Prevalence and Risk Factors for Hepatitis C Virus Infection in Mongolian Children: Findings From a Nationwide Survey

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Although the hepatitis C virus (HCV) infection is one of the major causes of chronic hepatitis and hepatocellular carcinoma (HCC) in Mongolia, its prevalence among children and routes of transmission are largely unknown. The aim of the study was to determine the prevalence of anti-HCV antibodies and the possible risk factors for transmission among school children using representative national data. A nationwide cross-sectional survey among elementary school children was conducted in four main geographical regions and the metropolitan area of Mongolia, through multi-stage, stratified, random cluster sampling. Serum samples from 1,145 children (response rate, 93%; 592 boys and 553 girls; age range, 7–12 years), which represented nearly 2% of the second grade population in Mongolia, were tested for HCV antibodies with a third-generation immunoradiometric assay (IRMA). Positive samples were further evaluated by a third-generation immunoblot assay (RIBA). A standardized questionnaire concerning the socio-demographic characteristics and potential risk factors was used. Overall, seven subjects were confirmed to be anti-HCV seropositive, giving a prevalence of 0.6% (95% CI: 0.15–1.0%). The prevalence of anti-HCV increased with age. In the multivariate logistic regression analysis, adjusted for age, sex, and residence, the history of dental manipulation (odds ratio [OR] = 15.4; 95% CI: 1.4–164.8) and surgery (OR = 8.3; 95% CI: 1.5–45.6) were associated independently with the presence of anti-HCV. These findings suggest that contaminated equipment used in the dental and surgical manipulations probably played a predominant role in HCV transmission among Mongolian children. Strict guidelines on disinfection and sterilization procedures of medical instruments have to be introduced and should be followed to improve the control of HCV infection in Mongolia. J. Med. Virol. 78:466–472, 2006. © 2006 Wiley-Liss, Inc.

KEY WORDS: HCV; epidemiology; iatrogenic transmission; children; Mongolia

INTRODUCTION

Hepatitis C virus (HCV) infection has become a major worldwide health problem because of the potential natural course of the disease, with more than 70% of patients progressing to cirrhosis and primary hepatocellular carcinoma (HCC). An estimated 170 million people, roughly 3% of the human population worldwide, are infected chronically with HCV [Heintges and Wands, 1997; Ramadori and Meier, 2001].

HCV is transmitted through percutaneous or percutaneous exposure to infectious blood or blood-derived body fluids. In developing countries, the primary sources of HCV infection include transfusion of blood and blood products from unscreened donors, parenteral exposure to blood through the use of contaminated or inadequately sterilized instruments and needles for in medical and dental procedures, the use of unsterilized objects for rituals (e.g., circumcision and scarification), traditional medicine (e.g., blood-letting and acupuncture), other activities that break the skin (e.g., tattooing and ear- or body-piercing), and intravenous drug abuse [Report of a WHO Consultation Organization in Collaboration with the Viral Hepatitis Prevention Board, Antwerp, Belgium, 1999; Alter, 2002].

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Although the transfusion of blood and blood products has been a major mode of HCV transmission, the introduction of universal blood donor screening virtually eliminated post-transfusion hepatitis C (Amarapurkar, 2000; Kao and Chen, 2000; Ramadori and Meier, 2001; Alter, 2002). Mother-to-infant transmission of HCV is uncommon and the overall risk of mothers transmitting the infection to their infants is approximately 4% to 7% of the total number of individuals undergoing pregnancy (Roberts and Yeung, 2002). Maternal co-infection with HIV (Gibb et al., 2000) and high viral load (Okamoto et al., 2000) has been associated consistently with a greater likelihood of transmission to their babies.

Located in the Northern part of Central Asia, Mongolia is a landlocked country bordered by Russia to the North and China to the South. With a total area of 1.6 million square kilometers and a population of 2.5 million as of 2004 (1.5 inhabitants per square kilometers), it is one of the most sparsely populated countries in the world. Although more than half of Mongolia’s population now live in urban areas (with 36% residing in the capital city of Ulaanbaatar), many Mongolians are semi-nomadic herders of horses, sheep, goats, cattle, and camels (Ministry of Health, The directorate of Medical Services, Mongolia, 2004 (available at http://www.moh.mn/)).

