Vaccine 33 (2015) 5723–5727

Vaccine

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

Long-term antibody persistence after vaccination with a 2-dose *Havrix*™ (inactivated hepatitis A vaccine): 20 years of observed data, and long-term model-based predictions

Heidi Theeten[a,1,2], Koen Van Herck[a,b,2], Olivier Van Der Meeren[c], Priya Crasta[c], Pierre Van Damme[a,*], Niel Hens[a,d]

[a] *Vaccine and Infectious Disease Institute, University of Antwerp, Antwerp, Belgium*
[b] *Department of Public Health, Ghent University, Ghent, Belgium*
[c] *GlaxoSmithKline Wavre, Belgium and Mumbai, India*
[d] *Interuniversity Institute for Biostatistics and Statistical Bioinformatics, Hasselt University, Diepenbeek, Belgium*

ARTICLE INFO

Article history:
Received 10 March 2015
Received in revised form 11 June 2015
Accepted 6 July 2015
Available online 16 July 2015

Keywords:
Immunogenicity
Inactivated hepatitis A vaccine
Long-term follow-up
Mathematical modelling

ABSTRACT

Antibody persistence in two cohorts of adults, who received inactivated hepatitis A (HAV) vaccine (1440El.U; *Havrix*™; GSK Vaccines) according to a 0–6 or 0–12 month schedule in 1992–1993, has been measured annually. After 20 years, >97% of the subjects in both studies were seropositive for anti-HAV antibodies. Geometric mean concentrations in the according-to-protocol cohorts were 312 mIU/ml in 34/36 subjects vaccinated initially at 0–6 months (NCT00289757) and 317 mIU/ml in 85/86 subjects vaccinated at 0–12 months (NCT00291876). Over the whole follow-up period, seven subjects (2 + 5, respectively) lost circulating anti-HAV antibodies but mounted a strong response after HAV booster administration (1440El.U). Mathematical modelling, which was applied to assess true persistence at Year 20 (accounting for drop-outs and missing data), and to predict longer-term persistence confirmed previous estimates that seropositive anti-HAV levels would persist in ≥95% vaccinees at Year 30 and ≥90% at Year 40.

ClinicalTrials.Gov number: NCT00289757/NCT00291876

1. Introduction

Hepatitis A virus (HAV) is responsible for the most common form of acute viral hepatitis [1,2] manifested as approximately 1.4 million clinical cases worldwide each year [3]. Nevertheless, HAV infections can be prevented by immunization with inactivated hepatitis A vaccines, such as *Havrix*™ (strain HM175; GSK, Belgium) which was first launched in 1992 and is now widely available [4].

Two primary vaccination studies, in which adults received inactivated hepatitis A vaccine either at 0, 6 months or 0, 12 months, were started in 1992–1993; the subjects have been subsequently followed up and long-term antibody persistence measured [5–7]. In the most recent publication describing the Year 17 time-point, all subjects who were vaccinated at 0, 6 months and 96.7% subjects vaccinated according to the 0, 12 month schedule remained seropositive [7]. In support of the clinical findings, mathematical modelling has predicted that detectable anti-HAV antibodies will persist for 20–25 years [8–10]. In the most recent modelling analysis, based on the serology data collected after 17 years of follow-up, it was predicted that ≥95% and ≥90% of subjects would remain seropositive after 30 and 40 years, respectively [11].

This brief report presents the serological data collected at the Year 20 time point. As results were only available for returning subjects, and as subjects who became seronegative received a booster HAV dose and were excluded from analysis after Year 17, mathematical modelling was applied to take into account all subjects who received the full 2-dose vaccination course.

Abbreviations: ATP, according to protocol; CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; GMC, geometric mean concentration; HAV, hepatitis A virus.

* Corresponding author at: Faculty of Medicine and Health Sciences, Centre for the Evaluation of Vaccination, Vaccine & Infectious Disease Institute, UNIVERSITEIT ANTWERPEN, 2610 Antwerpen (Wilrijk), Belgium. Tel.: +32 3 265 25 38.
E-mail address: pierre.vandamme@uantwerpen.be (P. Van Damme).

