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Long-term antibody persistence in children after vaccination with the pediatric formulation of an aluminum-free virosomal hepatitis A vaccine

Reference:

van Herck Koen, Hens Annick, De Coster Ilse, Vertruyen André, Tolboom Jeroen, Sarnecki Michal, van Damme Pierre.- Long-term antibody persistence in children after vaccination with the pediatric formulation of an aluminum-free virosomal hepatitis A vaccine

The pediatric infectious disease journal - ISSN 0891-3668 - 34:4(2015), p. 85-91

DOI: <http://dx.doi.org/doi:10.1097/INF.0000000000000616>

Handle: <http://hdl.handle.net/10067/1244600151162165141>

The Pediatric Infectious Disease Journal

LONG-TERM ANTIBODY PERSISTENCE IN CHILDREN AFTER VACCINATION WITH THE PEDIATRIC FORMULATION OF AN ALUMINUM-FREE VIROSOMAL HEPATITIS A VACCINE

--Manuscript Draft--

Manuscript Number:	PIDJ-214-484R1
Article Type:	Vaccine Reports
Keywords:	
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Manuscript Region of Origin:	BELGIUM
Abstract:	<p>Background: The pediatric dose of the virosomal hepatitis A vaccine Epaxal®, Epaxal® Junior, has been shown to be safe and immunogenic in children aged 1 to 17 years. The present study investigated the long-term immunogenicity of Epaxal® Junior. The standard dose of Epaxal® as well as an aluminum-adsorbed hepatitis A vaccine (Havrix® Junior) were used as comparators.</p> <p>Methods: A total of 271 children who had completed a 0/6 month immunization schedule (priming and booster dose) participated in this follow-up study. Anti-hepatitis A virus (HAV) antibody levels were measured using a microparticle enzyme immunoassay (HAVAB 2.0 Quantitative, Abbott Diagnostics) starting at 18 months following the second dose, and then yearly until 66 months (i.e. 5.5 years) after the second dose.</p> <p>Results: All subjects tested at Month 66 still had protective levels of anti-HAV antibodies (≥ 10 mIU/mL). Antibody levels were generally lower in subjects 1 to 7 years old than in subjects 8 to 17 years old and higher in females 11 to 17 years old than in males 11 to 17 years old. In addition, an age-dependent decay was observed, i.e. antibody levels decreased more rapidly in younger than in older children.</p> <p>Conclusion: Vaccination of children with two doses of Epaxal® Junior confers a real-time protection of at least 5.5 years. This protection is estimated to last approximately 25 years. Younger children showed lower antibody levels and a faster antibody decline than older children. Additional follow-up studies are needed beyond 5.5 years to further assess the long-term immunogenicity of Epaxal® Junior.</p>

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09th September 2014

John D. Nelson, MD
George H. McCracken Jr., MD
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Dear Drs Nelson and McCracken,

I would like to resubmit the manuscript entitled **Long-Term Antibody Persistence in Children after Vaccination with the Pediatric Formulation of an Aluminum-Free Virosomal Hepatitis A Vaccine** for publication in The Pediatric Infectious Disease Journal.

We would like to thank the reviewers for the detailed review. We have responded to all comments in our point-by-point response letter. Accordingly, we have made changes to the manuscript, renumbered the figures and amended table 1. We hope that the manuscript is now to your satisfaction.

Thank you for consideration of this manuscript.

With kind regards,

A handwritten signature in black ink, appearing to read 'Sarnecki', with a stylized, cursive script.

Dr Michal Sarnecki

MS No. PIDJ-214-484

Title: LONG-TERM ANTIBODY PERSISTENCE IN CHILDREN AFTER VACCINATION WITH THE PEDIATRIC FORMULATION OF AN ALUMINUM-FREE VIROSOMAL HEPATITIS A VACCINE

Responses to comments received on July 22, 2014

Reviewer #1:

This manuscript is the first study to Epaxal junior vaccine and 5.5 years follow-up.

Comment:

1. Figure 1 A, B, C is not useful, I recommend to delete it.

Response:

Thank you for this recommendation. We have considered again the information in Figure 1 and agree to remove it from the main section of the manuscript.

However, the authors consider Figure 1 useful and, for the following reasons, we wish to retain it as supporting data:

Figure 1 presents individual line plots of log-transformed anti-HAV concentrations for each of 3 study groups. This is informative as the data are not presented or described otherwise (anti-HAV GMCs in the tables cover only follow-up period – from 18 month onwards). Individual differences in antibody kinetics are visualized, in particular during the period of primary study (0-7 months), including outliers. This figure supports the information regarding sensitivity analyses presented in the text (e.g. analyses in subjects who had a decline and in those who had an increase in anti-HAV GMCs between Visit 3 and Visit 4). This figure also shows both the large variability between subjects, the very steady decline within subjects and thereby the low measurement variability of the anti-HAV antibody assay.

Comment:

2. Page 6 line 15-30 Had a statistical difference between Epaxal GM concentrations versus Epaxal junior and/or Havrix junior?

Response:

Thank you for this question, although please note that our paper focuses on long-term antibody persistence and not on the comparisons between treatments. However the authors agree on the addition of a short comment in this section.

