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Seroprevalence of IgG against conformational and linear capsid antigens of parvovirus B19 in Italian blood donors

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SUMMARY

Serum samples from 446 Italian blood donors between 18 and 65 years of age were analysed for the presence of IgG against parvovirus B19 capsid proteins VP1 and VP2 including conformational and linear epitopes. The overall prevalence of IgG against parvovirus B19 capsid proteins VP1 and VP2 against at least one antigen type was 79·1%. No significant difference was found between men and women. In the 18–27 years age group, 77·0% of the population had experienced infection with the virus, reaching 88·5% in the 48–57 years age group. The overall prevalence of IgG was 78·0% against conformational VP1 + VP2 antigens, 74·9% against conformational VP2, 70·9% against linear VP1 and 23·3% against linear VP2 in the analysis of the IgG response against different conformational and linear epitopes of VP1 and VP2. Although IgG against conformational VP1+VP2, conformational VP2 and linear VP1 was present in more than 60% of subjects in all age groups, IgG against VP2 linear antigens was present in only 32% of subjects in the 18–27 years age group and then decreased to 20·5% in the 28–37 years age group. A different trend was noted when IgG positivity against linear and conformational epitopes was analysed separately in men and women. A significant increase was found in seroprevalence of IgG against VP2 conformational antigens with increasing age in males and a significant decrease in seroprevalence of IgG against VP2 linear antigens in women with increasing age.

INTRODUCTION

Human parvovirus B19 can cause asymptomatic infections or acute self-limiting diseases such as erythema infectiosum in children, rash, fever and transient arthralgias in adults, as well as aplastic crises in patients with underlying haemolytic anaemias and hydrops fetalis after maternal infection during pregnancy. Parvovirus B19 has also been reported to cause persistent infections in individuals who are immunocompromised, causing chronic bone marrow failure and long lasting red cell aplasia. Moreover, some cases of persistent B19 infections associated with chronic arthropathy have been described in immunocompetent patients [1].

Infection with B19 has been reported worldwide, and the virus is usually spread by the respiratory

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route. The prevalence of immunoglobulin G (IgG) antibodies against B19 structural proteins increases with age; in Western countries about 15% of 5-year-old children have detectable IgG, approximately 50% of 5- to 20-year-old subjects are seropositive. In the adult population, over 50 years of age, seropositivity increases by up to 60–80% [2].

Specific IgG presence is a sign of past infection and gives protective immunity but reinfections have been described in the presence of a low level of specific IgG.

The parvovirus B19 immunological response is mainly directed against two structural proteins, VP1 (83 kDa) and VP2 (58 kDa), which represent approximately 4 and 96% respectively, of the B19 capsid. The VP1 and VP2 capsid proteins, coded from the same reading frame, are identical except VP1 contains 227 additional amino acids at the amino terminus. Several studies have demonstrated that parvovirus B19 IgG has a different reactivity against conformational and linear epitopes of VP1 and VP2 [3–5] and that the different reactivity against the various epitopes can be used as a marker of a recent or past B19 infection. Recombinant VP1 and VP2 proteins, expressed in both prokaryotic and eukaryotic systems, can be used to detect an immune response against conformational or linear epitopes when non-denatured or denatured antigens are used respectively. An updated serological survey, stratified by age and sex, in Italian blood donors was carried out to study the seroprevalence of IgG against B19 and the detailed seroprevalence against linear and conformational epitopes of both VP1 and VP2.

MATERIALS AND METHODS

Samples

A total of 446 serum samples were analysed from blood donors aged between 18 and 65 years, which had proved negative for B19 IgM and had been collected over 1 year in a non-epidemic period. The age and sex distribution of the blood donors is shown in the Table. The age distribution was not significantly different between males and females ($P = 0{\cdot}286$).

Serum samples, collected in aliquots, were kept frozen at $-20\,^{\circ}C$ until use. The samples were analysed for the presence of (*a*) IgG against VP1+VP2 using recombinant conformational antigens by ELISA; (*b*) IgG against VP2 using recombinant conformational antigens by ELISA; (*c*) IgG against VP1 using denatured linear antigens by Western blot; (*d*) IgG against VP2 using denatured linear antigens by Western blot.

