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Parvovirus B19 viraemia in Dutch blood donors

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SUMMARY

Blood, donated by asymptomatic donors, may contain and transmit parvovirus B19. To investigate the dynamics of parvovirus viraemia in asymptomatic blood donors, we studied the amounts of parvovirus DNA in pools of donor plasma, the prevalence of parvovirus antibodies among blood donors in relation to age, and the seasonal and year-to-year variation of the incidence of parvovirus infection in The Netherlands. The incidence of parvovirus infection follows a seasonal cycle and a cycle of several years. Among Dutch blood donors the incidence was estimated to be 0·56% per year. Forty seven out of 100 pools of 5000 plasma donations tested positive for parvovirus DNA. We inferred that the course of viraemia in asymptomatic donors shows a short peak ($>10^9$ copies parvovirus DNA/ml), followed by viraemia below 10^6 copies/ml for about 2 weeks.

INTRODUCTION

Parvovirus B19 is a small, non-enveloped, single-stranded DNA virus. Parvovirus B19 is the only member of the Erythrovirus genus, which was named because the virus replicates in the red cell precursors in the bone marrow, using the P blood group antigen as a receptor. Infection with parvovirus often has no or non-specific symptoms. In children it may present as erythema infectiosum or 'fifth disease' and in adults it may cause arthritis. Parvovirus infection causes aplastic crisis in people suffering from sickle-cell disease or chronic haemolytic anaemia. In immunodeficient patients the infection may cause chronic anaemia. During pregnancy parvovirus infection may affect the foetus, causing foetal anaemia; cardiac failure; oedema ('hydrops foetalis') and death.

The natural route of transmission is through respiratory secretions, but transmission via plasma products must occur frequently, considering the high rate of anti-parvovirus seroconversion observed among haemophiliacs [1, 2]. The identification by polymerase chain reaction and the subsequent removal of high-titred parvovirus viraemic donations from plasma pools significantly improves the viral safety of plasma products [3]. It is believed that in immunocompetent persons parvovirus infection is characterized by a highly viraemic phase of relative short duration. Nucleic acid testing on pooled samples of blood donors enabled us to further study this issue. To define more precisely the dynamics of parvovirus viraemia among blood donors we additionally studied the seasonal variation of the incidence of parvovirus infection in the general population, and the prevalence of parvovirus antibodies in blood donors of different age groups.

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METHODS

In a combined analysis of four data sources we estimated the incidence of parvovirus infection and the duration of parvovirus viraemia in Dutch blood donors. We also obtained estimates of the force of infection and the basic reproduction number for parvovirus infection.

Data sources and laboratory methods

To investigate the magnitude of year-to-year and seasonal variation of the incidence of parvovirus infection we collected the number of parvovirus infections as reported four-weekly by the major virology laboratories to the Dutch authorities (for results see ref. [4]). Although these data do not cover the whole of the country, it can be safely assumed that they reflect the relative magnitude of the seasonal and year-to-year variation of the number of parvovirus infections in The Netherlands.

We used data from an earlier study on the prevalence of antibodies to parvovirus B19 in 246 boys and male blood donors aged <1–69 years [2] (see Table), using an ELISA test based on a recombinant VP2 protein (Biotrin, Dublin, Ireland). We obtained the age distribution of Dutch blood donors who donated blood between October 2002 and September 2003 (we excluded two donors over 85 years, including one aged 100 years).

We undertook quantitative parvovirus DNA testing on 100 pools of donor plasma, each containing 5000 units of plasma collected from Dutch blood donors in 1999 and 2000. These 100 pools were randomly selected from 256 available pools of plasma. The test was performed using a validated assay, described in detail elsewhere [5]. In summary, DNA was obtained following Boom's procedure [6], using the NucliSens isolation reagents and the NucliSens extractor (bioMérieux, Marcy-l'Etoile, France). A total of 1 ml of plasma was added to lysis buffer containing guanidine thiocyanate and Triton X-100. Internal control DNA was added to each sample, enabling the monitoring of the efficiency of the extraction, amplification and detection procedure. This internal control was a synthetic double-stranded DNA molecule, featuring primer sites identical to the target parvoviral DNA, but with a unique hybridization probe binding region. Viral particles were disrupted and nucleic acids released. Silica particles were added to the lysate, binding released nucleic acid. The silica was washed several times. Nucleic acids were eluted from the solid phase. A total of 5 μl of the eluate was used for the quantitative detection of parvovirus DNA using a LightCycler apparatus (Roche Diagnostics Corporation, Indianapolis, IN, USA). During amplification of parvovirus and internal control DNA, the intensity of fluorescence reflects the amount of DNA produced. To determine the amount of parvovirus DNA in a sample, external standards with different amounts of parvovirus DNA were amplified, from which the LightCycler software constructed a calibration curve. The test was calibrated using the international parvovirus DNA standard. The sensitivity of the assay was determined to be 100 copies/ml (33 IU/ml) with a 95% hit rate; and 33 copies/ml (10 IU/ml) with a 50% hit rate.