[1] Current address: Post-doctoral Research Fellow of the Fund of Scientific Research (FWO), Belgium.

[2] Both authors equally contributed to this paper (shared first authorship).

http://dx.doi.org/10.1016/j.vaccine.2015.07.008

2. Materials and methods

Two double-blind, randomized primary vaccination studies started in 1992–1993, assessed the immunogenicity and safety of different lots of inactivated hepatitis A vaccine (1440El.U), administered either at 0, 6 months or 0, 12 months to healthy HAV-naïve adults [5]. Subjects who completed primary vaccination were invited to participate in the long-term follow-up phase (NCT00289757/NCT00291876) [6] whereby annual blood samples were collected up to Year 20. Anti-HAV antibodies were assessed using an in-house enzyme-linked immunosorbent assay (ELISA) assay (seropositivity cut-off: ≥20 mIU/ml) during the first 11 years and by a commercially available enzyme-linked immunoassay (Enzygost, Siemens Healthcare, Germany; seropositivity cut-off: ≥15 mIU/ml) from Year 11 onwards [6]. Full details of the immunogenicity and safety assessments are described elsewhere [5–7]. Subjects becoming seronegative (anti-HAV antibody concentration <15 mIU/ml) were eligible to receive a booster vaccine dose within one year of blood sampling and were subsequently excluded from further follow-up.

Both studies were approved by the Ethics Review Committee of the Antwerp University Hospital and were conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. Returning subjects provided written informed consent at each follow-up time-point before any interventions were performed.

2.1. Statistical analyses

The immunogenicity analyses were performed on the long-term according-to-protocol (LT-ATP) cohort and the long-term total cohort (LT-Total). All subjects belonging to the total cohort in the primary study and returning at the annual time points at Years 16–20 constituted the LT-Total cohort for that year. The LT-ATP cohort included all subjects from the ATP cohort in the primary study who had not received any additional HAV vaccine or recorded an abnormal increase in anti-HAV antibody concentrations since the previous time point [5–7]. An abnormal increase of anti-HAV antibody levels was defined as either a 4-fold or 2-fold increase if the previous anti-HAV level for that subject was <100 mIU/ml or ≥100 mIU/ml, respectively [7]. At each time point, seropositivity rates and geometric mean concentrations (GMCs) on seropositive subjects were calculated for anti-HAV antibodies with 95% confidence intervals (CI).

As lot-to-lot consistency was demonstrated in both primary studies, the immunogenicity results for the pooled groups have been used throughout.

The statistical analyses were performed using the Statistical Analysis Systems (SAS) version 9.2 (SAS Institute Inc., Cary, NC).

2.2. Mathematical modelling for long-term estimates

The populations included in the mathematical modelling were selected from the original LT-cohort at study start (modelling LT-cohort), as previously described by Hens et al. [11]. Exclusions from the modelling LT cohort at start of follow-up were mainly to avoid over-estimation of vaccine-induced immunity by suspected pre-existing immunity; furthermore some participants were excluded for not having received all vaccine doses in the primary study (see Fig. 1).

A linear mixed model that included an indicator variable for the Year 11 assay change was used to predict seropositivity rates (cut-off: ≥15 mIU/ml) at 30, 40 and 50 years after vaccination. The model was fitted using all available data for both studies. The Akaike Information Criterion (AIC) was used for model selection and goodness of fit was assessed using the standard diagnostic tools for linear mixed models.

For data analysis, a complete re-assessment of the optimal set of change-points was done, rather than re-applying those used in the previously fitted models [11]. Both one- and two-change-points models were evaluated. For both studies, the best 10 two change-point models yielded lower AIC-values than the best model with

