[a]	4.8–5.4
50–59 years	90	5.1[a]	4.7–5.4	5.2[a]	4.8–5.6	4.9[a]	4.5–5.2
60–69 years	107	5.1[a]	4.7–5.4	5.1[a]	4.7–5.4	5.2[a]	4.9–5.6
≥ 70 years	95	5.3[a]	4.9–5.7	5.4[a]	5.0–5.7	5.0[a]	4.7–5.4
Total	1064	5.6	5.5–5.7	5.6	5.5–5.7	5.1	5.0–5.2

[a]Significant difference between this age group and the groups with the highest GMT (3 months to 4 years).

largely dependent on humoral antibodies, but the production of intestinal IgA may also play a significant role [12].

As systematic polio vaccination was introduced in Greece in 1964, it can be assumed that the antibodies detected were mainly attributable to natural infection in individuals born before 1946, whereas those in individuals born after 1964, and in most individuals born between 1946 and 1964, were mainly vaccine-induced.

The seroprevalence of poliovirus types 1 and 2 was higher than that of type 3, and this finding might be attributable to the lower potency of poliovirus type 3 antigens. For all types, the highest prevalence and GMTs were found in children aged 1–9 years, indicating a good response to primary vaccination and the booster dose. In groups aged 10–19 years, high levels of immunity were found for poliovirus types 1 and 2, while lower but still high levels were found in groups aged 20–49 years. These results are consistent with those reported in other studies, and might be explained in some individuals by a booster effect caused by continued exposure to the wild virus [13,14]. The seroprevalence was also high, with stable antibody titres, in individuals aged ≥50 years, which probably represents predominantly natural immunity. Similar findings have been reported in some other countries [13,15–18].

The lowest seroprevalence was observed for type 3 virus in groups aged 10–29 years. This indicated an unsatisfactory level of immunity in adolescents and young adults. Significantly low GMTs were also found in these age groups. These results are consistent with those of some previous studies performed in other European countries [16,19]. The delays in receipt of oral polio vaccine doses, or the fact that a booster dose was not given since infancy, may have contributed to the decline in titres of neutralising antibodies.

In the present study, females aged 20–39 years had better protection than males in the same group (data not shown), possibly because of closer contact with small children vaccinated recently. The possible role of secondary spread of vaccine virus is supported by the findings of other studies [20].

In conclusion, the serological status of the population of northern Greece against poliovirus is at a high level for types 1 and 2. The low percentages of protective antibodies to poliovirus type 3 in adolescents and young adults suggest the need for a booster dose of vaccine to ensure personal and herd immunity and to minimise the risk of importation of wild poliovirus from endemic countries.

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RESEARCH NOTE

Isolation of an amikacin-resistant *Escherichia coli* strain after tobramycin treatment of previous recurrent episodes of respiratory tract infections caused by *Pseudomonas aeruginosa*

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ABSTRACT

Amikacin-resistant *Escherichia coli* strains are isolated rarely from clinical samples. In the present study, investigation of an amikacin-resistant clinical isolate of *E. coli* demonstrated the presence of two class 1 integrons carrying the *aacA4* gene plus the *aacA7* gene, and the *dfrA17* gene plus the *aadA5* gene, respectively. Resistance to amikacin in this *E. coli* isolate was related to the presence of both *aacA4* and *aacA7*.

Keywords *aac* genes, amikacin, *Escherichia coli*, integrons, resistance

During recent years, a dramatic increase has been observed in the number of multiresistant microorganisms. In *Escherichia coli*, high levels of resist-

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