HCV is one of the most important public health problems in Mongolia. Previous studies of HCV infection in the country have shown a high prevalence of anti-HCV antibodies among the adult population [Lvov et al., 1996; Oyunsuren et al., 1996; Fujioka et al., 1998; Takahashi et al., 2004]. In addition, HCV was estimated to be second only to hepatitis B virus (HBV) in causing HCC and chronic liver diseases in Mongolia. It was detected in approximately 60% of patients with chronic hepatitis and primary HCC [Oyunbileg et al., 1996; Konde et al., 1997; Davaalkham et al., 2004]. The incidence of HCC has increased in the country between 1973 and 2004 from 41.0 and 17.7 per 100,000 population to 61.8 and 43.4 in males and females, respectively. Currently, HCC is the leading malignancy in Mongolia, accounting for 44% of mortality and 38% of morbidity related to cancer [Ministry of Health, The directorate of Medical Services, Mongolia, 2002; Annual Health Report, 2004 (available at http://www.moh.mn/)].

Despite the fact that HCV infection is one of the major causes of chronic hepatitis and HCC in Mongolia, its prevalence among children and routes of transmission among children and in the general population are largely unknown. Thus a better epidemiological knowledge of distribution and transmission routes for HCV infection is needed urgently so that specific public health measures may be established to prevent and control this infection. The prevalence of HCV antibodies, stratified by age, sex, residence, ethnicity, and probable routes of transmission in the elementary school children is investigated, using representative national data covering all four geographical regions and the metropolitan areas of Mongolia.

**MATERIALS AND METHODS**

**Study Area and Subjects**

This survey was conducted as part of a collaborative study on the impact assessment of a hepatitis B vaccination program in Mongolia. A nationwide school-based cross-sectional survey was carried out in 25 schools selected at random from all public schools (n = 593) throughout Mongolia. Administratively, Mongolia is divided into the capital city and 21 provinces (Aimag in Mongolian), which are in turn divided into provincial centers and surrounding rural Soums (villages, lower rural administrative units). The territory of Mongolia is divided into four main geographical regions: Western (Altai and Khangai high mountains), Eastern (the Steppes), Southern (Khangai and Gobi Desert), and Central (Khentii mountains). To represent the whole country’s geographical and economic characteristics, Mongolia was divided into five initial strata: four main geographical regions and metropolitan areas, where the latter include the capital city, Ulaanbaatar, and the two largest cities, Durkhan and Erdenet. Each region was further divided into a province center (a central town in each province that is considered as urban) and rural Soums. The metropolitan area was divided into Ulaanbaatar and non-Ulaanbaatar: as a result, the country was divided into 10 strata. Next, the subjects were allocated to 25 clusters, with 40 students per cluster on average. A multi-stage random cluster sampling was then used to select classrooms; and to ensure the self-weighting of the survey, sampling in each stage was conducted with the probability proportional to a population size of second grade students. Schools were selected from a list of all public schools in Mongolia that was provided by the Ministry of Education, Culture, and Sciences [Ministry of Education, Technology, Sciences, and Culture of Mongolia, 2003]. As a result, 9 schools in the metropolitan areas, 4 in province centers, and 12 in rural Soums were selected for the study. Private schools were excluded because the children attending them comprised a small fraction of all second grade students (2%), and due to the possibility that their inclusion in the sample might create a bias. Classes were then selected from the sampled schools for inclusion in the study. For each school, a list of second grade classes was generated, and two classes in each school were randomly selected. To avoid a selection bias, logistical and ethical problems, all children in the selected classrooms were enrolled.