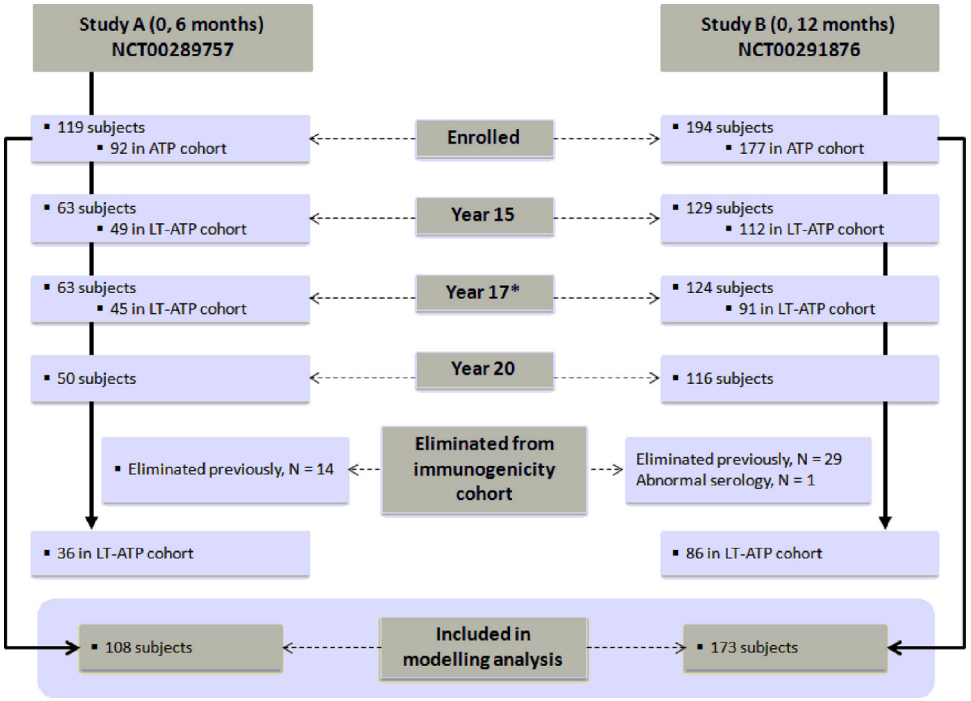

Fig. 1. Subject disposition in LT and LT-ATP cohorts. *Seven subjects (two from the 0, 6 month and five from the 0, 12 month schedules) who became seronegative received booster dose and were excluded from follow-up after Year 17. See references [6,7] for presentation of Year 15 and 17 follow-up results, respectively.

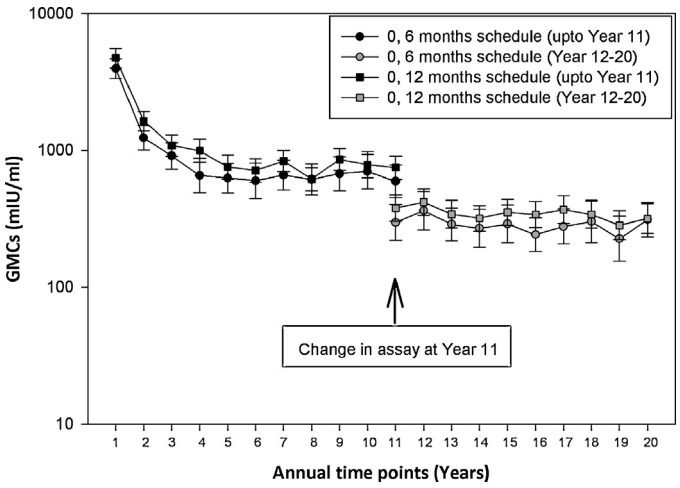

Fig. 2. Evolution of anti-HAV antibody GMCs over 20 years (Year 20 LT-ATP Cohort for Immunogenicity). The laboratory assay was changed at Year 11 from an in-house enzyme-linked immunoassay to a commercially-available one. Re-testing of the Year 11 samples with the new assay indicated that anti-HAV GMCs were slightly lower when tested using the new assay, but the anti-HAV antibody levels remained stable thereafter [6]. Anti-HAV seropositivity cut-offs: up to Year 11: ≥20 mIU/ml; Year 12 to Year 20: ≥15 mIU/ml

a single change-point. Because the model with change points at 6 and 24 months after the second dose was ranked second and first for the 0, 12 month schedule and 0, 6 month schedule, respectively, those change points were selected as the final set.