At all time points in Table 1, the GMC for Epaxal® was statistically significantly higher than both Epaxal® Junior and Havrix® Junior (all $p \leq 0.014$), except after 18 months where the difference between Epaxal® and Havrix® Junior was found to be just outside statistical significance ($p = 0.052$). There were no statistically significant differences between Epaxal® Junior and Havrix® Junior at any time point (all $p \geq 0.505$).

Comment:

3. Page 6 lines 39- 40: Is any explanation available for the trend for higher anti HAV antibody titer with increasing age?

Response:

Thank you for this enquiry. We have added a brief comment into the results section to bridge the reader to the discussion section.

This is a very first observation of the age effect that we have made for Epaxal and Epaxal Junior. We believe the trend for higher anti HAV antibody titers with increasing age is rather a reverse effect of

a faster decay and shorter periods of antibody persistence in younger subjects, whereas the relatively higher titers in older children are similar to the experience from adults. The reason for the age differences is not entirely clear, and we already present possible explanations in the discussion (e.g. shorter survival of antibody-secreting plasma cells in younger subjects).

Comment:

4. Line 51 in page 6: F test? typed mistake

Response:

No change is proposed in the manuscript.

The statistical test for treatment, gender, year since vaccination and age was indeed an F-test as is commonly used in ANOVA-like models. The test for the effect of gender, year since vaccination and age could alternatively also have been done by the t-test but not for the overall difference between the 3 treatments. We hope this explanation clarifies the situation.

Reviewer #2:

The study results presented here provide important follow-up data on the durability of the immune response for children/youth, ages 1 to 17 years, who had received a 0/6 month intramuscular immunization schedule of the Study EPA001B immunization study, including the Epaxal Junior, Epaxal and Havrix Junior Hepatitis A vaccine. A GMC of ≥ 10 mIU/ml and ≥ 20 mIU/ml were both appropriately used as correlates of protection for the analysis.

Comment:

1. The analysis is sound and the reported follow-up data, now until 5.5 years are relevant for clinical practice as well as modelling efforts for estimation of the overall duration of protection after two doses of the two dosage forms of the Epaxal vaccine and the Havrix vaccine.

Response:

No response required

Comment:

2. The manuscript can be further strengthened by discussion of the evidence from adult studies on different immunization routes (intradermal and subcutaneous) of virosomal hepatitis A vaccine, with particularly the subcutaneous application being of potential public health relevance, due to the significantly reduced dosing volume.

Response:

Thank you for this suggestion, and we agree in principle that this would be an important addition. However there is very limited experience with different administration routes. Therefore, due to limited scope of the data available, it would be difficult to include any meaningful extension to the manuscript.

There is very limited experience with Epaxal administered with alternative immunization routes with only one pediatric study with intradermal administration and one study including both subcutaneous and intradermal administration in adults. The number of subjects participating in these studies was limited and, more importantly, there was no long term follow up, which would be of relevance to the discussed manuscript.

Comment:

3. The observation of higher antibody GMC in female versus male participants, as also pointed out by the authors has also been observed for other vaccines.

Response:

No response required

Comment:

3 (continued)

The data presented for the age dependence of the GMC decrease should also be presented stratified by the individual vaccine administered. Currently Figure 3 shows the regression lines for the scatter plot of all data, stratified by age ranges.

Response:

Thank you for this request, however the authors feel that the age-dependent decrease of antibodies over time, although described in the manuscript, is not large enough that it can be easily seen in Figure 3 when plotted for each vaccine separately.

We attach a file to this response [file: titercorr3.rtf] for clarification. Per vaccine the regression lines are drawn both with and without 95% confidence bands. The large overlap of the confidence bands shows that the differences between regression lines are for a large part just statistical variability. Only in the figure for the 3 vaccines together the differences between regression lines suggest that the age dependency might be real because the overlap of confidence bands now based on the total number of subjects is much less. For this reason we only presented the figure for all vaccines together. The formal test of the age dependency is done later in the paper as part of the statistical modeling.

Comment:

3 (continued)

Since Influenza virosomes are being used in the Epaxal vaccine, it would be of interest to assess if the immune response to repeated annual seasonal influenza vaccines can be potentially correlated with the GMC decrease.

Response:

Thank you for this question. We are unable to comment on the potential influence of the influenza vaccinations on the long term persistence of anti-HAV antibodies after vaccination with Epaxal or Epaxal Junior.

Unfortunately within the scope of the described clinical study and other studies which follow up Epaxal-vaccinated subjects, we have not collected information regarding seasonal influenza vaccinations administered to the study subjects.

Comment:

3 (continued)

Also the initial GMC after the second dose, should be discussed for the three vaccines.

Response:

Thank you for this request. The authors agree on addition of the GMCs at 1 month after the second dose of Epaxal to Table 3.

The initial GMCs at 1 month after second dose were presented in the publication from the primary study (Van Der Wielen, 2007). It should be noted that these data are part of a different population and different laboratory and statistical analysis, and so should be interpreted only with caution alongside the follow up data in the rest of the table.