Table. *Seroprevalence of antibodies to parvovirus B19 among 246 male blood donors and boys*

Age (years)	Total (n)	Positive	
		n	(%)
<1–10	48	11	(23)
11–20	19	10	(53)
21–30	28	16	(57)
31–40	47	33	(70)
41–50	55	44	(80)
51–60	32	27	(84)
61–70	17	15	(88)

Statistical methods

We used the serological survey data, grouped in fourteen 5-year age bands, to estimate the force of infection for parvovirus. We investigated several models, including a constant force of infection model, a piecewise constant model, and the gamma model $\lambda(x) = \alpha x \exp(-x/\beta) + \gamma$, which allows for unimodal departure from the constant force of infection model [7]. Goodness of fit was evaluated using the deviance [8].

We calculated the average annual incidence rate of parvovirus in Dutch blood donors as

$$\mu = \sum_{x=18}^{85} \pi(x)[S(x) - S(x+1)],$$

where $\pi(x)$ is the proportion of blood donors of age x and $S(x) = \exp - \int_0^x \lambda(u)\mathrm{d}u$ is the estimated proportion susceptible at age x.

The probability that a single donation is negative is $\exp(-\mu \times s \times V)$, where s is a factor allowing for the lower than average incidence in 1999 and 2000 when

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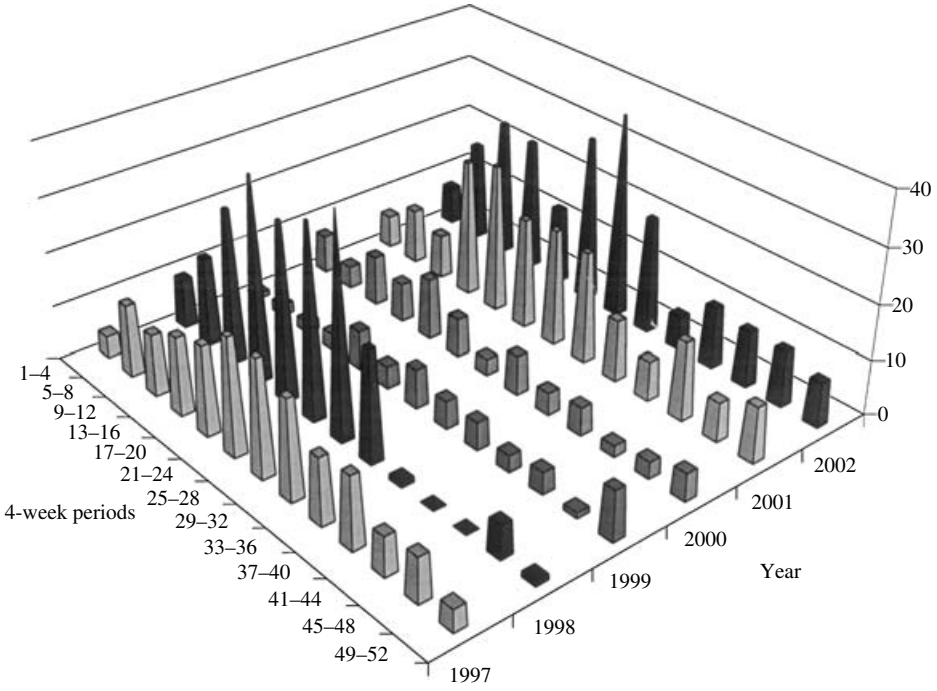

Fig. 1. Reported cases of parvovirus infection in The Netherlands.

the pools were formed, and V is the duration of detectable parvovirus viraemia. Assuming that the donations are independent and that dilution effects are ignorable, a pool is negative exactly when all constituent donations are negative. The probability that a pool of 5000 donations is negative is, therefore $(\exp(-\mu \times s \times V))^{5000} = \exp(-5000 \times \mu \times s \times V)$. Hence the probability that a pool is positive is

$$p = 1 - \exp(-5000 \times \mu \times s \times V).$$

This model was fitted as binomial with complementary log–log link [9]. The model parameters, including V, were estimated by maximum likelihood.