**Ethical and Consent Procedures**

This study was approved by the Ethical Review Committee (ERC) of the World Health Organization, Geneva, Switzerland, the ERC of the Ministry of Health, Mongolia, the ERC of the Health Sciences, University, Mongolia, and by the ERC of Jichi Medical School, Japan. A written informed consent form was presented to the parent/guardian of each participant and a signed form was received prior to enrolling each participant.
Interview With the Parents and Blood Specimen Collection

Field work was conducted in October and November 2004. The study team, which consisted of physicians and nurses, was first involved in a training seminar regarding the survey to standardize the data to be collected, interviewing, blood drawing, and the packaging, handling, and shipping of serum samples to the laboratory in accordance to the manual instruction. The students' demographic information was obtained from the school rosters and included date of birth, gender, place of residence, and ethnicity. Age was calculated from the subject's reported date of birth. Information on the potential risks for HCV transmission was obtained by interviewing the parents, using a structured questionnaire, which dealt with information on socio-demographic characteristics and potential risk factors for exposure to HCV. The latter included a history of invasive medical procedures [e.g., dental treatment, surgery, injections at the hospital, injections at home by medical and non-professionals (persons who have learned their trade by practical experience), blood tests, and acupuncture] and information on any history of hospitalization, living with persons infected chronically with hepatitis viruses or chronic liver diseases, and sharing tooth brushes with family members.

Blood samples were collected from an antecubital vein, centrifuged on site within 1.5–2 hr and the sera were separated following a standard protocol. All serum specimens were stored at –80°C in the capital-Ulaanbaatar until transported to Japan under the cold-chain.

Laboratory Assays

The serum specimens taken from the study subjects were tested for the antibodies to HCV by using a third-generation immunoradiometric assay (IRMA HCV Ab 3.0, Ortho-Clinical Diagnostics, Tokyo, Japan), which detects antibodies directed to core, NS3, NS4, and NS5 antigens. Samples with a cut-off index (COI) of ≤ 1.0 were judged as negative. The seropositive samples by IRMA were further confirmed by a recombinant immunoblot assay (RIBA 3.0, Chiron Corp., Emeryville, CA) for the final diagnosis. Only samples found to be positive by the immunoblot assay were considered anti-HCV positive.

Liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT) were also evaluated by standard methods using kits from CicaLiquid (Kanto Kagaku Co. Ltd., Japan). All laboratory analyses were performed at the SRL Laboratory, Tokyo, Japan.

Statistical Methods

The overall prevalence of HCV markers among school children was calculated with a 95% confidence interval (CI). The chi-square test (or Fisher exact test where applicable) was used to evaluate the prevalence of an HCV infection among those of various ages, sex, ethnic, and residential groups. The crude odds ratios (OR) were estimated by univariate analysis to observe the association of each variable with the presence of anti-HCV. The adjusted OR was generated to determine the independent contribution of risk factors to HCV infection, using multivariate logistic regression models. All statistical tests were two-sided; and a level of P < 0.05 was used to indicate statistical significance. Statistical analyses were performed using the SPSS (Version 12.01) software.

RESULTS

A total of 1,271 students from 46 classes of the 25 schools chosen at random (9 schools in the Metropolitan area, 4 in the Provincial Centers, and 12 in rural Soums) were selected across the country for this study. Four schools in remote areas with a small population had only one second grade class: thus there were included 46 classes in the study. Among these 1,271, 89 children were absent during the study or refused to participate, so that 1,182 children participated (participation rate, 93%). The level of participation was higher in rural (95.6%) than in urban areas (89.5%). Of the total number of students enrolled in the study, blood was not taken from 37 students (3.1%) despite the parents' agreement. Finally, the prevalence of an HCV infection was determined for 1,145 students (592 boys and 553 girls; age, mean ± standard deviation, 8.5 ± 0.7; range, 7–12 years), who constituted nearly 2.0% of the total Mongolian primary school children in the second grade.

Approximately 55% of the students were from urban areas (living in the metropolitan area or central towns of the provinces), whereas 45.3% were children living in rural Soums. One thousand and seven students (87.9%) were Khalkh, which is a major ethnic group in Mongolia, and 138 (12.1%) were non-Khalkh from minority ethnic groups [Burjad (N = 86), Uzemchin (N = 24), Durvid (N = 8), and others (N = 20)]. Because of an insufficient number in each category, minority ethnic group children were classified as non-Khalkh for the purpose of data analysis.

