AUAMelkonian Zara

Age as a risk factor for Hepatitis A among group of children 0 - 4 years old

(Research grant proposal)

Department of Public Health

Table of Content

Executive Summary

Hepatitis A is an infectious disease with a low level of sanitary and hygiene control is approved to be a main predisposing factors. Hepatitis A is the most prevalent type of hepatitis. In Yerevan Hepatitis A cases compose about 90% of all hepatitis cases (1117 / 1237) in 1998 year whereas children under 14 compose 67% (748 / 1117) of this number. The actual number of cases is likely much higher due to the high percent of subclinical disease manifestation among children under years of 5.

However the age -specific prevalence varies among different countries considerably. In the poorest developing countries, all children become infected by age 10 years whereas in northern European countries and Japan prevalence may be 30-60 % in older adults (above age 40) but is less than 10 % in young adults and virtually nil in children (1).

According to CDC definition Armenia is among countries with high - intermediate prevalence of the Hepatitis A. The shift from being one of the former USSR republic to the Independence Declaration in 1991 year rapidly worsen the social and economic situation in the country. Blockade of the republic, great number of refugees from Azerbaijan, unemployment, lack of electricity provide basis for high prevalence of the disease. Not much improvement has been done for recent years.

For effective improvement of the situation it is necessary to allocate prevention resources at the target group of the population and control main risk variables responsible for the disease. However no study have been conducted in the Republic before to define the age-specific prevalence of the disease and the mean age at which hepatitis A virus infection first occurs. The proposed study is attempts to resolve this problem and has a goal to provide data for educational program and health policy implementation. The objectives of the study are: 1) determination of the mean age at which HAV virus first occurs among children aged 1-4 and 2) investigation of age-specific risk variables among group of children 1-4 years old. A number of observational (cross- sectional) studies have been done worldwide to reveal the prevalence of the disease among children at different ages (3, 5). However these studies did not specify the age at which children are at the most risk for getting the disease. Only ongoing study of disease incidence during some period of time (cohort study) will give us the answer to this question and allow to annually control for any changes in risk variables associated with the disease. In the project the author describes the proposed study, justifies design and analysis methods along with general information about Hepatitis A.

Background

According to the data from WHO (World Health Organization) the liver disease are among the leading causes of mortality and morbidity all over the world. Data from the fourth Russian conference dedicated to the liver diseases that took place on 08.17.1999 in Moscow shows that there are about 30 million people with liver disease in Russia and only virus hepatitis of different forms is revealed among more than a million Russians.

There are several strains of Hepatitis but currently we distinguish five main types of Hepatitis. Hepatitis A and hepatitis E are mainly transmitted through the fecal-oral route, while hepatitis B, C, and D are spread through blood or other body fluids. Transmission of HAV by blood is recognized to occur infrequently because appearance of antibody limits viremia.

Hepatitis A is the most prevalent type of Hepatitis. Each year approximately 1.4 million people worldwide become infected with hepatitis A. However the real number is likely much higher taking into account high rate of subclinical form of disease manifestation. Data for Armenia show that in 1998 year 1237 people were infected with hepatitis and 90% of them (1117 / 1237) composed cases with Hepatitis A. Currently two main types of Hepatitis A cases are registered in the san-epid station of the republic - type A and type B. Though the real numbers could be a little differ from those reported, the general picture won't change much taking into account the great number of subclinical, unreported cases with Hepatitis A.

Major risk factors for the disease (1) are:

Person -to - person contact

Poor personal hygiene

Poor sanitation

Crowded living condition.

Unsafe sexual practices

Employees of day care centers and health care institutions such as nursing homes

Traveling to the areas with poor sanitation

Street drug use

Specific occupational risk factors for HAV are not well documented though some studies of sewage workers in Europe have suggested two - to threefold elevated risk of infection (4)

(Appendix # 1)

Low maternal education level has been found to be also an important risk factor in some studies (2).

Virus Description

"The hepatitis A virus is a small nonenveloped RNA virus belonging to the family Picornaviridael. The HAV is more heat stable than other picornaviruses. Able to retain infectivity in feces for at least 2 weeks, and loses only 100-fold infectivity over 4 weeks at room temperature. The virus is stable to extraction by nonionic detergents, chloroform, or ether, and to pH 3 for 3 hr and remains viable for many years at – 20 o C. It is completely inactivated by formalin (0.02% at 37oC for 72 hr), hypochlorite (1.0-1.5 ppm for 30 min), and heating at (100oC for 5 min) but is only partially inactivated at 60oC for 1 hr.

The HAV is excreted via the biliary system into the feces, where it appears from 1 to 2 weeks before illness in highest quantities. Virus excretion begins to decrease at the onset of clinical illness and in most persons has decreased substantially by the first week after onset" (4). (Appendix # 1)

Clinical picture

Clinical picture of hepatitis A range from few or no symptoms to fulminant hepatitis. The disease is characterized by the age dependance of clinical illness, low rates of fulminant disease and absence of chronic disease .The common symptoms are: fever / chills, jaundice, pain in the liver area, dark urine, light-colored stools, abdominal pain. The symptoms can be presented in all this range or partially. Rapid symptom onset and diarrhea are more common characteristic of hepatitis A than hepatitis B, but symptoms should not be used to distinguish types of hepatitis. Extrahepatic manifestations are uncommon, although arthritis, meningoencephalitis, and aplastic anemia are reported to be rarely associated with HAV infection (1).

The clinical expression of HAV infection is highly age dependent. Serological studies have conformed that infection in children under age 6 rarely causes jaundice (fewer than one in ten infections), whereas in adults, and probably in older children above age 5, infection usually causes typical symptoms, with 50-90% of cases having jaundice (3).

Usually no specific treatment is needed besides vitamins and digestive ferments. In many cases proper diet and rest are enough to avoid any serious damage of the liver. Nevertheless with a mean incubation period of 28 days many individuals can spread the disease well before they are even aware they have it. Children are considered a main source of spreading the infection due to high rate of subclinical manifestation of the disease among children under age of 5. Approximately 45% of persons with HAV cannot identify a recognized risk factor associated with their disease, but about half of them have children under five years of age living in their households (1)

In individuals with pre-existing chronic liver disease acute Hepatitis A infection may cause severe illness and fulminant hepatic failure. According to data the disease accounts for 1.5-27% of fulminate hepatitis cases in developed areas. The risk of fulminant outcome among clinical cases is estimated to be 0.1-0.5% (1). Hepatitis surveillance in the United States has shown the fatality rate among clinically ill cases to be age dependent, from about 0.3% among children and young adults to 1.3-2.2% in those over age 40 years (5).

Moreover, according to the recent study published in New England Journal of Medicine the team lead by Dr.Sandro Vento of the University of Verona, found that in the alphabet soup of hepatitis infections, adding A to C can be a deadly mix (6). Taking into account that only in United States each year an estimated average of 35000 people got infected with Hepatitis C (5) the study could have a great scientific potential though these preliminary findings needs further careful investigation.