In a further analysis, we assumed that the viraemic period includes two phases: a first period V_{high} of high replication and a subsequent period V_{low} of low levels of parvovirus DNA. We classified positive pools into 'high' and 'low', and assumed that a pool is classified as 'high' exactly when one or more constituent donations is made during the high replication phase. We fitted this model using a trinomial likelihood with the following probabilities of 'high' and 'low' positive pools:

$$p_{\text{high}} = 1 - \exp(-5000 \times \mu \times s \times V_{\text{high}})$$

$$p_{\text{low}} = \{1 - \exp(-5000 \times \mu \times s \times V_{\text{low}})\} \times \exp(-5000 \times \mu \times s \times V_{\text{high}}).$$

Confidence limits were obtained by bootstrapping the serological survey, the annual incidence of parvovirus infection (used to estimate s), and the distribution of 'high' and 'low' positive pools (2500 independent samples of each) [10].

RESULTS

Seasonal and year-to-year variation

Figure 1 shows the number of cases of parvovirus infection per 4-week periods reported by virology laboratories in The Netherlands from 1997 to 2002. A considerable seasonal and year-to-year variation of the incidence of parvovirus infection is present. For example, in 1998, 41 cases were reported in weeks 25–28, but no cases were reported in weeks 37–44; in 1999 the total number of cases was four times lower than in 1998. In 1999 and 2000, no marked seasonal elevation of parvovirus infections occurred and the absolute number of reported cases was low. This finding is in line with the observation that in The Netherlands parvovirus infection shows a 4-year cycle, with peaks in 1990, 1994, 1998 and 2002 [11].

The plasma pools were formed in 1999 and 2000, during a low incidence period. In these two years, 127 parvovirus infections were reported, compared to 226 in 1998 and 186 in 2001. We assumed, therefore, that the incidence in 1999 and 2000 was a

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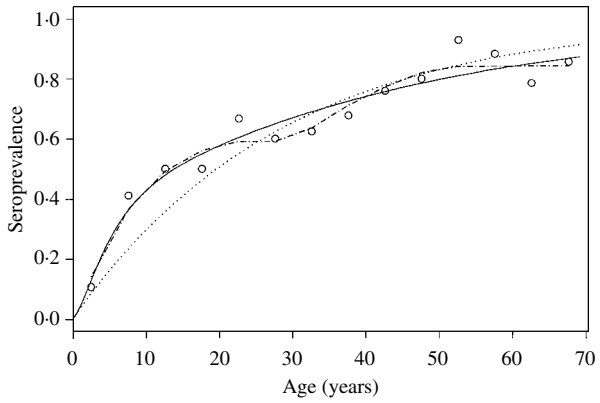

Fig. 2. Seroprevalence of parvovirus antibodies in The Netherlands. ○, Data; ·····, constant force of infection; –·–·–, piecewise constant model; ——, gamma model.

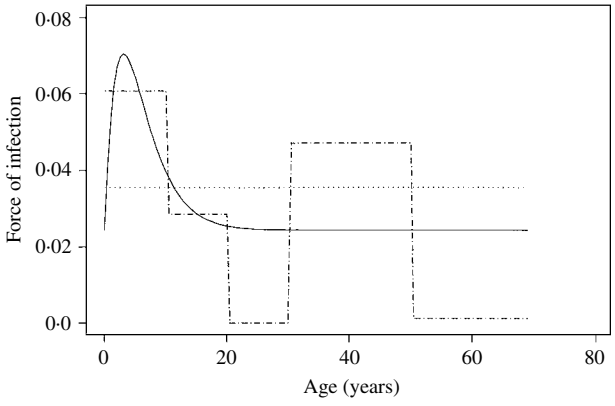

Fig. 3. Force of parvovirus infection in The Netherlands. ·····, constant force of infection model; –·–·–, piecewise constant model; ——, gamma model.

factor $s=(2\times127)/539=0{\cdot}4712$ less than the average value.

Force of infection and incidence of infection in blood donors

Figure 2 shows the proportions of persons testing positive for antibodies to parvovirus in 5-year age groups from ages <1–69 years, together with the fitted curves corresponding to a constant force of infection (deviance $D=8{\cdot}21$, 13 D.F.), a piecewise constant force of infection ($D=2{\cdot}60$, 9 D.F.) and a gamma force of infection ($D=3{\cdot}84$, 11 D.F.). The estimated forces of infection are shown in Figure 3. The gamma force of infection peaks at 3·0 years. Based on the deviance criterion, all three models provide a reasonable fit to the data. The gamma model provides perhaps the best compromise between goodness of fit and parsimony. The calculated force of infection is similar to that in previous estimates [12].