Additional comment from the authors:

In the process of responding to the journal comments and updating the manuscript, an error was noticed in the version that we had submitted. The error, highlighted in grey below, was typographical, and occurred in the “Data Analysis” section:

at the five follow-up visits. A real-time assessment of seroprotection 18 to 66 months after the second dose and a computer model analysis to estimate long-term seroprotection in all subjects and – for GMCs – in subjects who completed all five follow-up visits were performed. The predicted duration of endpoint was computer-based modelling analysis of long-term protection. Seroprotection (i.e. the persistence of antibodies) was analyzed using the seroprotective threshold of 10 mIU/mL, as well as the more stringent threshold of 20 mIU/mL, as both the 10 and 20 mIU/mL antibody concentrations can be considered as correlates of seroprotection after

This have been corrected to read:

second dose and a computer model analysis to estimate long-term seroprotection in all subjects and – for GMCs – in subjects who completed all five follow-up visits were performed. The predicted duration of seroprotection (i.e. the persistence of antibodies) was analyzed using the seroprotective threshold of 10 mIU/mL, as well as the more stringent threshold of 20 mIU/mL, as both the 10 and 20 mIU/mL antibody concentrations can be considered as correlates of seroprotection after immunization against HAV.¹⁸ Descriptive statistics were used to present the

Treatment group=Group A Epaxal 0.25ml

Treatment group=Group B Epaxal 0.5ml

Treatment group=Group C Havrix Junior 0.5ml

Treatment group=Group A Epaxal 0.25ml

Treatment group=Group B Epaxal 0.5ml

Treatment group=Group C Havrix Junior 0.5ml

LONG-TERM ANTIBODY PERSISTENCE IN CHILDREN AFTER VACCINATION WITH
THE PEDIATRIC FORMULATION OF AN ALUMINUM-FREE VIROSOMAL HEPATITIS
A VACCINE

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Key words: hepatitis A; virosomes; pediatric; long-term protection

Abbreviated title: Anti-hepatitis A antibody kinetics in children

Running head: Virosomal hepatitis A vaccine

Conflicts of interest and source of funding: This study was funded by Crucell Switzerland AG,
Bern, Switzerland (Study EPA001FU). Jeroen Tolboom is an employee of Crucell Holland BV
and Michal Sarnecki of Crucell Switzerland AG. Michal Sarnecki was a previous employee of
Biotie Therapies Corp and held stock or had stock options in this company. Annick Hens declares
a past financial relationship with GSK and Gentcel. Pierre Van Damme declares that he was
principal investigator of the present study and received monies from Janssen and Crucell for this

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4 work. He also declares a past relationship with GSK Vaccines, Merck, Novartis, Gentice, and
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7 SP-MSD. Ilse De Coster declares a past financial relationship with Gentice (for a congress).
8
9
10 Koen Van Herck declares a grant to his institution from Crucell Switzerland AG, a consultancy
11
12 with a grant to his institution from Pfizer, grants/pending grants from GSK and fees for lectures
13
14 from several vaccine manufacturers. Andre Vertruyen declares no conflicts of interest.
15
16

17 **Acknowledgments:** We would like to thank Dr Sonja Basta (funding provided by Crucell
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19
20 Switzerland AG) for writing the manuscript, and also all participants in the study.
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ABSTRACT

Background: The pediatric dose of the virosomal hepatitis A vaccine Epaxal[®], Epaxal[®] Junior, has been shown to be safe and immunogenic in children aged 1 to 17 years. The present study investigated the long-term immunogenicity of Epaxal[®] Junior. The standard dose of Epaxal[®] as well as an aluminum-adsorbed hepatitis A vaccine (Havrix[®] Junior) were used as comparators.

Methods: A total of 271 children who had completed a 0/6-month immunization schedule (priming and booster dose) participated in this follow-up study. Anti-hepatitis A virus (HAV) antibody levels were measured using a microparticle enzyme immunoassay (HAVAB 2.0 Quantitative, Abbott Diagnostics) starting at 18 months following the second dose, and then yearly until 66 months (i.e. 5.5 years) after the second dose.

Results: All subjects tested at Month 66 still had protective levels of anti-HAV antibodies (≥ 10 mIU/mL). Antibody levels were generally lower in subjects 1 to 7 years old than in subjects 8 to 17 years old and higher in females 11 to 17 years old than in males 11 to 17 years old. In addition, an age-dependent decay was observed, i.e. antibody levels decreased more rapidly in younger than in older children.

Conclusion: Vaccination of children with two doses of Epaxal[®] Junior confers a real-time protection of at least 5.5 years. This protection is estimated to last approximately 25 years. Younger children showed lower antibody levels and a faster antibody decline than older children. Additional follow-up studies are needed beyond 5.5 years to further assess the long-term immunogenicity of Epaxal[®] Junior.