Table. *Age and sex distribution among the studied blood donors*

Age groups (years)	Males	Females	Total
18–27	50 (23·2%)	50 (21·7%)	100 (22·4%)
28–37	39 (18·1%)	49 (21·3%)	88 (19·7%)
38–47	45 (20·8%)	32 (13·9%)	77 (17·3%)
48–57	37 (17·1%)	50 (21·7%)	87 (19·5%)
58–65	45 (20·8%)	49 (21·3%)	94 (21·1%)
Total	216	230	446

Detection of IgG against VP1 and against VP2 conformational antigens by commercial enzyme immunoassay

Specific IgG against VP1+VP2 conformational epitopes was detected by an enzyme immunoassay using both parvovirus B19 VP1 and VP2 proteins expressed by recombinant baculovirus as antigens (Medac, Hamburg, Germany). Specific IgG against only VP2 conformational epitopes was detected by an enzyme immunoassay using parvovirus B19 VP2 capsids expressed by recombinant baculovirus as antigen (Biotrin, Dublin, Ireland). The performance of the ELISA assays and the interpretation of the results were carried out according to manufacturers' instructions.

Detection of IgG against VP1 and against VP2 linear epitopes by Western blot

Recombinant capsid proteins VP1 and VP2 of human parvovirus B19 were cloned and expressed in *E. coli* as fusion proteins with a biotinylated 13 kDa peptide as described previously [5] and were then purified by metal chelate affinity chromatography in the presence of 8 M urea. Bands of approximately 97 and 71 kDa, corresponding to the expected size of VP1 and VP2 proteins fused with the 13 kDa peptide, were visualized by SDS–PAGE analysis. The purified capsid proteins (VP1 and VP2) were loaded in equal amounts and were separated under denaturing conditions by SDS–PAGE (8% acrylamide). They were then transferred to a nitrocellulose membrane which was cut longitudinally into 2-mm-wide strips. In the Western blot assay, each strip was treated with blocking

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buffer [1% dried milk in 150 mM NaCl, 100 mM Tris–Cl (pH 7·5)] for 2 h and then incubated for 1 h with human sera (1:100 in blocking buffer). After washing with PBS 0·3% Tween-20, the strips were incubated for 1 h with peroxidase-conjugated anti-human IgG (Dako A/S, Glostrup, Denmark) diluted 1:1000 in blocking buffer. The membranes were then incubated in the dark with the peroxidase enzyme substrate which consisted of a solution of 2 ml methanol containing 6 mg 4-chloro-1-naphtol (Bio-Rad Laboratories, Milan, Italy) added to a solution of 10 ml PBS containing 6 μl of 10% hydrogen peroxide immediately before use. Membranes were washed in distilled water, air dried and stored.

Statistical analysis

Frequencies were used as descriptive statistics. Data were analysed by means of the χ^2, Pearson and Mantel–Haenszel test for linear association, the McNemar test and the kappa statistic. Two-tailed P values less than 0·05 were considered statistically significant. Statistical analysis was performed by running the SPSS/PC+ statistical package [6].

RESULTS

The overall prevalence of parvovirus B19 IgG to capsid proteins VP1 and VP2 in at least one assay against conformational or linear antigens in samples from 18- to 65-year-old Italian blood donors was 79·1%. At 18–27 years of age, 77·0% of the population had experienced infection with the virus, reaching 88·5% in the 48–57 years age group. No significant trend was found between IgG seroprevalence and age ($P=0·124$).

When the B19 IgG prevalence was studied in men and women separately (Fig. 1), the overall prevalence was 78·2% in men and 80·0% in women and showed no significant difference ($P=0·734$). Although no significant relationship was found between prevalence of IgG and age in men ($P=0·329$), a significant increase in seroprevalence with age was found in women ($P=0·002$). Comparing gender within each age group, the seroprevalence in men was significantly higher in the 28–37 years age group ($P=0·046$) while in women it was statistically higher in the 48–57 years age group ($P=0·003$).