Studies of age-specific prevalence of anti-HAV in many countries have defined that in the majority of developing countries, including most of Africa, Asia, South and Central America, almost all adults and older children have antibodies indicative of prior infection. In the poorest countries all children become infected by age 10 years. The highest incidence of hepatitis A in such countries is in children. Nearly 30 % of the reported cases occur in children younger than 15. In these countries, infection may be delayed to later childhood among children in upper socioeconomic classes (1).

Data for Yerevan show that children under 14 compose 67% of all the cases with hepatitis A in 1998 year. The highest number of incidence cases occurred among children aged 7-13 (717/1117). However most of children aged 0-6 do not show symptoms, so the unreported number for this group is likely much higher.

In contrast, in more developed countries in Europe and Asia, HAV prevalence in adults varies widely. In advanced developing countries, such as Greece and Taiwan, HAV prevalence in young adults reaches 80-90%, but prevalence in children under age 10 is only 20-30%, with the major increase in infection prevalence occurring between ages 10 and 19 years (1).

In Europe and the United States, prevalence in young adults varies from 30 to 70% but less than 10% in young children. During recent years in the USA, 23 000 to 36 000 clinical cases of HAV infection have been reported annually to the CDC (6). Nevertheless, because of the frequent occurrence of subclinical infections, the actual number of HAV infections occurring each year probably is far higher than the number reported.

In northern European countries and Japan, HAV infection appears to be disappearing; prevalence may be 30-60 % in older adults (above age 40) but is less than 10 % in young adults and virtually nil in children.

Data on disease incidence, although often limited by lack of availability of serological tests, confirms these patterns. From these data rates of clinical Hepatitis A are estimated to vary from 5/100 per year in poorest countries to more than 100 cases per 100 in more advanced countries.(1)

In the least-developed countries, HAV transmission occurs exclusively during early childhood, and the entire population becomes infected before age 10 years; in these areas, clinical disease is recognized only in children. As hygiene /sanitation standards improve, the age of infection shifts progressively to older children and young adults, particularly in upper socio-economic groups. This may paradoxically result in higher rates of clinical disease, and common source outbreak (1).

As sanitation conditions improve further, the infection frequency in all age group and overall rates of disease decrease; clinical disease is recognized in older children and young adults, and localized outbreaks may

occur. Finally, in the most developed countries, infection rates decline in all age groups, endemic disease becomes rare, and cases of disease occur primarily as importation from areas having a high endemicity of disease.

In the regions of high endemicity with low to high rate of disease the peak age of infection is at early childhood and transmission occurs via person to person contacts .The infection outbreaks in such situation are uncommon. In the regions of low-moderate endemicity with high disease rate the peak age of the infection occur at late childhood/young adults and transmission occurs via person to person contacts and trough food and waterborne outbreaks (Appendix # 1).

Cost

Direct and indirect costs of Hepatitis A have been estimated to approximate $1000 per clinical case for persons 18 years or younger, and $ 2100 per case for persons older than 18 years (8) resulting in a projected cost in the United States of over $200 million annually (7). Additional economic costs are incurred when adults who contract the disease miss an average of 27 days of work, which translates into approximately $2600 in lost wages for each adult case (8). These estimate do not include business losses in the restaurant or tourist industries related to outbreaks of the disease.

Diagnosis

Currently the disease is diagnosed by serologic tests for total HAV (both immunoglobuline M[IgM] and immunoglobuline G[IgG] and IgM-specific HAV antibodies to the capsid proteins. The presence of IgM HAV antibodies usually indicates recent infection. IgM antibodies are present at the onset of illness and usually disappear within 3 months, but may persist for 6 month or longer. IgG HAV antibodies develop shortly after IgM HAV antibodies, persist for the lifetime of the individual, and are associated with protection against the disease.Besides, a variety of biochemical tests that are indicative of hepatocellular damage [such as Leanne transaminase (ALT)] are routinely used to screen for evidence of hepatic inflammation and for clinical management of disease. These tests remain important for assessing liver damage in humans and in animal transmission studies. However, for epidemiologic studies they have been replaced by the specific serological tests (1,9).

Methods of Prophylactics

There is no specific treatment for hepatitis A other than supportive care. Therefore, the best therapy is prevention. Prevention takes forms of:

1. Stopping transmission of virus and/or
2. Active or passive immunization of susceptible individuals

Immune Globulin for intramuscular injection remains the main method of postexposure disease prophylactic Fortunately two inactivated vaccines (Vacta and Havrix) were developed for hepatitis A recently and licensed in 1995-1996, both approved for children 2 through 18 years and for adults. In the preliminary studies conducted in endemic region of Tailand (10) the efficacy of Havrix was estimated to be 94 % after two doses, 100 % after three doses. The results of other recently conducted studies in Alaskan native villages (11) Slovak Republic (12) and New York State (13) are relevant. Studies are in progress evaluating simultaneous administration of HAV, Diphtheria and tetanus toxoids and pertusis, and Haemophilus influenza type b vaccines to children (9). Vaccines are the most effective methods of disease prophylactic and immunization should be directed to the population at risk and in a case of large community outbreaks (Appendix # 1).

Specific Aims and Objectives of the Study

The age and geographical distribution are well - known risk factors for Hepatitis "A". In a poor countries with high-intermediate prevalence of the disease the majority of the population are tested seropositive by the age of 10-14 (1). A number of cross- sectional studies have been conducted (2, 3) to determine age-specific prevalence of the Hepatitis A the in different countries but still did not provide any clue for understanding the natural history of the disease and association with risk factors at different ages.

According to data from the CDC (see the map in the Appendix) Armenia is among countries with high - intermediate prevalence of disease. The shift from being one of the former USSR republic to the Independence Declaration in 1991 year rapidly worsen the social and economic situation in the country. Blockade of the republic, great number of refugees from Azerbaijan, unemployment, lack of electricity provide basis for low level of sanitary and hygiene control that in turn is a predisposing factor for high prevalence of hepatitis "A". Not much improvement has been done in socioeconomic status of the population for recent years. The interventions are needed to decrease the high prevalence of the disease in the republic.

Currently only two main types of Hepatitis is registered the san-epid station of Yerevan – type A and B. Data for Yerevan City show that children under 14 compose 60 % (808 / 1339) and 67% (748 / 1117) of overall number of cases with Hepatitis A in 1997 and 1998 years accordingly (Appendixes #2, 3). However these numbers are fail to tell us much about the distribution of infection among children at various ages due to the following reasons:

1.Low number of incidence cases among group of people aged 14 and older may be the result of past disease experience (e.g. the highest prevalence of disease in this group of people).

2.Within the age category of 0-14 also may be various explanations of the results. For example low number of incidence cases among children aged 0-6 can be due to high percent of subclinical form of disease manifestation among this group of children.