Seventeen children (1.5%) tested positive for the anti-HCV antibodies by IRMA, 7 of whom were confirmed positive by recombinant immunoblot assay, giving a seroprevalence of 0.6% [95% CI: 0.15–1.05] (Table I). The anti-HCV seropositivity was higher for those coming from the metropolitan area (1.2%), followed by the provincial centers (0.5%) and lowest (0.2%) in the rural Soums.

The prevalence of HCV infection according to demographic and risk-related characteristics in Mongolian elementary school children is shown in Tables II and III. The prevalence of anti-HCV antibodies had a tendency to increase with age. Children with histories of dental manipulation and surgery were more likely to be anti-HCV seropositive. No significant differences were found in regards of gender, ethnicity, residence, or other risk-related variables.
The confirmation HCV seroprevalence among elementary school children in this study was 0.6% (7 out of 1,145). There have been limited and inconsistent data on anti-HCV prevalence among the children in Mongolia. Some studies of anti-HCV have documented a seropositivity rate of 3.3% among 251 children aged 4–11 years in the country, which was increased to 53.5% among the population aged 61 and over [Baatarkhuu et al., 2004]. However, another study revealed a high prevalence (15.8%) of HCV infection among the 127 children of comparable age (80–11 years) residing in the capital—Ulaanbaatar city [Dagwadorj et al., 1999].

The prevalence of HCV infection found in the present survey was lower than that had been reported in Mongolia. Nevertheless, the study design and laboratory method limit comparability of HCV seroprevalence results with the other surveys. The lower level of HCV infection in this study may be explained by several factors: (1) Use of a highly specific third-generation immunoblot assay (RIBA 3.0) that excludes false positive results. A supplemental immunoblot assay has not been used in the earlier studies in Mongolia; (2) Random sampling of the study population may have reduced the selection bias. The sample population in this study represents the general Mongolian elementary school children, particularly the second grade students residing in different geographical areas throughout the country. In addition, these children may reflect the general population of their age, because the school attendance rate is high in Mongolia. By international standards, literacy in Mongolia is very high (amounting to 97.8% of the population). According to the last Population and Housing Census of Mongolia, by age 8, 84.8% of the children are attending primary school; and the number rises to 93.3% by age 9 [National Statistical Office of Mongolia, United Nations Population Fund, United Nations Statistics Division, 2001]. (3) Difference in study years: The situation in infection-control may have improved in this

**DISCUSSION**

The prevalence of HCV and probable routes for transmission among Mongolian elementary school children were examined by using representative national data. This information is critical to a better understanding of the burden of the disease and developing appropriate preventive programs to control the spread of HCV infection in Mongolia.
country due to some new steps that have been taken (e.g., the introduction of disposable syringes).

It is assumed that HCV infection is not common in Mongolian elementary school children. The prevalence of anti-HCV in this study was similar to that reported for children of comparable age (0.8% by ELISA and RIBA) in Italy where chronic HCV infection is highly endemic among the adult population [Ansaldi et al., 2005]. In addition, it has previously been demonstrated that HCV infection is rarely seen in children [Kao and Chen, 2000], reflecting a low risk of infection [Alter et al., 1999]. The increasing trend of anti-HCV seropositivity by age in the present study was in concordance to that previously found in Mongolia [Baatarkhuu et al., 2004; Takahashi et al., 2004], as well as in other countries [Alter et al., 1999; Sun et al., 1999; Frank et al., 2000; Nerriniet et al., 2005]. This increase could indicate an accumulated risk of infection over time and at the same time suggests a cohort effect with reduced transmission in recent years. Since the middle of the 1990s, locally produced disposable syringes have been introduced in Mongolia [Ebright et al., 2003], which might have reduced the rate of transmission of blood-borne infections via unsafe injections. Nosocomial transmission of HCV is well documented and it appears to be a major source of infection in many developing countries. Epidemiological and genetic studies have indicated that transmission occurs because of a lack of attention to infection-control practices, such as sterilization of medical, surgical, and dental equipment.