Data were processed using Microsoft Excel and SAS procedures MIXED and NLMIXED, symmetrized (antibody levels were log 10-transformed) and the mean trend of the observed antibody levels was represented as described by Hens et al. [11]. The coefficient of simple determination (R^2) and D index of agreement were also calculated [11].

3. Results

3.1. Immunogenicity in subjects returning at Year 20

At Year 20, 50 subjects who had received HAV vaccination at 0, 6 months and 116 subjects who received the vaccine at 0, 12 months returned for follow-up, of whom 36 and 86 subjects, respectively, constituted the LT-ATP cohorts (Fig. 1). The mean age was 47.6 years (range: 37–59 years) and 50.5 years (range: 42–60 years) in the LT-ATP cohorts, respectively; 77.8% and 74.4% of subjects were female, respectively, and all subjects were Caucasian. The study cohorts at each time point were comparable to the original cohorts in terms of demographic characteristics and post-dose 2 anti-HAV antibody concentrations.

After 20 years, all subjects in the LT-ATP cohorts in both studies were seropositive for anti-HAV antibodies. GMCs in the LT-ATP cohorts at Year 20 were 312 mIU/ml (95%CI: 233.2–416.8) and 317 mIU/ml (95%CI: 247.4–407.1) in subjects vaccinated initially at 0, 6 months and 0, 12 months, respectively. Following primary vaccination, a steep decline in GMC was observed in both studies up to Month 24, after which it appeared to stabilize (Fig. 2).

During 20 years of follow-up, seven subjects (two from the 0, 6 months and five from the 0, 12 months schedules) lost circulating anti-HAV antibodies. All, except one, mounted a strong anamnestic response following HAV booster administration (1440El.U). The pre-booster antibody concentration in the subject who became seronegative in the current time period was 16 mIU/ml, which rose to 4894 mIU/ml on Day 30 post-booster. This subject reported grade 1 pain on the day of vaccination and the following day.

3.2. Modelling results (modelling LT-cohort)

A total of 281 subjects were included in the modelling analysis (0, 6 month schedule: 173 and 0, 12 month schedule: 108 [11]); of whom 22 and 17 participants, respectively had data excluded from the analysis from a specific visit onwards due to: booster dose received within/outside the study (7 and 4, respectively); abnormal rise in consecutive measurements after dose 2 (12 and 12, respectively); obvious outlier in observations (3 and 1, respectively).

Using ≥15 mIU/ml as the seropositivity cut-off value, these model predictions corroborate the existing claim [11] that ≥95% subjects will remain seropositive at Year 25 in both studies. The seropositivity rates are estimated to remain consistently ≥90% up to Year 40 after which they are expected to decline but remain ≥85% for both studies with 88.9% (78.7–95.3%) at Year 45 and 86.1% (75.9–93.5%) at Year 50 for the 0, 6 month schedule and 93.1% (83.8–95.9%) and 89.6% (80.3–92.5%), respectively for the 0, 12 month schedule (Fig. 3).

4. Discussion

The current studies are unique in terms of assessing the long-term immune response to inactivated HAV vaccine for 20 years. Indeed, the vaccine induced high levels of immunogenicity in both studies and detectable anti-HAV antibodies persisted up to 20 years in more than 97% of subjects. Following primary vaccination, a steep decline in GMC was observed in both studies up to Month 24, after which the GMC appeared to stabilize. Although our data suggest that a 0–12 month schedule would induce higher antibody levels resulting in higher predicted long-term antibody levels than a 0–6 month schedule, it should be noted that the study populations were recruited independently. This observation may therefore also relate to differences in known confounders such as age, gender or BMI.