INTRODUCTION

Infection with the hepatitis A virus (HAV) causes an acute, self-limiting inflammation of the liver. The severity of the illness depends on the age of the patient: while young children are usually asymptomatic, older children and adults generally develop symptomatic disease.^{1, 2}

Several HAV vaccines have been developed that are highly immunogenic and provide rapid and long-lasting protection from infection.¹ One of the vaccines available is Epaxal[®] which is aluminum-free and contains formalin-inactivated HAV associated with virosomes as adjuvant.^{3, 4}

The vaccine produces an excellent antibody response after the first dose and is well tolerated.³⁻⁸

A second (booster) dose is usually given between 6 and 12 months. After the booster response anti-HAV antibody concentrations decline over time.⁹

Real-time follow-up of subjects vaccinated with the standard dose of Epaxal[®] (≥ 24 IU/0.5 mL) or aluminum-adsorbed HAV vaccines has shown that anti-HAV protection persists for up to 14 years.¹⁰ In a published consensus statement, a group of experts concluded that – based on observed evidence and model calculations – the 0/6 to 18 month immunization schedule currently used in adults will provide a long-lasting protection against HAV infection and most likely no further booster is needed in immunocompetent persons.¹¹

Long-term follow-up of adult subjects vaccinated with two doses of an aluminum-adsorbed vaccine (schedule 0/6 month and 0/12 month) showed persistence of vaccine-induced antibodies against HAV for at least 17 years. Mathematical models based on these 17 year data sets estimated that seropositive levels of anti-HAV antibodies persist in more than 95% of adult subjects for at least 25 years.^{12, 13} A first analysis based on mathematical modelling of data

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4 obtained 5 years after the second dose has shown that the standard dose of Epaxal[®] confers at
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6 least 20 years of long-term immunogenicity in adult subjects whereby the median duration of
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8 antibody persistence was estimated to be at least 46.8 years (95% confidence interval [CI]: 41.2-
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10 56.6).⁹ This projected duration of antibody persistence was again tested with real-time data from
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12 a follow-up of the same study population 9 to 11 years after the second dose: seroprotection
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14 persisted in all subjects and the data provided now an estimated duration of antibody persistence
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16 of at least 30 years (cut-off level ≥ 10 mIU/mL). The median predicted duration of antibody
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18 persistence was 52.1 years (95% CI: 50.8-54.2). The more stringent cut-off for seroprotection of
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20 ≥ 20 mIU/mL resulted in a median predicted duration of antibody persistence of 45.0 years (95%
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22 CI: 43.7-46.3).¹⁴ Results from a 15-year follow-up of this cohort have shown that all subjects
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24 maintained protective levels of anti-HAV antibodies (unpublished data).
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32 A lower-dosed pediatric presentation of Epaxal[®], Epaxal[®] Junior (≥ 12 IU/0.25 mL), has been
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34 tested in three clinical trials in children up to the age of 17 years and was found safe and
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36 immunogenic enough to elicit a protective immune response.¹⁵⁻¹⁷ However, long-term studies are
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38 lacking to determine the duration of this protection.
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42 The present report describes the results of a 5.5-year follow-up of Study EPA001B, which
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44 investigated the immunogenicity of Epaxal[®] Junior compared with Epaxal[®] and Havrix[®] Junior
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46 (720 EU) in children aged 1 to 17 years.¹⁷ The aim of the follow-up was to determine if the
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48 persistence of anti-HAV antibodies induced by Epaxal[®] Junior was comparable with that
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50 observed with Epaxal[®] and with Havrix[®] Junior.
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MATERIALS AND METHODS

Study Participants and Design. This was a 5.5-year follow-up of Study EPA001B (NCT01405677), which was an open Phase II study conducted at two centers in Belgium.¹⁷ Healthy subjects 12 months to 17 years of age and seronegative for anti-HAV antibodies had been enrolled in the primary study. Subjects who had received two intramuscular (i.m.) doses of the vaccines (Epaxal[®] Junior, Epaxal[®], or Havrix[®] Junior) according to a 0/6-month schedule and who had completed the primary study period were eligible to continue the follow-up period.

The study had been approved by the Ethics Committee of each study center. Parents/legal guardians and subjects (if applicable) provided written informed consent for the follow-up.

Assessment of Immunogenicity. Serum samples were obtained at 18 months after receipt of the second (booster) dose of vaccine and then yearly for up to 66 months (i.e. 5.5 years after the second dose). The sera of all five follow-up time points were stored and tested in parallel at the end of the study using the same test kit batch. Anti-HAV antibody levels were measured with the microparticle enzyme immunoassay HAVAB 2.0 Quantitative for the AxSYM system (Abbott Diagnostics Division, Wiesbaden, Germany). The detection limit was an anti-HAV concentration of 6.0 mIU/mL. Levels below 6.0 mIU/mL were arbitrarily set to half of the limit (3.0 mIU/mL) for calculation of geometric mean concentrations (GMCs). Seroprotection was defined as an antibody concentration of ≥ 10 mIU/mL.¹⁸

Data Analysis. The primary endpoints for the analysis of this follow-up study are the individual measurements of the anti-HAV antibody concentrations at 18, 30, 42, 54 and 66 months after the second dose. Secondary endpoints included GMCs and the proportion of seroprotected subjects

at the five follow-up visits. A real-time assessment of seroprotection 18 to 66 months after the second dose and a computer model analysis to estimate long-term seroprotection in all subjects and – for GMCs – in subjects who completed all five follow-up visits were performed. The predicted duration of seroprotection (i.e. the persistence of antibodies) was analyzed using the seroprotective threshold of 10 mIU/mL, as well as the more stringent threshold of 20 mIU/mL, as both the 10 and 20 mIU/mL antibody concentrations can be considered as correlates of seroprotection after immunization against HAV.¹⁸ Descriptive statistics were used to present the serological data. GMCs with 95% CI were calculated from log-transformed anti-HAV antibody concentrations. Subgroup analyses of GMCs were performed by age, and - in children ≥ 11 years - by gender. The persistence of individual serum antibodies was estimated based on published methods using a linear mixed model for the log-transformed concentration values.¹⁴ The age effect was estimated in a similar model but with treatment and gender as explanatory factors, and age, year and the age-year interaction as covariables.