<p>| TABLE III. Prevalence of HCV Infection in Mongolian Elementary School Children According to Risk-Related Variables |</p>
<table>
<thead>
<tr>
<th>Variables</th>
<th>Yes</th>
<th>No</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure to potential risks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dental manipulation</td>
<td>1.7% (6/358)</td>
<td>0.1% (1/695)</td>
<td>0.007</td>
</tr>
<tr>
<td>Hospitalization</td>
<td>1.2% (6/504)</td>
<td>0.2% (1/542)</td>
<td>0.061</td>
</tr>
<tr>
<td>Surgery</td>
<td>4.5% (2/44)</td>
<td>0.5% (5/1003)</td>
<td>0.030</td>
</tr>
<tr>
<td>Blood test</td>
<td>0.8% (2/255)</td>
<td>0.5% (4/743)</td>
<td>0.034</td>
</tr>
<tr>
<td>Injection at the hospital</td>
<td>0.7% (3/435)</td>
<td>0.2% (1/529)</td>
<td>0.033</td>
</tr>
<tr>
<td>Injection in the last year</td>
<td>2.0% (2/102)</td>
<td>0.4% (4/926)</td>
<td>0.112</td>
</tr>
<tr>
<td>Injection at home (by professionals)</td>
<td>0.8% (1/132)</td>
<td>0.6% (5/882)</td>
<td>0.568</td>
</tr>
<tr>
<td>Sharing of toothbrush</td>
<td>1.4% (4/295)</td>
<td>0.4% (3/757)</td>
<td>0.101</td>
</tr>
<tr>
<td>Family history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic hepatitis C</td>
<td>2.4% (1/41)</td>
<td>0.6% (5/909)</td>
<td>0.233</td>
</tr>
<tr>
<td>Chronic hepatitis B</td>
<td>0.9% (1/115)</td>
<td>0.6% (5/837)</td>
<td>0.539</td>
</tr>
</tbody>
</table>

*Prevalence of anti-HCV is shown as % (N-positive/N-tested) in the table.

**Acupuncture, injection at home by non-professionals, liver cancer, and liver cirrhosis of household members were not included in the table due to no anti-HCV positive cases among children with the above-listed history.

<p>| TABLE IV. Univariate and Multivariate Logistic Regression Analyses for Potential Risk Factors of HCV Infection |</p>
<table>
<thead>
<tr>
<th>Variables</th>
<th>Crude OR (95% CI)</th>
<th>P-value</th>
<th>Adjusted OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Socio-demographic variables</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (year)</td>
<td>1.9 (0.9–4.1)</td>
<td>0.111</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Sex (girls/boys)</td>
<td>1.4 (0.3–6.4)</td>
<td>0.717</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Residence (urban/rural)</td>
<td>5.0 (0.6–41.8)</td>
<td>0.135</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Father’s occupation (professional or skilled/others)</td>
<td>2.3 (0.4–11.8)</td>
<td>0.286</td>
<td>2.1 (0.4–11.7)</td>
<td>0.388</td>
</tr>
<tr>
<td>Mother’s occupation (professional or skilled/others)</td>
<td>0.9 (0.2–4.4)</td>
<td>1.0</td>
<td>0.8 (0.1–4.2)</td>
<td>0.764</td>
</tr>
<tr>
<td>Housing type (apartment/others)</td>
<td>3.9 (0.8–17.5)</td>
<td>0.091</td>
<td>3.2 (0.6–16.4)</td>
<td>0.265</td>
</tr>
<tr>
<td>Number of household members (equal or more than 5/less than 5)</td>
<td>1.3 (0.3–5.9)</td>
<td>1.0</td>
<td>1.3 (0.3–6.1)</td>
<td>0.727</td>
</tr>
<tr>
<td>Exposure to potential risks (yes/no)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dental manipulation</td>
<td>11.8 (1.4–98.6)</td>
<td>0.007</td>
<td>15.4 (1.4–164.8)</td>
<td>0.024</td>
</tr>
<tr>
<td>Hospitalization</td>
<td>6.5 (0.8–54.3)</td>
<td>0.061</td>
<td>6.2 (0.7–51.9)</td>
<td>0.093</td>
</tr>
<tr>
<td>Surgery</td>
<td>9.5 (1.8–50.4)</td>
<td>0.030</td>
<td>8.3 (1.5–45.6)</td>
<td>0.015</td>
</tr>
<tr>
<td>Blood test</td>
<td>1.5 (0.3–8.0)</td>
<td>0.649</td>
<td>1.6 (0.3–9.0)</td>
<td>0.586</td>
</tr>
<tr>
<td>Injection at the hospital</td>
<td>3.7 (0.4–55.4)</td>
<td>0.333</td>
<td>3.9 (0.4–37.6)</td>
<td>0.244</td>
</tr>
<tr>
<td>Injection in the last year</td>
<td>4.6 (0.8–25.5)</td>
<td>0.112</td>
<td>3.8 (0.7–21.7)</td>
<td>0.131</td>
</tr>
<tr>
<td>Injection at home (by professionals)</td>
<td>1.3 (0.2–11.6)</td>
<td>0.568</td>
<td>1.3 (0.1–11.8)</td>
<td>0.814</td>
</tr>
<tr>
<td>Sharing of tooth brush</td>
<td>3.5 (0.8–15.5)</td>
<td>0.101</td>
<td>3.4 (0.7–15.3)</td>
<td>0.115</td>
</tr>
<tr>
<td>Family history (yes/no)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic hepatitis C</td>
<td>4.5 (0.5–39.6)</td>
<td>0.233</td>
<td>4.2 (0.5–38.1)</td>
<td>0.198</td>
</tr>
<tr>
<td>Chronic hepatitis B</td>
<td>1.5 (0.2–12.6)</td>
<td>0.539</td>
<td>1.7 (0.2–14.7)</td>
<td>0.648</td>
</tr>
</tbody>
</table>