Our first modelled estimation of long-term persistence, based on extrapolating the linear trend in 5 years of observed data, was that circulating antibodies would last on average 20–25 years after the second vaccine dose [10]. Using linear mixed models on 6–10 years of observed data, we concluded that circulating antibodies would persist in ≥95% of the vaccinees at year 25 [4]. Our current results, observed 20 years after vaccination, confirm that the vast majority of subjects still present circulating antibodies at that time-point, and suggest that the long-term decline in GMC seems to have levelled off. However, whether a true plateau in GMCs has been achieved remains to be confirmed. Indeed, Wiedermann et al. in their 7-year follow-up study of subjects vaccinated at 0, 1–2 and 12 months, reported the initial steep decline followed by a second slower reduction in anti-HAV antibodies, whereby seroprotective persistence was estimated to vary between 24 and 47 years [12]. Nevertheless, our results are consistent with other studies of long-term anti-HAV persistence in adults [13–15] and children [14,16].

Seven seronegative subjects who received additional vaccine as part of the study [6,7] were excluded from follow-up after Year 17, which could have potentially biased the persistence rates upwards. To overcome these limitations, a mathematical model was applied to assess true persistence at Year 20, whereby the statistical analysis took into account all available test results since the study start from all subjects who received the full vaccination course irrespective of their anti-HAV antibody serostatus, up until they were eliminated for any reason. The modelling results corroborate the previous estimates made up to Year 40 [11] and suggest that persistence extends beyond that time-point.

As well as being unique amongst clinical trials for such prolonged follow-up, these studies have allowed us to compare actual long-term clinical results with early model predictions. Inactivated HAV vaccine confers a high level of seroprotection against HAV for at least 20 years and current modelling predicts that seropositive