To assess the robustness of the results, several sensitivity analyses were performed: To assess the impact of drop-outs, GMCs were calculated separately in subjects who attended all follow-up visits (“completers”) and in “non-completers”. To look for possible explanations, the age effect on GMC kinetics was analyzed with the change in antibody titer between Visit 3 (1 month after the first dose) and Visit 4 (before the second dose) as predictor; furthermore, to see if the age effect is subgroup related, analyses were done separately in subjects who had a decline and in those who had an increase in anti-HAV GMCs between Visit 3 and Visit 4. To assess the impact of outliers, the age effect was further analyzed excluding subjects with extreme titers as well as

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4 excluding Visit 6 (18 months after the second dose) from the analysis where antibody decline
5 might still be more rapid.
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10 11 12 13 RESULTS 14

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16 *Study population.* Subjects were eligible for enrolment in the follow-up study if they had
17 received two doses of the study vaccines and had completed the core study period of Study
18 EPA001B (Visits 1–5). All 308 subjects enrolled originally in Study EPA001B met these
19 criteria, however, 271 (88.0%) subjects agreed to participate in the follow-up part of the study
20 (Epaxal[®] Junior, 108 subjects; Epaxal[®], 111 subjects; Havrix[®] Junior, 52 subjects). Serological
21 data for 66 months after the second dose were available for a total of 213 subjects: 85 (78.7%)
22 subjects in the Epaxal[®] Junior group, 87 (78.4%) subjects in the Epaxal[®] group, and 41 (78.8%)
23 subjects in the Havrix[®] Junior group. Most subjects who discontinued the follow-up part of the
24 study were either lost to follow-up or did not react to invitations to attend the visits.
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39 The median age of the 271 subjects in the follow-up was 9 years (range, 1 to 17 years) at the
40 time of enrollment in the primary study. An equal number of females and males was included
41 (135 females, 136 males). The 271 subjects had similar characteristics in terms of gender and age
42 distribution as the 308 subjects enrolled in the original study.¹⁷
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49 *Immunogenicity.* All subjects tested 66 months after they had received the second dose of
50 Epaxal[®] Junior, Epaxal[®], or Havrix[®] Junior, still had protective anti-HAV antibody levels
51 (≥ 10 mIU/mL). Of the 213 subjects assessed at 66 months after the second dose, all but one
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subject in the Epaxal[®] Junior group and one subject in the Havrix[®] Junior group had antibody concentrations ≥ 20 mIU/mL.

Individual line plots of log-transformed concentrations during the follow-up are shown in the Figure, Supplemental Digital Content 1. Overall, GMCs showed a steady decline over time (Figure 1). At 66 months after the second dose GMCs had decreased from 305 mIU/mL (95% CI: 260-358) to 171 mIU/mL (95% CI: 141-207) in the Epaxal[®] Junior group, from 440 mIU/mL (95% CI: 364-531) to 241 mIU/mL (95% CI: 201-287) in the Epaxal[®] group, and from 326 mIU/mL (95% CI: 247-431) to 152 mIU/mL (95% CI: 109-213) in the Havrix[®] Junior group (Table 1).

At all time points in Table 1, the GMC for Epaxal[®] was statistically significantly higher than both Epaxal[®] Junior and Havrix[®] Junior (all $p \leq 0.014$), except after 18 months where the difference between Epaxal[®] and Havrix[®] Junior was found to be just outside statistical significance ($p = 0.052$). There were no statistically significant differences between Epaxal[®] Junior and Havrix[®] Junior at any time point (all $p \geq 0.505$).

Post booster GMCs (1 month after second dose) in subjects who did not attend all visits were very similar to those found in subjects who came to all follow-up visits (data not shown). GMCs were in general higher in subjects vaccinated with the standard dose of Epaxal[®] than in the other two groups and also higher in females than in males (Table 2). In addition, an age-dependent decrease could be seen, i.e. GMCs decreased more rapidly in younger than in older children, possibly caused by limited survival of antibody-secreting plasma cells in younger subjects, as described more fully in the discussion section. A scatter plot of individual log titers 5.5 years

after the second dose against log titers at 1 month after the second dose is shown in Figure 2. The regression lines by age group indicate a trend for higher anti-HAV antibody titers with increasing age. This trend could be seen at the beginning of the follow-up and increased even further during the observation period. A statistical analysis modeling the factors affecting anti-HAV GMCs during the follow-up revealed an influence of treatment, gender, year since vaccination, and age (Figure 3). Each of these factors had a statistically significant effect (F test with degrees of freedom for the fixed effects in the mixed model: all $P \leq 0.002$). The age effect remained statistically significant both for the subgroup of subjects ($N = 165$, $p < 0.001$) with increasing titers between Visit 3 (post first dose) and Visit 4 (before the second dose) and for the subgroup ($N = 106$, $p = 0.001$) with decreasing titers. Also, when three subjects with outlying series of titers were excluded from the analysis the age effect remained statistically significant ($P < 0.001$). Furthermore, excluding Visit 6 (18 months after the second dose) from the analysis still revealed a statistically significant age-dependent trend ($P < 0.001$).