Thus based on the available data of disease incidence by years it is impossible to define the specific age group at which children are at the most risk of getting the disease. Meanwhile having these data we can compare the association of known risk variables with the disease for this specific group of children and directly allocate the disease prevention strategies for this targeted population of children.

Most cases among children under 6 are assymptomatic. For Yerevan children of 3-6 age interval composed only 20% (164 / 808) of cases with hepatitis A among children under 14 in 1997 year. However according to the literature the main vulnerable group of the population for the regions with high prevalence of disease are children aged 3-6 (2). We proposed to test the idea by the and targeted preventive strategies to the population at risk. The objectives of the study are as follows:

Current research:

1.To determine the proportion of infected children (incident cases) at different ages, taken from the same cohort during 5-years interval.

2.To determine the most vulnerable age group for Hepatitis A according to the highest proportion of incident cases.

3.To conduct annually the investigation of the risk factors for the children at different ages associated with the disease and look at the strength of the association (Risk Ratio) The purpose is ongoing control for the contribution of different risk variables to the disease at different ages.

Education program and health policy implementation:

Educational program for parents and/or prophylactics methods based upon the results of the previous study. The purpose is to control specific risk variables (with the highest RR) at particular most vulnerable age group of children.

The design of the research (retrospective, cohort) is chosen in accordance with relevant studies conducted before (14) and is the most appropriate to reach the objectives.

Methodology.

Inclusive criteria. The cohort will consist of all live births reaching the age for enrollment at the beginning of the study (11-13 months for the first blood test). It is expected to observe the lowest proportion of HAV infected cases among these group of children. The initial test will be conducted to define the status of

enrolled infants at the time of their first anniversary. Since that the regular screening serologic test will be conducted annually to reveal the incidence of disease. All incidence cases will be counted at the end of particular age interval. Data will be analyzed and registered in the table below.

Age Interval (in months)	1 #entering interval HAV free	2 #acquiring HAV during the interval	3 #lost to follow-up	4 effective number observed (#entering-0.5#lost)	5 proportion acquiring HAV during the interval (#HAV/effect#)	6 proportion HAV free 1-proportion of HAV infected	7 Cumulative proportion of HAV free
12-24							
25-36							
37-48							
49-60							

Data collection.

Face-to face interview with the parents or other responsible subjects of the cohort children will be used for data collection. The questionnaires will be assigned annually. The questionnaire includes introduction part with general information about the study purpose and sponsor organization. Interviewers at the homes of the respondents will complete the questionnaires. Information about study risk variables will be gathered along with some demographic data. As the study will take place during 5-year interval it is desirable to collect data on as many well-known personal, behavioral and environmental risk variables as it is possible. The proposed study will investigate risk variables listed below.

	Measure		
Risk variables	Nominal	Numerical	Categorical
Maternal education level			**X**
Overcrowded living place		**X**	
Quality of drinking water			**X**
Duration of water ditsribution		**X**	
Frequency of water distribution			**X**
Feeding practice			**X**
Hands washing practice			**X**
Washing hands with soap	**X**		
Having domestic animal at home	**X**		
Having an access to any other domestic animal	**X**		
Location of closet	**X**		
Living address			**X**
# of kindergarten		**X**	

Presence of children under age of 5 at home is considered a possible risk variable for comparably elder population. However it is not reasonable to test this variable for the group of children aged 1-4.Maternal education level is ordered by a) school level b) college level, d) graduate and postgraduate level. Questions about child's hygiene maintaining behavioral practice will ask about usual hand washing practice of a child. The living address and number of kindergarten is important to reveal water or food common source outcomes. Living address is a categorical variable since will be grouped by districts during the analysis. Currently the living standard in Yerevan is 19 square meters / a person. However according to literature (3) it seems more reasonable to use another standard for assessing overcrowded living place that is a special coefficient derived from dividing the number of family by the number of room. The living place is

considered overcrowded when this coefficient is equal or more than 1. Thus we ordered the coefficient by<1, >=1.Another important questions concern the average frequency of drinking water distribution (currently it is a big problem in Yerevan, in many districts drinking water is present once, for only some hours during a day). The proposed questionnaire is attached.(see Appendix # 6)

Serologic tests**.** Cohort children will be tested annually by serologic test started at their first anniversary. Blood will be tested for the presence of IgG. Since the tests will be conducted annually there is no need for the additional test on the presence of IgM. Trained specialists either from the local polyclinic or from Diagnostic Center can conduct tests at the local polyclinic in the biochemical laboratory. In case of need blood will be taken at respondent's home. The specific biochemical assays should be provided.

Analysis of the results will be done annually by multiple regression analysis (Stata) and by calculating of the RR for the risk variables. Comparing the changes in proportion of incident cases and looking at the RR of risk factors we'll be able to reveal the general trend in contribution of specific predisposing factor at different ages. Once we'll observe the highest proportion of incident cases well be able to determine the risk factor responsible for such an increase and allocate prevention resources at targeted group of children (at particular age with highest proportion of incident cases). For the subgroup group of enrolled children tested HAV positive at the beginning of the study case-control approach with OR calculations (retrospective study) can be used since no survey is expected to be conducted a year before.

Sampling Calculation

The direct calculation of the sample size is complicated due to following reasons: since the measurements are repeated on the same individuals. Thus we cannot assume any independence. The calculation becomes more complicated since we are looking at incidence over time and the same disease cannot be an incident within a child twice. The older children become the less will be our sample sized since the diseased children won't participate in the study twice. Making direct estimation of that lost in advance is impossible. The case may be treated with the same tools as any time-to-event things such as Kaplan-Meier estimators or more complicated Nelson-Aalen estimators but still does not resolve the problem with sample size. To overcome all the problems mentioned above it is proposed to take a cohort of 500 children (number is reasonably large, but affordable and manageable) borne during 1999. The number is adjusted for lost to follow up (20%) and percent of eligible children (70%). Data will be analyzed in order to define the presence of the association of the risk variables with the disease and the strength of that association that is measured by RR. Our cases will be incident cases during a year interval and controls will be children without the disease. Relating to the proposed study we assume to observe the minimal number of cases at the beginning of the study and maximal number at the end of the study. Test will be conducted at alhpa=0.05. The power of the test will be calculated using Stata Statistical Package.

Method of Sampling

In order to make the result of the study generalizable representative sample will be conducted using Stratified-sampling technique. We will stratified children in alphabetic order by different districts of Yereven and take the appropriate proportion from each of them that will represent the strata in the general population of newborn children. The list of children will be gathered at the local pediatric polyclinic. Children names will be taken along with their addresses, phone numbers and birthday data. Then children will be chosen from the each strata using the systematic random technique that will provide the participants with equal chance to be chosen.