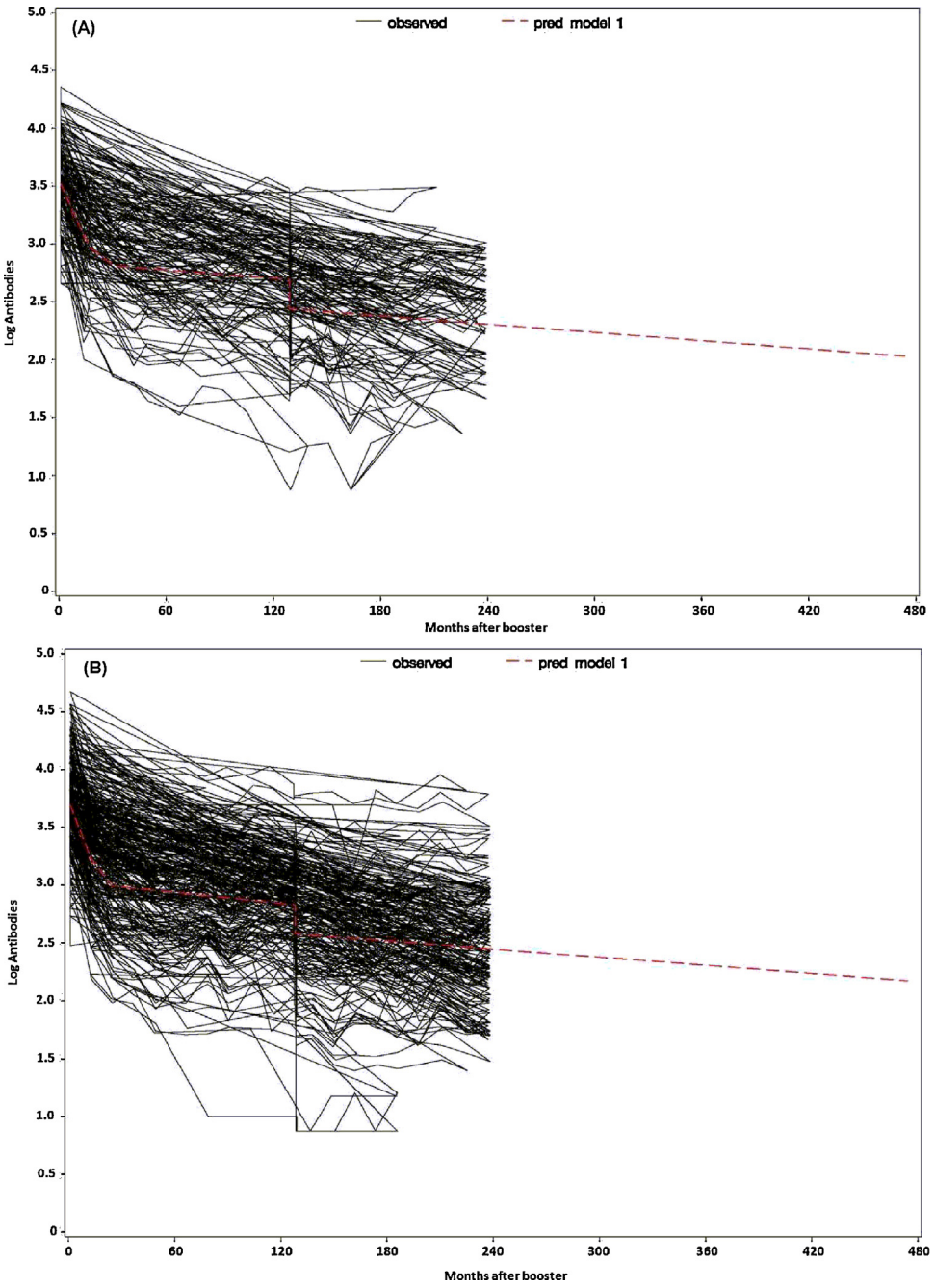

Fig. 3. Observed individual profiles and population-averaged estimation of anti-HAV levels using all available test results and a model with linear time trend (modelling LT cohort; 0, 6 months: 108 subjects, 0, 12 months: 173 subjects). The selected set of 2 change points at 6 and 24 months after the last vaccine dose for 0, 6 month schedule (panel A) and 0, 12 month schedule (panel B). Black lines show the observed individual antibody levels and the red line shows population-averaged estimation of anti-HAV levels.

anti-HAV levels will persist in ≥95% vaccinees at Year 30, and ≥90% at Year 40.

Havrix is a trademark of the GSK group of companies.

Contributors

PVD was the principal investigator; KVH and HT were co-investigators. PVD, OVDM, KVH, HT, PC and NH contributed to the conception, design, analysis and interpretation of the study. NH, KVH and PC provided statistical expertise. All authors participated in the development of this manuscript.

Conflict of interest

OVDM and PC are employees of GlaxoSmithKline Vaccines and OVDM declares having GlaxoSmithKline stocks. PVD conducts clinical trials for which the University obtains research grants from Glaxo SmithKline and other companies. KVH conducts clinical trials and undertakes modelling for which the University obtains research grants from Glaxo SmithKline and other companies and has received honoraria from GlaxoSmithKline and other companies for consultancy, lecturing and advisory board membership. HT conducts clinical trials for which the University obtains research grants from Glaxo SmithKline. NH has received research grants

from GlaxoSmithKline for undertaking analyses on behalf of his institution.

Funding source

GlaxoSmithKline Biologicals SA was the funding source for the analysis. GlaxoSmithKline Biologicals SA also funded all costs associated with the development and publication of the manuscript. All authors had full access to the data and had final responsibility to submit for publication.

Acknowledgements

The authors would like to thank all subjects who participated in this study. The authors would also like to acknowledge Brigitte Cheuvart (employed by GlaxoSmithKline group of companies) for statistical input, Ramandeep Singh (employed by GlaxoSmithKline group of companies) for medical writing assistance and Julia Donnelly (freelance on behalf of GlaxoSmithKline Vaccines) for publication coordination.

Appendix. Model description and parameters

We used a linear mixed model with two change points (6 and 24 months) after the second dose, without additional covariates. The model was applied on all available data from Year 1 to Year 20. The following model structure and parameters were used:

$$\log_{10}(\text{titre}_{ij}) = (\beta_0 + \beta_1 I_{\text{newtest},j} + b_{0i}) + (\beta_2 + b_{2i})\text{time}_{ij} + (\beta_3 + b_{3i})(\text{time}_{ij} - cp_1)_+ + (\beta_4 + \beta_5 I_{\text{newtest},j} + b_{4i})(\text{time}_{ij} - cp_2)_+ + \in_{ij},$$

with $\log_{10}(\text{titre}_{ij})$, the basis 10 log antibody level of subject i at time j; time in months after the second dose; cp_i, $i = 1, 2$ the two change-points, $(\ldots)_+$ the heavisde function taking value 0 if the argument is negative and value 1 if the argument is positive, and I_{newtest} the indicator function rendering value 1 if the new test is used and 0 otherwise. Whereas the β parameters represent fixed effects, the b parameters represent random effects and $\in_{ij} \sim N(0, \sigma^2)$ represent the independent and normally distributed error terms.