The median predicted duration of antibody persistence (cut-off ≥ 10 mIU/mL) was 25.1 years (95% CI: 22.5-27.3) for Epaxal[®] Junior, 28.3 years (95% CI: 26.4-31.0) for Epaxal[®] and 24.5 years (95% CI: 22.1-28.6) for Havrix[®] Junior (Figure 5). The median predicted duration was generally lower in subjects ≤ 7 years of age than in subjects ≥ 8 years (Table 3). The exceptions were males aged 11 to 13 years in both the Epaxal[®] group and the Havrix[®] Junior group. A higher median predicted duration was found in females than in males, except for subjects aged 14 to 17 years in the Epaxal[®] group.

Applying the more stringent cut-off of ≥ 20 mIU/mL shortened the median predicted duration of antibody persistence to 20.4 years (95% CI: 18.8-22.0) with Epaxal[®] Junior, 22.8 years (95% CI:

21.7-26.4) with Epaxal[®] and 20.7 years (95% CI 16.6-24.9) with Havrix[®] Junior. Age and gender had the same influence on the median predicted duration of antibody persistence as seen for the ≥ 10 mIU/mL cut-off (data not shown).

DISCUSSION

The present study included 271 subjects whose anti-HAV antibody levels were monitored during a real-time annual follow-up for 66 months after receiving two doses of Epaxal[®] Junior, Epaxal[®], or Havrix[®] Junior.

All subjects tested at Month 66, i.e. 5.5 years after they had received the second dose of Epaxal[®] Junior, Epaxal[®], or Havrix[®] Junior, maintained protective anti-HAV antibody levels (cut-off ≥ 10 mIU/mL). The only published clinical data comparable to this observation come from experience with a pediatric aluminum-adsorbed HAV vaccine (Avaxim 80U Pediatric) for which a 97.9% seroprotection rate was found 10 years after a two-dose immunization course.¹⁹ Another follow-up study reports on long-term immunogenicity of Havrix[®] (360 EU, the former pediatric formulation of Havrix) 17 years after a three-dose childhood immunization schedule.²⁰ Several long-term follow-up studies were also performed with the current higher-dosed pediatric formulation of Havrix[®] (Havrix[®] Junior, 720 EU) at a two-dose immunization schedule - the formulation and schedule used as a comparator in the present study. In these studies, children were found to be still seroprotected after 10 to 14 years.^{21, 22} Furthermore, persistence of anti-HAV antibodies for 5 to 10 years in children has been shown in several studies after vaccination with the adult formulation of aluminum-adsorbed HAV vaccine or with combined hepatitis A and B vaccines.^{21, 23-26}

The linear mixed mathematical model predicted a median duration of antibody persistence (≥ 10 mIU/mL) of approximately 25 years in subjects vaccinated with Epaxal[®] Junior. This estimate was similar to the duration estimated for Havrix[®] Junior, and slightly lower than the

projected duration of antibody persistence after vaccination with the standard dose of Epaxal®. These results confirm the findings of similar follow-up studies which projected protective anti-HAV antibody levels (cut-off ≥ 10 or ≥ 20 IU/mL) lasting for at least 20 years in children vaccinated with aluminum-adsorbed HAV vaccines.^{20, 23, 27-32} Overall, the results of the present and previous studies are in line with the recent systematic review indicating that, after a full two or three-dose primary immunization course, protective anti-HAV antibody levels persist for over 15 years.¹⁰ Expert consensus concluded that underlying immune memory provides protection far beyond the presence of vaccine-induced antibodies and a booster vaccination against HAV is not considered necessary in fully immunized healthy individuals.¹¹ However, further monitoring is needed to determine if individuals vaccinated as children are still protected throughout adulthood. An evaluation of an anamnestic response has not been performed in long-term studies with Epaxal® or Epaxal® Junior and needs to be investigated. Also, the follow-up period in the present study might have been too short to detect a change in decline rate and a constant yearly antibody decline rate may not have been reached yet. Racznik *et al.* found that anti-HAV antibody levels stabilized between 10 and 17 years after immunization of children and suggested that the rates of antibody decline diminish over time.²⁰ Consequently, based on the data and the mathematical analyses presented after completion of the 5.5-year follow-up, no definite claim can be made on how long two immunizations with Epaxal® Junior will provide protection against hepatitis A in healthy children. Therefore, the follow-up should be further prolonged to be able to draw a more definitive conclusion.