Advantages and limitations of the study

The proposed study has a number of advantages as:

1. Study design (retrospective, cohort) allows
   - to investigate the natural history of the disease
   - to define the mean risk age of the children population
   - to define the specific risk variables responsible for the disease at different ages
2. To effectively allocate prevention resources
3. To improve available statistical data

Although proposed research will give lots of benefits it has both usual disadvantages of the cohort study and its special limitations as following:

Lost to follow up. Usually we assume that the lost participants experience the same practice as the present ones. Nevertheless high percent of the population migrates abroad. If to some extent we can assume that in some regions of the former Soviet Union the prevalence of the disease at younger ages is not much differ from the local situation, the prevalence in other developed countries is entirely different (e.g. considerable part of the population migrates to USA). Thus trying to follow the participants and collect the necessary information from wherever they are can be of no use. The increase of the sample size is alternative method to deal with the problem.

High cost. Though the research requires much costs than other types of the study (case-control or cross-sectional) the cost will decrease considerably as the study will progress. Besides, targeted and most effective allocation of the prevention resources that is the eventual purpose of the proposed study will exceed its cost.

Long time duration (5-year interval). During this time interval some changes may occur concerning the investigators' general life plans or their health status that can not make their participation possible any more. The relevant changes concerning participated children will be captured by annual survey.

Special limitations

1. Infants who die prior to age 1 and probably also have a high chance of having HAV (not necessarily as a cause of death but as part of a collection of exposures) will be missing out.

2.For the subgroup of enrolled infants tested positive the case-control approach will be utilized since no preliminary data on risk variables will be available.

3.The number of incidence cases may be not sufficient to conduct statistically powerful tests (at p=0.9 or 0.8), especially at the beginning of the study (the lowest prevalence of the disease). Such a case my be considered as a pilot study. Alternative approach is an increase of the sample size that may be not feasible due to limited financial resources.

Ethical consideration

1. Some difficulties are expected related to the willingness of the children or their parents to participate in the procedure of blood sample taking. The procedure is a painful one and it could be difficult to persuade children or their parents to go through.
2. Concern about the proper use of clean ,disinfected syringes and needles
3. Possible difficulties with transportation of children and their parents to the local clinic or Diagnostic Center for taking blood samples.

3.Ethical issues connected with survey procedure:

- Disturbance of a family privacy
- Disturbance of a family time-management
- Face-to-face interview with an eligible youth when other members of the household are not present
- Reluctance of the respondents to be open and honest while answer to the behavior related questions

In order to resolve these problems it is proposed to:

- Encourage children by giving them little presents or/and create opportunity for free of charge simultaneous general blood analysis that can prevent or signal about possible health problems in the nearest future .

- Allow the parents of the children be present at the blood taking procedure in order to insure them that only new clean needles and syringes are used for taking the blood sample and all the things are run properly.
- For the reason of convenience the blood sample may be drawn on the basis of the regular check up.

In case of need the blood taking procedure may be organized at respondents' home

- To organize a preliminary contact with respondents by phone in order to explain the purpose of the interview and provide the information about sponsor organization. Set the convenient time for personal, face-to-face interview
- Train interviewer to build a good interpersonal contact, do not force the respondent to give the expected answer

Challenging the ethical issues at the beginning is very important since build the basis for the future open and friendly relations during next years of study.

Time framework

2000 year

January-February. Office rendering, installation of the equipment, hiring the staff personnel, sharing responsibilities.

March-May. Sample design based on the list of new -borne children for 1999.

June-December. Contacting the participants, distribution of questionnaires, taking blood samples for serotests, maintaining Database system

2001 year

January-March. Analyzing data for the year 2000

January-December. Contacting the participants, distribution of questionnaires, taking blood samples for serotests

2002 year

January -March. Analyzing data for the year 2001

January-December. Contacting the participants, distribution of questionnaires, taking blood samples for serotests

2003 year

January -March. Analyzing data for the year 2002

January-December. Contacting the participants, distribution of questionnaires, taking blood samples for serotests

2004year

January -March. Analyzing data for the year 2003

April. Making a decision of whether to continue the study for the next five years or start a case-control study

References:

1. Battegay M., Gust I.D., McFeinstone S.
Hepatitis A Virus
Pirnciple and Practice of Infectious Disease, Vol 2, 150:1636-1651

2. Redlinger, Thomas, PHD; O'Rouske, Kathleen, PhD, MPH;Van Desslice, James, PhD, MSSE
Hepatitis A among Schoolchildren in a US-Mexico Border Community
American Journal of Public Health
Vol.87 (10), October 1997, pp.1715-1717

3. (5.V. M. Zhdanov, V.A. Ananiev, V.M. Stakhanova
Viral Hepatitis
USSR. AMS. M.: Meditsina, 1986 256 pp., ill

4.Sanchez Y., LaBelle R., Hejkal T. et al. Identification of hepatitis A antigen among water sewage workers prior to an outbreak of water borne infectious hepatitis.

5. Centers for Disease Control and Prevention. Hepatitis Surveillance Report No.55.. Atlanta, GGGA: Centers for Disease Control and Prevention; 19994:1-34.).

6.Neu Reuters News, 1998

7. C. Shapiro, CDC, written communicatio, July 1995

8. Hadler SC. Global impact of hepatitis A virus infection: changing patterns. In: Hollinger FB, Lemon SM, Margolis H, eds. Viral Hepatitis and Liver Disease: Proceedings of the 1990 International Symposium. Baltimore, MD: Williams & Wilkins, 1991: 14-20)Gust ID, Lehmann NE, Lucas CR.

9 Prevention of Hepatitis A Infections: Guidelines for use of Hepatitis Vaccine and Immune Globulin
Pediatrics, Vol. 98(6), Dec.1996, pp1207-1215

10. A.Kompanietz, N.Doroshenko, V.Stachanova and others

The regulations of the epydemiologic process for Hepatitis A and B on the areas with different prevalence of diseases.
J Microbiol., 1984, 5, pages 70-74

11. McMahonB.J., WilliamsJ, Beller M., et al.
A program to control an outbreak of hepatitis A in Alaska using an inactivated hepatitis vaccine.
Abstracts of the 34th Conference on Antimicrobial Agents and Chemotherapy.1995; 35: 1980

12. PrikazskyV., OlearV., CernochA., Safary A., AndreF.
Interruption of an outbreak of hepatitis A in two villages by vaccination
J Med Virol. 1994; 44: 457-459 Shapiro C.N., Margolis H.S.,
Worldwide epidemiology of Hepatitis A virus infection
J Hepatol. 1993; 18: S11-S14

13. Wezberger A., Mensch B., Kuter B., et al
A controlled trial of formalin-inactivated hepatitis A vaccine in healthy children
N Eng J Med. 1992; 327: 453-457 Cleland l., Ginneken J.V.
Natural education and child survival in developing countries: the search for pathways of influence
Soc Sci Med. 1988; 27 (12) 1357-1368

14. Relationship between the prevalence of antibody to hepatitis A antigen and age: a cohort effect?
J Infect. Dis., 1978; 138: 425-6

Budget

The estimated expenditures for implementing the proposed research are given in the Appendix # 8.# Income taxes and pension/social security taxes are estimated in accordance to ROA taxation regulations for NGO (non-govermantal) non-porfit organizations.