References

[1] Franco E, Meleleo C, Serino L, Sorbara D, Zaratti L. Hepatitis A: epidemiology and prevention in developing countries. World J Hepatol 2012;4:68–73.

[2] Martinez A, Broner S, Sala MR, Manzanares-Laya S, Godoy P, Planas C, et al. Changes in the epidemiology of hepatitis A outbreaks 13 years after the introduction of a mass vaccination program. Hum Vaccin Immunother 2015;11:192–7.

[3] World Health Organization. Viral hepatitis – report by the Secretariat; 2010. Available at http://apps.who.int/gb/ebwha/pdf_files/WHA63/A63_15-en.pdf (accessed on 16.01.15).

[4] Van Herck K, Van Damme P. Prevention of hepatitis A by Havrix: a review. Expert Rev Vac 2005;4:459–71.

[5] Van Damme P, Mathei C, Thoelen S, Meheus A, Safary A, Andre FE. Single dose inactivated hepatitis A vaccine: rationale and clinical assessment of the safety and immunogenicity. J Med Virol 1994;44:435–41.

[6] Van Herck K, Jacquet JM, Van Damme P. Antibody persistence and immune memory in healthy adults following vaccination with a two-dose inactivated hepatitis A vaccine: long-term follow-up at 15 years. J Med Virol 2011;83:1885–91.

[7] Van Herck K, Crasta PD, Messier M, Hardt K, Van Damme P. Seventeen-year antibody persistence in adults primed with two doses of an inactivated hepatitis A vaccine. Hum Vaccin Immunother 2012;8:323–7.

[8] Van Damme P, Thoelen S, Cramm M, De Groote K, Safary A, Meheus A. Inactivated hepatitis A vaccine: reactogenicity, immunogenicity, and long-term antibody persistence. J Med Virol 1994;44:446–51.

[9] Van Herck K, Beutels P, Van Damme P, Beutels M, Van den Dries J, Briantais P, et al. Mathematical models for assessment of long-term persistence of antibodies after vaccination with two inactivated hepatitis A vaccines. J Med Virol 2000;60:1–7.

[10] Van Herck K, Van Damme P. Inactivated hepatitis A vaccine-induced antibodies: follow-up and estimates of long-term persistence. J Med Virol 2001;63:1–7.

[11] Hens N, Ghebretinsae AH, Hardt K, Van Damme P, Van Herck K. Model based estimates of long-term persistence of inactivated hepatitis A vaccine-induced antibodies in adults. Vaccine 2014;32:1507–13.

[12] Wiedermann G, Kundi M, Ambrosch F, Safary A, D'Hondt E, Delem A. Inactivated hepatitis A vaccine: long-term antibody persistence. Vaccine 1997;15:612–5.

[13] Rendi-Wagner P, Korinek M, Winkler B, Kundi M, Kollaritsch H, Wiedermann U. Persistence of seroprotection 10 years after primary hepatitis A vaccination in an unselected study population. Vaccine 2007;25:927–31.

[14] Hammitt LL, Bulkow L, Hennessy TW, Zanis C, Snowball M, Williams JL, et al. Persistence of antibody to hepatitis A virus 10 years after vaccination among children and adults. J Infect Dis 2008;198:1776–82.

[15] Ott JJ, Irving G, Wiersma ST. Long-term protective effects of hepatitis A vaccines. A systematic review. Vaccine 2012;31:3–11.

[16] Lopez EL, Contrini MM, Mistchenko A, Debbaq R. Long-term immunity after two doses of inactivated hepatitis A vaccine, in Argentinean children. Pediatr Infect Dis J 2010;29:568–70.