In the analysis of IgG response against different conformational and linear epitopes of VP1 and VP2, the overall prevalence of IgG was 78·0% against

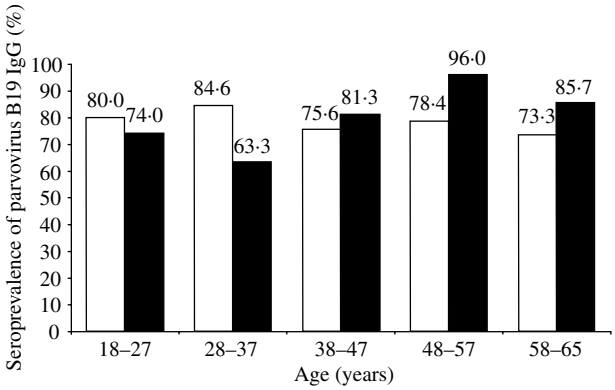

Fig. 1. Seroprevalence of B19 IgG stratified for different age groups and sex. □, Males; ■, females.

conformational VP1+VP2, 74·9% against conformational VP2, 70·9% against linear VP1 and 23·3% against linear VP2. The IgG prevalence against each antigen was significant when compared with each other (P values ranging from $<0·001$ to 0·007).

A good concordance in seroprevalence between IgG against conformational VP1+VP2 and IgG against conformational VP2 was found ($\kappa=0·851$), as well as a good concordance between IgG against linear VP1 and IgG against conformational VP1+VP2 and conformational VP2 ($\kappa=0·754$ and 0·898 respectively). However, a poor concordance was found between IgG against VP2 linear antigens and the other three types of IgG ($\kappa=0·144$, 0·185 and 0·222 respectively). No significant difference in seroprevalence of IgG against B19 conformational or linear antigens was found between males and females ($P=1·000$, 0·071, 0·075; $P=0·846$ respectively).

The IgG response against different conformational and linear epitopes of VP1 and VP2, stratified by age group is shown in Figure 2. While IgG against conformational VP1+VP2, conformational VP2 and linear VP1 was present in more than 60% of subjects in all age groups, IgG against VP2 linear antigens was present in only 32% of subjects in the 18–27 years age group and then decreased to 20·5% in the 28–37 years age group remaining stable throughout the older age groups. A significant positive relationship between the seroprevalence of IgG against conformational VP2 and age was found ($P=0·008$).

The distribution of IgG response against different conformational and linear epitopes of VP1 and VP2 in males (Fig. 3*a*) and females (Fig. 3*b*) of different ages showed a significant increase in IgG prevalence against VP2 conformational antigens in males with

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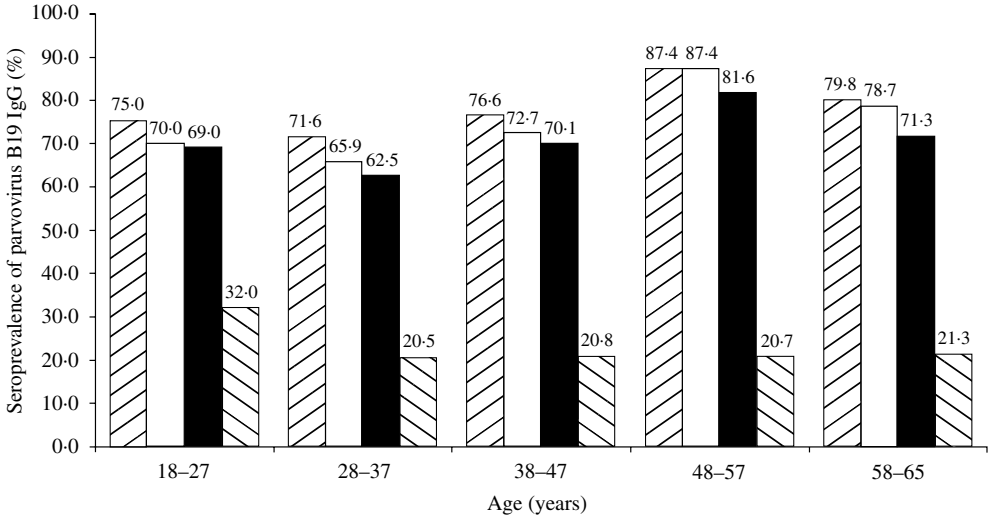

Fig. 2. Seroprevalence of B19 IgG against VP1 and VP2 conformational and linear epitopes stratified for different age groups. ⊿, VP1 + VP2 conformational; □, VP2 conformational; ■, VP1 linear; ◺, VP2 linear.