Consultation is needed with the main specialist of the Republic on infectious diseases and with the chief of the Laboratories Service at the Republican Diagnostic Center. Consultation may be needed for both the overall research and specific parts. The proposed cost is the direct cost for the consultation

Operating cost

The cost includes rent of the space (1room) with fixed equipment, update cost of fuel for traveling by 2 cars. Bank fees of approximately 1 % of the office rental cost are included as well.

Cost for blood testing

The cost was calculated after consultation with the chief of the Laboratories Service at the Republican Diagnostic Center.

One box of the chemical agents for blood testing is for testing 96-100 people. In average 15% of cases are questionable and should be tested again. After adjustment the estimated number will be 82-85children.

It is impossible to calculated tests cost for the remaining 4 years in advance since it is impossible to estimate the number of HAV free participants at the end of each age interval. The only assumptions that we can make are as follows:

1. The number of study participants will decrease, as the study will progress being maximal and minimal at the beginning and the end of the study accordingly.
2. At least half of the enrolled participants will be tested HAV positive (prevalence of 0.5) at the end of the study. Thus the cots for blood testing and survey will be half of that estimated at the beginning. Assuming the prevalence of disease will increase gradually from 0.1-during the first year till 0.4-during the fourth year of the study accordingly we can approximate cost for these years.

Procurement Cost

This includes the cost of the boxes for testing on the presence of IgG, cost of the syringes, needles and other supplies.

Incentives

Giving incentives to the study participants is a worldwide used practice. It is especially important for proposed study since it will encourage children go through the painful procedure of blood testing. It is proposed to buy toys for children or give incentives directly to the parents.

Indirect cost

Cost estimated for that number of activities that can not be planned in advance. The cost is estimated at 12 % of overall budged.

Acknowledgment

I express my great acknowledgment to M. Tompson that is the main supervisor of the project for his constant assistance and encouraging me during the work process. I am very thankful to Dr. H. Armenyan for his comments and notes regarding the research. I also acknowledge to the chief of the Laboratories Service of the Republican Diagnostic Center, Mr. L R. Badalyan for assistance related to Diagnoses and Budget parts of the project.

Appendix # 6

Questionnaire

1.Location

2.Interviewer's name_____

3.Date of interview _____(day/month/year)

4.Time when interview was started ___

5.Time when it was ended___

6. Name of the child

7. Birthday (day/month/year)

8. Living Address:

_____

_____-

_____

9.Phone number_____

10. Test result(IgG)

-Introduction

My name is _____. I'm the representative of the _____ organization that conduct a research project on Hepatitis A. The purpose of the project is determination of children age that is most vulnerable for the disease and investigation of the specific risk factors associated with the disease at this age. The research is sponsored by_____ and will last for 5 years. Research consists of two main parts: survey of participated infants' parents or other responsible subjects and infants' blood testing. The latter is needed to validate the diagnose of hepatitis A. Both procedures will be conducted annually at the same time of child anniversary. Once the child is tested positive he is taken out from the study.

I assure the confidentiality of the survey information. That is your choice to refuse or agree to participate at any stage of the study.

1.Who is the main person responsible for the child?

a) Father •

b) Relative •

c) Neighbor •

d) Others •

2.Can I talk with that person?

a) Yes •

b) No •

If yes, go to the question #4

3.What are the reasons?

1.Not at home •

2.Refuse •

3.Others (Indicate_____)

4.How many people live at child's home? ___

5.How many rooms are there? ___

6.What are the usual frequency of water distribution at child's home?

a) Once a day? from___(a.m., p.m.) till_____(a.m., p.m.)

b) Two or more times a day? from____(a.m., p.m.) till_____(a.m., p.m)

from____(a.m., p.m.) till_____(a.m., p.m)