Age had a significant influence on anti-HAV antibody decline over the 5.5 years of follow-up and on the predicted duration of antibody persistence with younger subjects showing a faster

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4 decay and shorter periods of antibody persistence. This age effect seemed to be independent of
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6 gender and vaccine received. Several sensitivity analyses showed that the age effect was robust.
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8 A similar influence of age has been observed during a real-time follow-up of children aged
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10 18 months to 15 years vaccinated with a pediatric HAV vaccine (Avaxim 80U Pediatric) in
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12 which younger subjects showed a more rapid antibody decline over the 3 years of observation.³³
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14 Shorter-lived antibody responses can also be observed after early-life immunization with other
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16 vaccines and might be caused by a limited survival of antibody-secreting plasma cells in younger
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18 subjects.³⁴ A delayed and deficient establishment of long-term bone marrow plasma cells during
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20 early life was recently demonstrated in mice due to the inability of early life bone-marrow
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22 stromal cells to provide sufficient survival signals for plasmablast survival and differentiation
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24 into surviving plasma cells.^{35, 36} Given these age-related limitations, statistical models to predict
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26 the duration of anti-HAV antibody persistence in adults may not apply to children of all age
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28 groups. Further studies are therefore needed to examine the differences in long-term antibody
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30 responses in children of various age groups. Of particular interest will be to investigate if the
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32 faster antibody decline rate in younger children persists or slows down and stabilizes as children
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34 get older. To address this question, the current follow-up should be extended so that more real-
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36 time data on anti-HAV antibody kinetics in children can be collected and evaluated.
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46 The GMCs of anti-HAV antibodies were higher in females than in males throughout the follow-
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48 up period. This gender difference was previously observed for Epaxal[®] as well as for aluminum-
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50 adsorbed hepatitis A vaccines and for many other types of vaccines.^{27, 37-39} The differences in the
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52 immune responses of males and females can be attributed not only to differences in sex
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hormones, but are also related to X chromosome gene contributions and possibly to the effects of environmental factors.^{40, 41}

Several sensitivity analyses were performed to assess the robustness of the results. Applying the more stringent cut-off for seroprotection of ≥ 20 mIU/mL resulted in a median predicted duration of antibody persistence that was approximately 5 years shorter as compared to values achieved when the ≥ 10 mIU/mL cut-off was used. As with any long-term follow-up study, some subjects did not attend all visits or dropped out as the study progressed. An analysis comparing the GMCs of subjects who missed visits to those of subjects who completed the entire follow-up revealed very similar post booster GMCs. It is therefore unlikely that the antibody kinetics in drop-outs would have been different from values observed in study completers.

In conclusion, the data from the present follow-up show that Epaxal[®] Junior given to children at a 0/6 months schedule confers a real-time protection of at least 5.5 years. A mixed linear model estimated that this protection lasts approximately 25 years. Anti-HAV antibody kinetics were age-dependent with younger subjects showing lower antibody levels and a faster decline. Further follow-up studies are needed to determine how long protective anti-HAV antibody levels persist in children of different age groups.

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FIGURE LEGENDS

FIGURE 1. Anti-HAV geometric mean concentrations with 95% confidence intervals at Visit 6 (18 months after the second dose), Visit 7 (30 months after the second dose), Visit 8 (42 months after the second dose), Visit 9 (54 months after the second dose), and Visit 10 (66 months after the second dose).

FIGURE 2. Scatter plot of log titers 5.5 years after the second dose against log titers 1 month after the second dose with regression line by age group.

FIGURE 3. Fitted log titers against year from the statistical model with treatment, gender, age, year and the age-year interaction as predictors. A. Epaxal[®] Junior, B. Epaxal[®], C. Havrix[®] Junior. Upper bundle for females, lower bundle for males.

FIGURE 5. Estimated duration of protection (cut-off ≥ 10 mIU/mL) after two doses of hepatitis A vaccine using a linear mixed model for the log-transformed concentration values (Kaplan–Meier curves). The time axis is defined as the number of years after the primary dose.

SUPPLEMENTAL FIGURE. Individual anti-HAV log-transformed concentrations at Visit 1 (baseline), Visit 3 (post first dose), Visit 4 (before second dose), Visit 5 (1 month after the second dose), Visit 6 (18 months after the second dose), Visit 7 (30 months after the second

dose), Visit 8 (42 months after the second dose), Visit 9 (54 months after the second dose), and Visit 10 (66 months after the second dose). A. Epaxal[®] Junior, B. Epaxal[®], C. Havrix[®] Junior.

Table 1. Geometric Mean Concentrations of Anti-HAV Antibodies Following Two Doses of Hepatitis A Vaccine (all available subjects)

Time after the second dose	Epaxal [®] Junior		Epaxal [®]		Havrix [®] Junior	
	N	GMC (95% CI)	N	GMC (95% CI)	N	GMC (95% CI)
1 month*	121	1714 (1490-1970)	121	2690 (2355-3072)	60	2024 (1552-2641)
18 months	108	305 (260-358)	111	440 (364-531)	51	326 (247-431)
30 months	107	260 (221-305)	108	388 (324-463)	47	257 (190-348)
42 months	98	214 (180-255)	96	342 (289-404)	47	227 (166-309)
54 months	90	189 (158-226)	92	290 (244-345)	44	186 (136-255)
66 months	85	171 (141-207)	87	241 (201-287)	41	152 (109-213)

*From Van der Wielen et al., 2007¹⁷. Note the 1 month data are derived from a different analysis and different population to the data presented for the other timepoints.