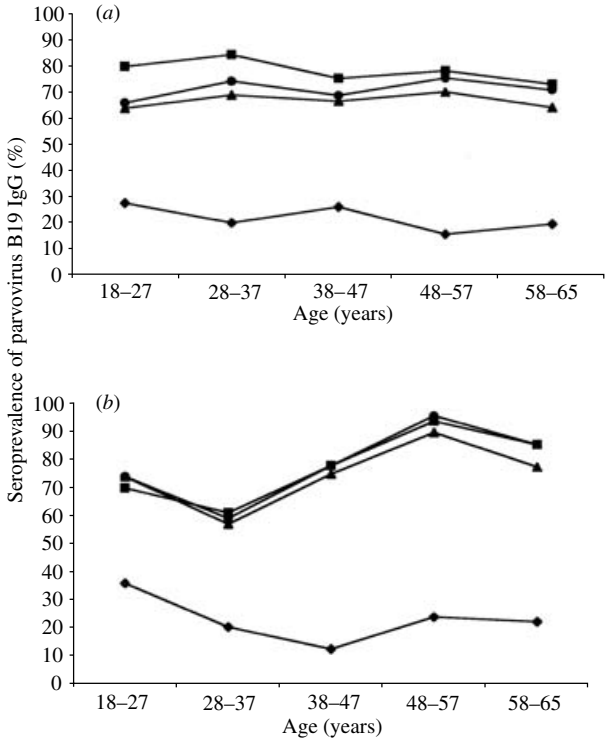

Fig. 3. Seroprevalence of B19 IgG against VP1 and VP2 conformational and linear epitopes stratified for different age groups in (*a*) males and (*b*) females. –■–, VP1 + VP2 conformational; –●–, VP2 conformational; –▲–, VP1 linear; –◆–, VP2 linear.

increasing age ($P = 0{\cdot}016$) and a significant decrease in IgG prevalence against VP2 linear antigens in women with increasing age ($P = 0{\cdot}027$).

DISCUSSION

Comparing Italian data on IgG prevalence against parvovirus structural proteins with data obtained in other studies [2, 7, 8–11] in different parts of the world stratified by age (Fig. 4), it seems clear that in continental Europe contact with B19 occurs at lower ages. However, in Asiatic countries the distribution of the virus seems more limited, the contact is postponed, and therefore a progressive age dependency of seroprevalence of IgG to B19 seems more evident. The low distribution of B19 in Asiatic countries is confirmed by the low overall seroprevalence of IgG in Singapore [12] and Thailand [13] (16·2 and 20·16% respectively). The B19 IgG prevalence in the Italian study seems higher than in other countries probably due to the use of very sensitive ELISA tests of the last generation using baculovirus-generated viral particles with VP1 + VP2 antigens. However, most epidemiological studies used ELISA assays employing baculovirus-generated viral particles with only VP2 antigens [2, 8–10, 14–17]. The higher prevalence of B19 immunity in the present study could also be explained by the high density of the population given that B19 is transmitted after close contact exposure. In a study performed in Rio de Janeiro, 80% of children between 11 and 15 years of age were positive for B19 IgG [18], while in another Bazilian study, between 5 and 11% of members of Brazilian tribes were parvovirus IgG positive [19]. However, a report from Hong Kong [9] which has a population of 6 million in an area of 1000 km² and an annual