c) 24hours/day •

7. What is the average duration of water supply?

__ (in min. or hours)

8. How much time in average from this number are you present at home?

____(min., hours)

9. What type of water the child usually drinks:

a) Boiled •

b) Raw •

c) Filtered •

10. How does the child usually wash the hands?

a) Himself / herself without any help from the parents •

b) Parents wash his/her hands •

c) Himself /herself under the parents' control •

If the answer is a) go to the question #12

11. Do you always use the soap or any other substitution during the procedure?

a) Yes •

b) No •

12. What is the feeding practice of your baby?

a) Breast-feeding •

b) Formula prepared with raw water •

c) Prepared with boiled water •

d) Supplemented with raw food •

13. Have you any domestic (cat, dog) animal at home?

a) Yes •

b) No •

14. Does your child have an access to any domestic animal ?

a) Yes •

b) No •

c) Do not know •

15. What is the location of the closet at your living place?

a) At home •

b) In the yard •

16. Your education level

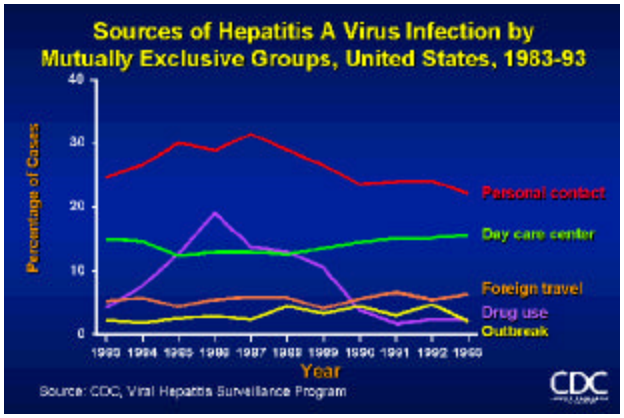
a. School level •

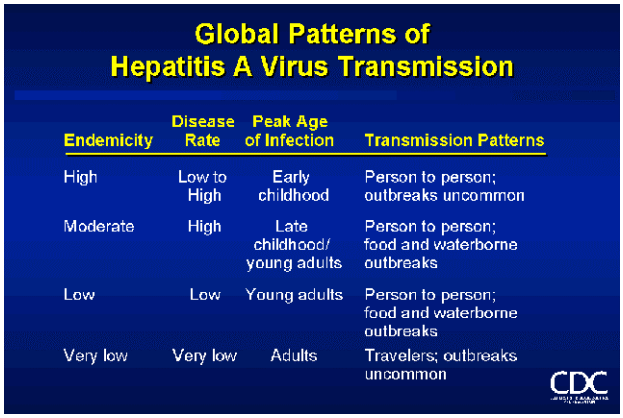
2 Graduate •

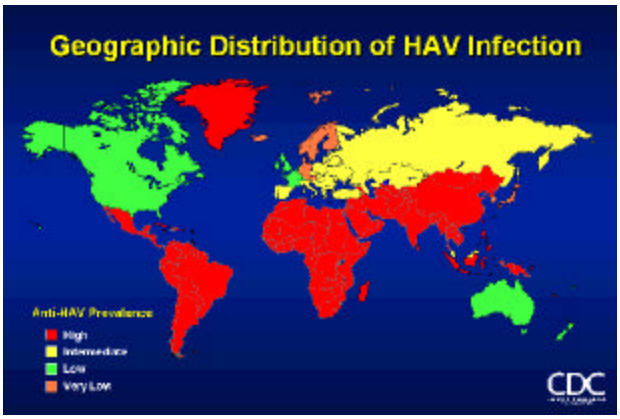
3 Post Graduate •

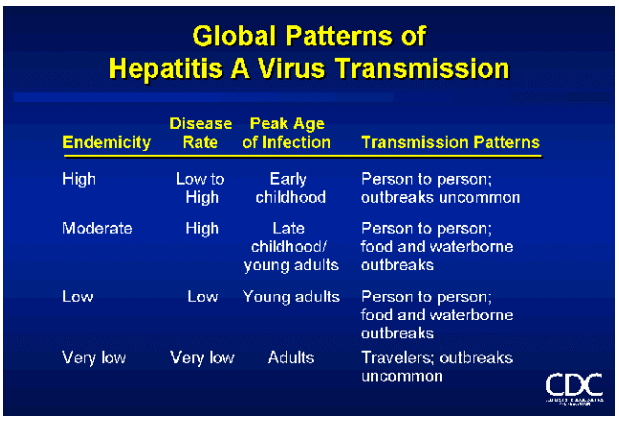
Thank you very much for the participation.

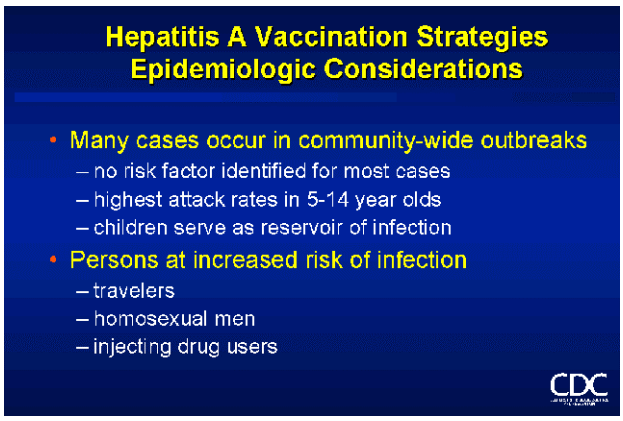
Appendix #1


Sources of Hepatitis A Virus Infection by Mutually Exclusive Groups, United States, 1983-93
Percentage of Cases
40
30
20
10
0
Personal contact
Day care center
Foreign travel
Drug use
Outbreak
1983 1984 1985 1986 1987 1988 1989 1990 1991 1992 1993
Year
Source: CDC, Viral Hepatitis Surveillance Program
CDC


Global Patterns of Hepatitis A Virus Transmission
Endemicity | Disease Rate | Peak Age of Infection | Transmission Patterns
High | Low to High | Early childhood | Person to person; outbreaks uncommon
Moderate | High | Late childhood/ young adults | Person to person; food and waterborne outbreaks
Low | Low | Young adults | Person to person; food and waterborne outbreaks
Very low | Very low | Adults | Travelers; outbreaks uncommon
CDC


Geographic Distribution of HAV Infection
Anti-HAV Prevalence
High
Intermediate
Low
Very Low
CDC


Global Patterns of Hepatitis A Virus Transmission
Endemicity | Disease Rate | Peak Age of Infection | Transmission Patterns
High | Low to High | Early childhood | Person to person; outbreaks uncommon
Moderate | High | Late childhood/ young adults | Person to person; food and waterborne outbreaks
Low | Low | Young adults | Person to person; food and waterborne outbreaks
Very low | Very low | Adults | Travelers; outbreaks uncommon
CDC


Hepatitis A Vaccination Strategies Epidemiologic Considerations
• Many cases occur in community-wide outbreaks
– no risk factor identified for most cases
– highest attack rates in 5-14 year olds
– children serve as reservoir of infection
• Persons at increased risk of infection
– travelers
– homosexual men
– injecting drug users
CDC

Appendix #2

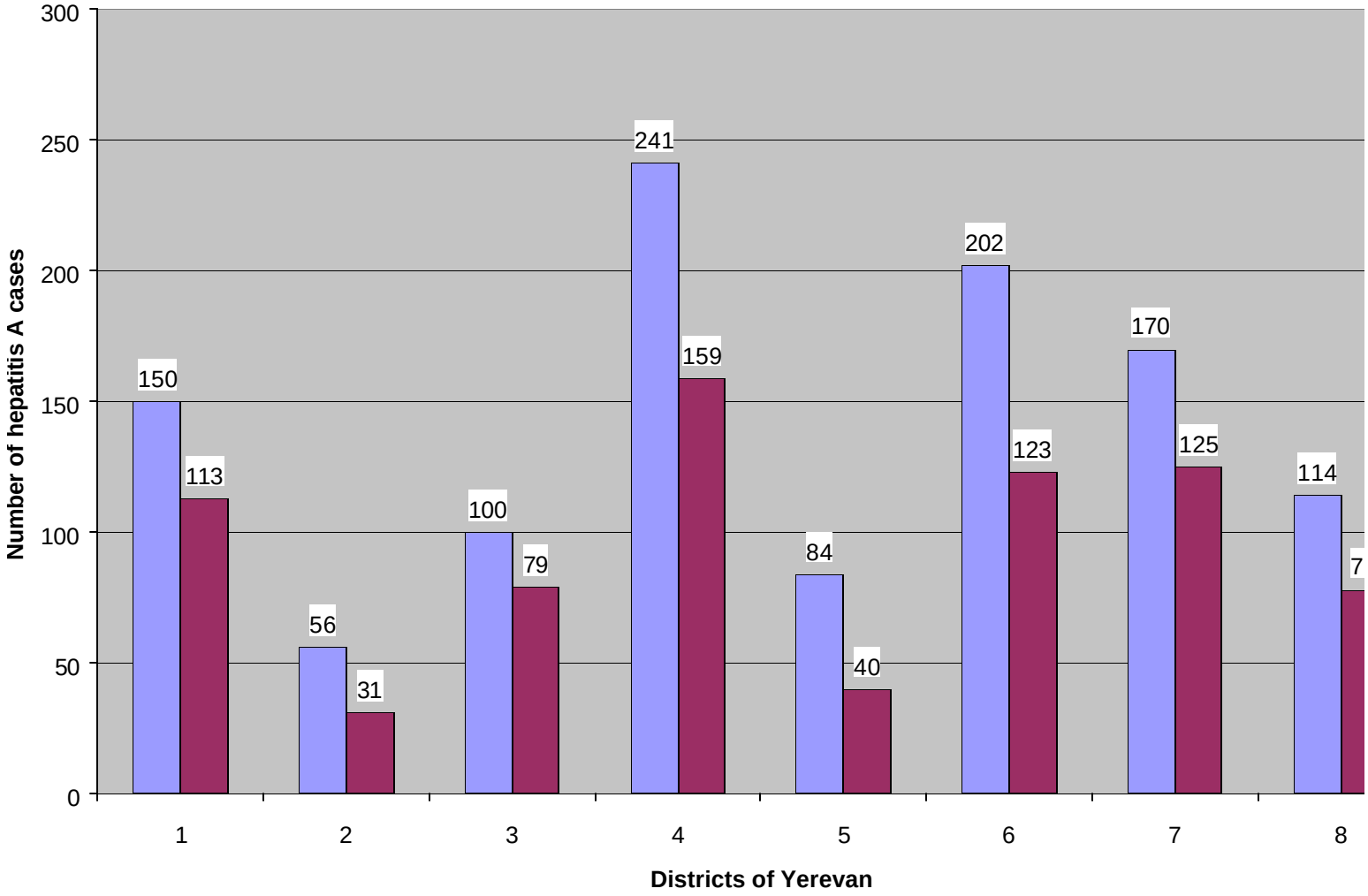
Registered cases of Hepatitis A in 1997 and 1998 years