N indicates number of subjects included in analysis; GMC, geometric mean anti-HAV concentrations (mIU/mL); 95% CI, two-sided 95% confidence interval.

Table 2. Geometric Mean Concentrations of Anti-HAV Antibodies Following Two Doses of Hepatitis A Vaccine by Age (years) and Gender

A Epaxal[®] Junior

Stratum	Time after the second dose									
	18 months		30 months		42 months		54 months		66 months	
	N	GMC	N	GMC	N	GMC	N	GMC	N	GMC
Age 1	8	288	8	242	8	206	8	162	7	138
Age 2-4	22	265	21	234	20	194	19	158	17	129
Age 5-7	19	213	19	180	19	143	19	122	19	104
Age 8-10	18	390	18	328	17	284	15	246	14	207
Age 11-13 female	11	392	11	370	11	322	10	292	11	296
Age 11-13 male	10	262	10	213	7	128	6	199	5	213
Age 14-17 female	10	548	10	430	8	346	7	354	6	406
Age 14-17 male	10	272	10	226	8	229	6	191	6	186

B Epaxal®

Stratum	Time after the second dose									
	18 months		30 months		42 months		54 months		66 months	
	N	GMC	N	GMC	N	GMC	N	GMC	N	GMC
Age 1	8	366	8	309	8	278	8	234	8	189
Age 2-4	20	524	19	392	18	391	18	330	18	262
Age 5-7	21	452	21	408	20	358	20	308	18	213
Age 8-10	21	352	21	303	20	280	17	256	17	227
Age 11-13 female	9	715	9	595	8	398	8	370	8	323
Age 11-13 male	10	350	10	298	7	208	6	176	5	185
Age 14-17 female	10	619	10	585	8	470	7	351	8	340
Age 14-17 male	12	351	10	400	7	455	8	293	5	228

C Havrix® Junior

Stratum	Time after the second dose									
	18 months		30 months		42 months		54 months		66 months	
	N	GMC	N	GMC	N	GMC	N	GMC	N	GMC
Age 1	3	355	2	173	2	137	2	101	2	80
Age 2-4	9	330	8	315	8	274	8	206	7	176
Age 5-7	9	338	8	241	8	207	8	171	8	139
Age 8-10	11	486	11	388	11	334	10	264	10	229
Age 11-13 female	5	418	4	257	4	244	4	208	4	209
Age 11-13 male	5	141	5	110	5	98	5	102	5	85
Age 14-17 female	5	303	5	258	5	272	4	213	3	128
Age 14-17 male	4	211	4	219	4	173	3	167	2	91

N indicates number of subjects included in analysis; GMC, geometric mean anti-HAV concentration (mIU/mL).

Table 3. Estimated Duration of Protection (Years) After Two Doses of Hepatitis A Vaccine (cut-off ≥ 10 mIU/mL)

	Epaxal [®] Junior		Epaxal [®]		Havrix [®] Junior	
	N	Median (95% CI)	N	Median (95% CI)	N	Median (95% CI)
Total	107	25.1 (22.5-27.3)	108	28.3 (26.4-31.0)	47	24.5 (22.1-28.6)
Stratum						
Age 1-4	28	20.6 (18.0-22.3)	27	23.3 (20.1-28.2)	10	22.0 (19.7-26.6)
Age 5-7	20	20.9 (16.8-24.0)	21	27.1 (24.2-28.4)	8	18.6 (13.9-28.2)
Age 8-10	18	27.5 (23.5-34.2)	21	31.3 (29.4-37.1)	11	28.6 (16.5-37.3)
Age 11-13 female	11	38.9 (36.3-44.2)	9	30.9 (25.3-37.9)	4	32.0 (15.9-50.0)
Age 11-13 male	10	28.5 (18.7-34.9)	9	22.8 (21.0-26.7)	5	22.1 (14.3-45.4)
Age 14-17 female	10	34.8 (22.3-39.8)	10	31.2 (24.9-44.5)	5	47.7 (6.9-50.0)
Age 14-17 male	10	25.9 (23.9-39.7)	11	32.6 (26.9-43.3)	4	29.0 (24.0-42.4)

N indicates number of subjects included in analysis; 95% CI, two-sided 95% confidence interval.

Figure 1

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Supplemental Figure

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SUPPLEMENTAL FIGURE. Individual anti-HAV log-transformed concentrations at Visit 1 (baseline), Visit 3 (post first dose), Visit 4 (before second dose), Visit 5 (1 month after the second dose), Visit 6 (18 months after the second dose), Visit 7 (30 months after the second dose), Visit 8 (42 months after the second dose), Visit 9 (54 months after the second dose), and Visit 10 (66 months after the second dose). A. Epaxal[®] Junior, B. Epaxal[®], C. Havrix[®] Junior.