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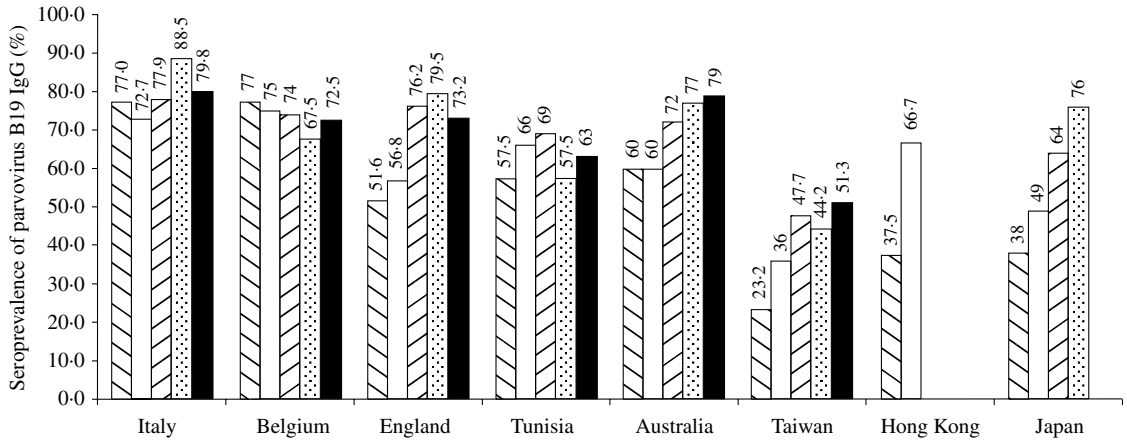

Fig. 4. Comparison of the prevalence of IgG to B19 in different countries stratified for different age groups. ⊠, 18–27 years; □, 28–37 years; ▨, 38–47 years; ⊡, 48–57 years; ■, 58–65 years.

number of 10 million visitors showed a lower prevalence rate in young adults. This may suggest that other factors than population density may play an important role in the infectivity of the virus.

Overall, there was no significant difference in B19 IgG prevalence in Italian men and women although seroprevalence in men was significantly higher in the 28–37 years age group, while in women it was higher in the 48–57 years age group. The prevalence of IgG in females was found to be higher than in males in various studies performed in the United States, Brazil and Taiwan [10, 19, 20] and this situation was explained by the more frequent close contact of adult women with children. On the other hand, studies performed in Brazil, Australia, and Chile [2, 18, 21], as well as the present study, showed no gender differences in the prevalence. Since parvovirus B19 infection during pregnancy has been associated with hydrops fetalis and fetal death, the study of susceptibility to B19 in fertile women would help estimate the risk of infection with B19 during pregnancy in epidemic and endemic situations.

In the present Italian study, the seroprevalence of IgG against linear VP2 (23·3%) was particularly low in comparison with the prevalence of IgG against conformational VP1 + VP2, conformational VP2 and linear VP1 (78·0, 74·9 and 70·9%). With respect to B19 structural proteins, most neutralizing epitopes on VP2 are conformational while those on VP1 appear to be linear [22]. Therefore, a long-lasting immune response against VP2 conformational and VP1 linear antigens is consistent with the protective role of this immunoglobulin against B19 reinfection. Since IgG against VP2 linear antigens was present in only 32·0% of subjects in the 18–27 years age group and then significantly decreased to 20·5% in the 28–37 years age group, the present data appear to be consistent with findings that IgG reactivity toward linear epitopes on VP2 is of short duration. IgG was present in almost all the subjects with a very recent B19 infection which then undergoes a drastic temporal diminution [5]. The higher prevalence of antibody against linear VP2 in the 18–27 years age group in the present study may reflect a recent infection.

When IgG positivity against linear and conformational epitopes was analysed separately in men and women a different trend was noted, with a significant increase in IgG prevalence against VP2 conformational antigens in males with increasing age, and a significant decrease in seroprevalence of IgG against VP2 linear antigens in women with increasing age. The different immune response in men and women may reflect different clinical features in men and women. Adult women are especially prone to B19 arthropathy [23], which is thought to be immunologically mediated, and the causes of this gender prevalence are still unknown. Further studies are necessary to investigate whether the different reactivity of B19 IgG in men and women is of clinical significance.

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