1998 year

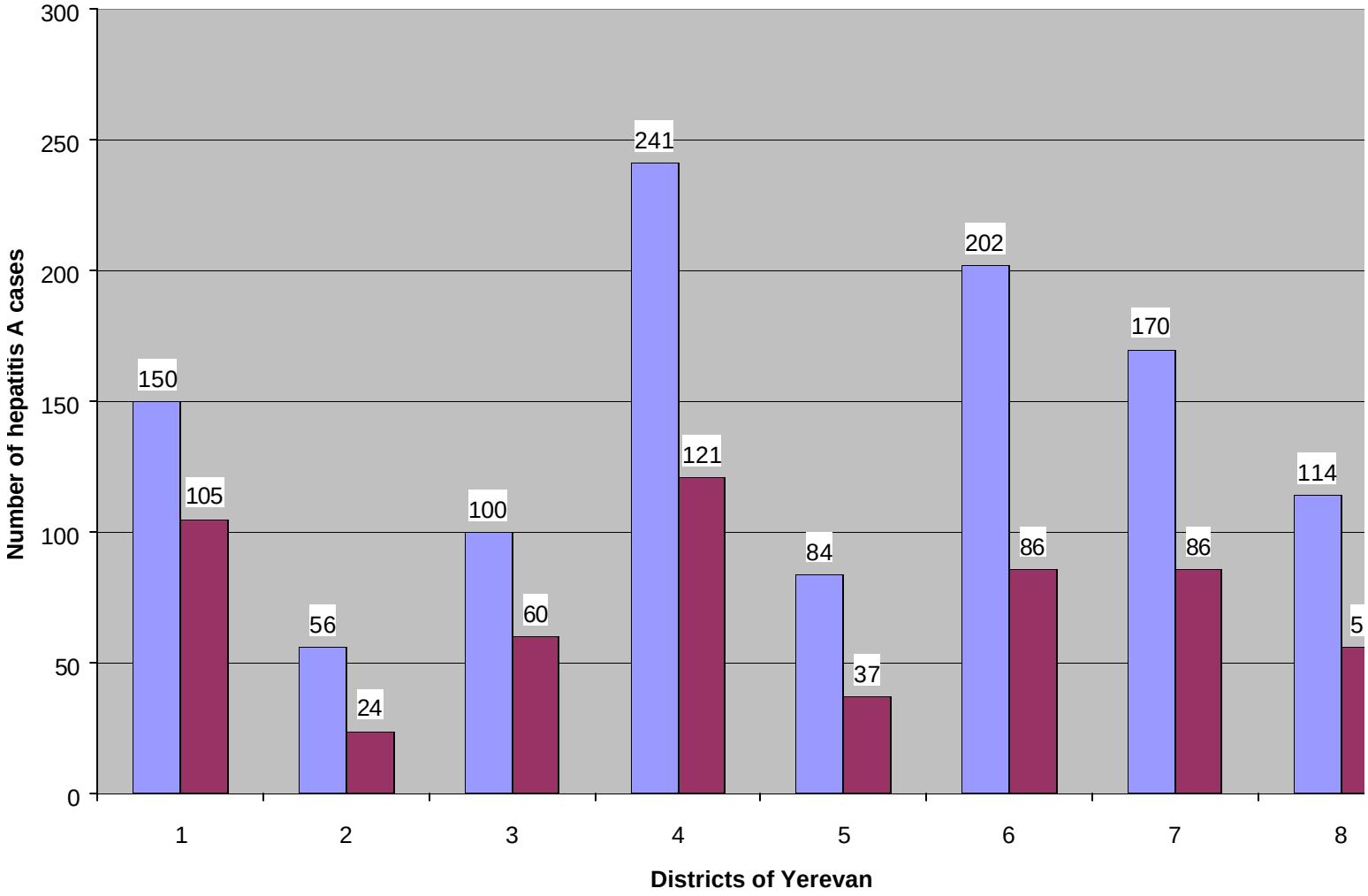
	Hepatitis A			
Towns and reagions of Armenia	Overall #	Before14	3-6	7-13
Yerevan	1117	748	164	717
Shengavit	150	113	7	105
Miasnikian	56	31	7	24
Erebouny	100	79	17	60
Sovietski	241	159	37	121
Spandarian	84	40	3	37
Arabkir	202	123	36	86
Shaumian	170	125	36	86
Mashtotsi	114	78	21	56
Kotaik reagion (the most)	155	139	54	82
Bagramian region (the least)	1	0	0	0
Overall # for the republic	2983	2137	678	1305

	All hepatitis		Hepatitis A	
Towns and regions of Armenia	0-2	3-6	0-2	3-6
Yerevan	12	165	11	156
Shengavit	2	31	2	30
Miasnikian	1	8	1	8
Erebouni	0	12	0	12
Sovetski	5	48	4	43
Spandarian	0	8	0	7
Arabkir	2	34	2	37
Kotaik	2	20	2	17
Bagramian	0	2	0	2
Overall# for republic	56	631	51	617