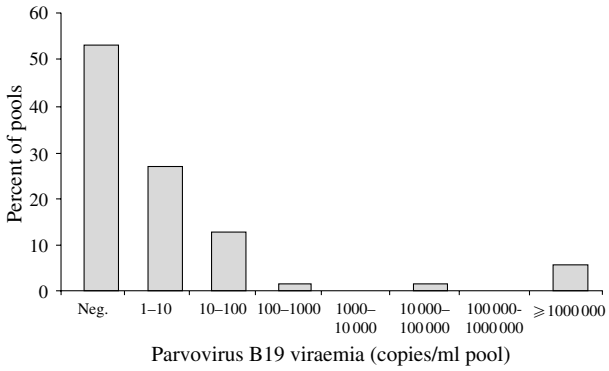

Fig. 4. Levels of parvovirus B19 viraemia in 100 plasma pools (5000 donations/pool).

A total 428 139 persons aged between 18 and 85 years donated blood during 2002–2003. Using the gamma model for the force of infection, the average annual incidence of infection in blood donors is estimated to be $\mu=0{\cdot}00563$ (95% CI 0·00188–0·00744).

Assuming homogeneous mixing (consistent with a constant force of infection), the basic reproduction number for parvovirus infection is estimated to be 3·0 (95% CI 2·6–3·5).

Duration of parvovirus B19 viraemia

Among the 100 plasma pools of 5000 donations, 47 tested positive for parvovirus DNA. We estimated the duration of detectable parvovirus B19 viraemia to be $V=17{\cdot}5$ days (95% CI 11·0–53·0).

Figure 4 shows the distribution of the amount of parvovirus DNA in the pools. This reveals the existence of two discrete groups of viraemic donations. Five of the 47 parvovirus-positive pools contained more than 10^6 copies of parvovirus DNA/ml (=more than 5×10^9 copies/ml donor plasma); 40 pools showed viral loads less than 100 copies/ml of pooled plasma (=below 500 000 copies/ml donor plasma). We classified pools with more than 10^6 copies of parvovirus DNA/ml as 'high', and the remainder as 'low'. We estimated the phase of high levels of parvovirus DNA to last $V_{\text{high}}=1{\cdot}4$ days (95% CI 0·3–4·9), and the phase of low levels to last $V_{\text{low}}=16{\cdot}1$ days (95% CI 10·2–48·9).

DISCUSSION

In our analysis, some assumptions were made of unknown certainty. For example, in our estimation of the yearly incidence of parvovirus infection among blood donors (Fig. 2), we assumed that the increase of

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seroprevalence with age is caused by new infections occurring in all age groups, not by an age-cohort effect. (An age-cohort effect shows increasing seroprevalence in older people because infections were more prevalent in the past.) Inevitably the confidence of our estimations is limited: our calculations were based on the seroprevalence as determined in 246 children and donors; on the number of reported cases of parvovirus infection in clinical patients; and on parvovirus levels as determined in 100 large plasma pools. The confidence intervals we obtain are wide, reflecting the uncertainty in these quantities, most particularly the uncertainty in the estimated incidence of parvovirus in blood donors.

These 100 plasma pools represent approximately half the total amount of plasma donated during one year in The Netherlands, and is thus likely to be a representative sample of the donor population.

Our seroprevalence data are consistent with studies in Australia and Great Britain, reporting anti-parvo seroprevalences increasing from 51% in people aged 10–19 years to 78% in people over 50 years respectively, from 50–60% in young adults to more then 85% in people over 70 years [13, 14]. We demonstrated a significant seasonal and year-to-year variation of the incidence of parvovirus infection, and found some evidence that the force of infection is highest in young children.

We estimate the average duration of peak-level viraemia exceeding 10^9 copies/ml donor plasma to last for only 1 or 2 days. Levels between 5000 and 500 000 copies/ml donor plasma are estimated to last for approximately 16 days, although the confidence limits extend from 10 days to 7 weeks. The subsequent duration of viraemia below 5000 copies/ml remains unknown. An early study reported undetectable parvovirus DNA in samples from six recalled donors, 60–100 days after the viraemic donation [15]. However, following 14 symptomatic and asymptomatic persons with parvovirus infection, Musiani et al. reported the presence of low levels of parvovirus DNA for 2 or 3 months in 7 out of 14 persons; during 4–6 months in 6 out of 14 persons; and persistence of parvovirus DNA in one patient suffering from chronic arthritis [16]. Studying 128 patients with erythema infectiosum, Erdman et al. found 98 and 67% of the patients parvovirus DNA-positive in the first and second month respectively after onset of symptoms [17].

Prolonged detection of parvovirus DNA in healthy persons is possibly caused by lingering, non-infectious parvovirus DNA after acute infection. However, if indeed complete parvovirus particles remain present for up to 6 months after infection, even small pools of donor plasma will often contain small amounts of parvovirus, and transmission of parvovirus via blood transfusion may be relatively common.

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