*Adjusted for age, sex, and residence.

**Acupuncture, injection at home by non-professionals, liver cancer, and liver cirrhosis of the household members were not included in the table due to no positive cases among children with the above histories. No positive cases were found among non-khalkh minority ethnic group children.

*Ger-housing group was combined into others category in the regression analysis, due to the similar rate of anti-HCV positivity.
Although the transfusion of blood and blood products has been a major mode of HCV transmission, the recognition of HCV led to the development of diagnostic tests for screening blood supplies and donors, which virtually eliminated post-transfusion hepatitis C. [Major et al., 2001; Ramadori and Meier, 2001]. Thus, information regarding the blood transfusion history of the children who were born after blood donor screening was not obtained. According to the statistical indicators from the Blood Center, post-transfusion viral hepatitis has been virtually eliminated (to 0.25 per 1,000 unit products, a total 3 cases) in the country; and since the late 1990s, no case has been detected in Mongolia [Blood Center of Mongolia, 2003].

One of the potential limitations of this study is that the subjects were limited to elementary school children, specifically second-grade students. In addition, information regarding the potential risk factors (chronic hepatitis of household members and other medical histories) was obtained through interviews with their parents so it was difficult to thoroughly estimate the HCV transmission routes in the study population. Considering these issues, the estimates on HCV infection and routes for transmission among the general population for all age groups should be further verified so that the current situation in the country may be fully understood.

Despite these limitations, the significant risk factors for HCV infection in Mongolia are described for the first time. The strengths of this study include an accurate representation of the elementary school children in this sample, as well as the larger sample size obtained through random cluster sampling from all four geographical regions and the metropolitan areas of Mongolia in comparison with the previous studies. In addition, all laboratory examinations for this survey were carried out in an accredited high quality laboratory (“SRL”) in Japan, and a highly specific immunoblot assay (RIBA 3.0) was performed to confirm the anti-HCV-positive results, which has not thus far been used in the other studies in Mongolia.

In conclusion, the observations suggest that HCV infections do not appear to be common among elementary school children in Mongolia. Contaminated equipment used in health-related procedures, particularly in dental and surgical manipulations, probably play predominant roles in HCV transmission among Mongolian children. Strict guidelines on disinfection and sterilization procedures for medical instruments in these sectors are required urgently and must be followed to improve the control of HCV infection in Mongolia.

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