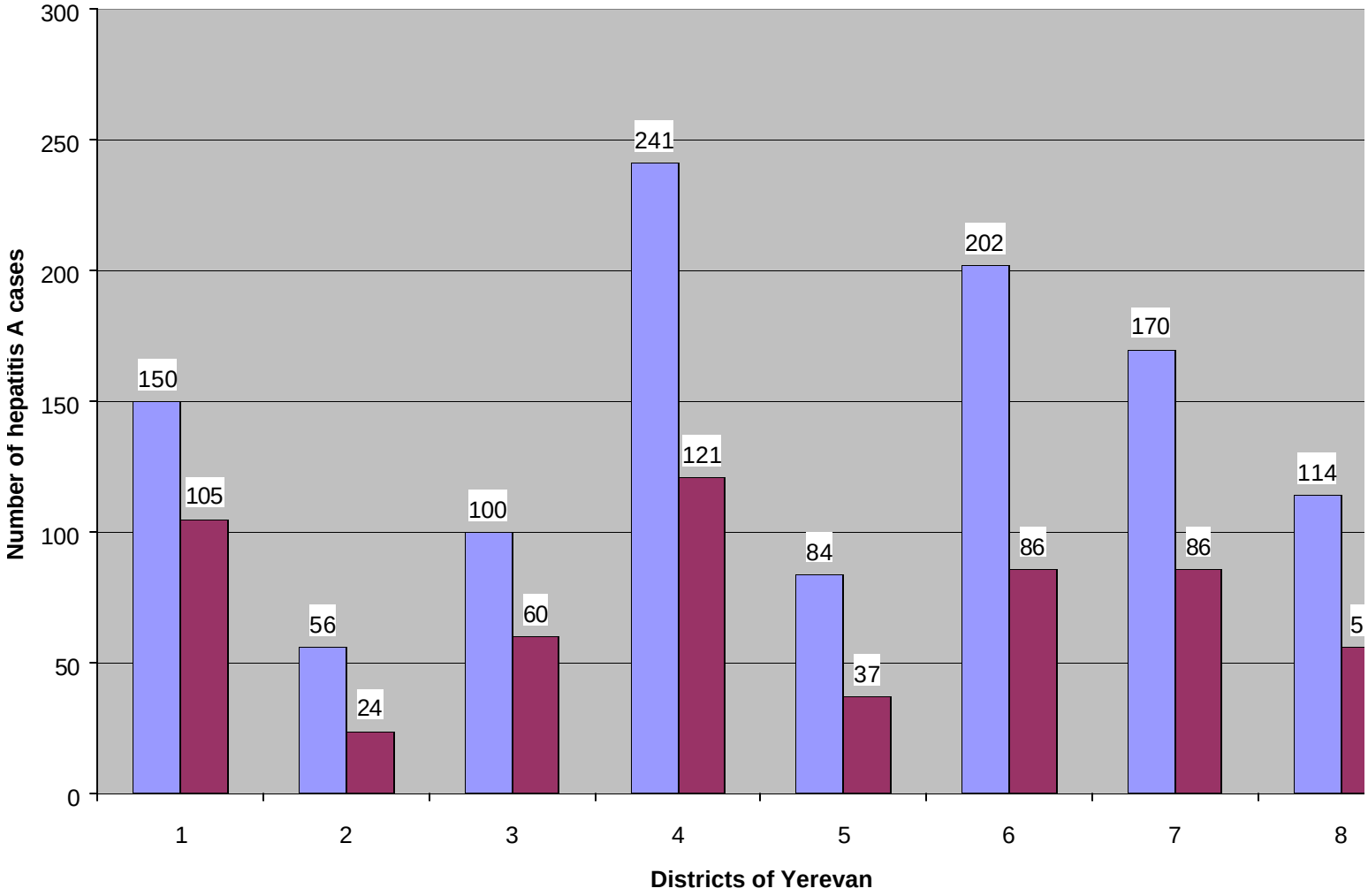
Appendix. The contribution of hepatitis A cases among children before 14 to the overall number of hepatitis A cases in Yerevan



Appendix . The contribution of hepatitis A cases among children aged 7-13 to the overall number of Hepatitis A in Yerevan



Appendix . The contribution of hepatitis A cases among children aged 7-13 to the overall number of Hepatitis A in Yerevan



Appendix #7

Time framework of the project

Month	Office rendering	Equipment installation	Hiring personnel, sharing responsibilities	Sample design	Contacting participants, distribution of questionnaires	Blood tests	Data analysis
January	A	A	A		BCD	BCD	BCDE
Fabular	A	A	A		BCD	BCD	BCDE
March				A	BCD	BCD	BCDE
April				A	BCD	BCD	
May				A	BCD	BCD	
June					ABCD	ABCD	
July					ABCD	ABCD	
August					ABCD	ABCD	
September					ABCD	ABCD	
October					ABCD	ABCD	
November					ABCD	ABCD	
December					ABCD	ABCD	

*A-2000 year; B-2001 year; C-2002 year; D-2003 year; E-2004 year.

Appendix #8

Budget.

Personnel	**2000 year**	**2001 year**	**2002 year**	**2003 year**	**2004 year**
Project Coordinator1@300/mo	**$ 3600**	**$ 3600**	**$ 3600**	**$ 3,600**	**$ 900**
Project Assistants2@200/mo	**$ 2400**	**$ 2400**	**$ 2400**	**$ 2400**	**$ 600**
Office Manager 1@150/mo	**$ 1800**	**$ 1800**	**$ 1800**	**$ 1800**	**$ 450**
Financial Manager1@300/mo	**$3600**	**$3600**	**$3600**	**$3600**	**$ 900**
Technician1@50/mo	**$ 600**	**$ 600**	**$ 600**	**$ 600**	**$ 150**
Total Personnel	**$12000**	**$12000**	**$12000**	**$12000**	**3000**
Consaltation	**$ 2400**	**$ 2400**	**$ 2400**	**$ 2400**	**0**
Operating Cost					
Office rental	**$1200**	**$1200**	**$1200**	**$1200**	**$300**
Transportation					
Car rental and maintenance2@$200/mo/12	**$ 4800**	**$ 4800**	**$ 4800**	**$ 4800**	**$1200**
Fuel 2@$0.4@10lit./day@22@12	**$ 2112**	**$ 2112**	**$ 2112**	**$ 2112**	**$528**
Office supplies (papers, pens, etc.)$100/mo@12	**$1200**	**$1200**	**$1200**	**$1200**	**$300**
Communication $200/mo/12	**$ 2400**	**$ 2400**	**$ 2400**	**$ 2400**	**$600**
Bank charges (1%)	**$120**	**$120**	**$120**	**$120**	**$3**
Total operating cost	**$11832**	**$11832**	**$11832**	**$11832**	**$2931**
Office Equipment					
Computers (Pentium 2) 2@$1000	**$2000**	**0**	**0**	**0**	**0**
Laser Printer 1@$500	**$500**	**0**	**0**	**0**	**0**
Copier 1@$1000	**$1000**	**0**	**0**	**0**	**0**
Total equipment	**$3500**	**0**	**0**	**0**	**0**
Training					**0**
Training in data-base 5days@10/day	**$ 50$**	**0**	**0**	**0**	**0**
Blood Testing					
Testing on IgG 500 @$10	**$ 5000**	**$4750**	**$4250**	**$3500**	**0**

Procurements					
Chemical Assays 1boxes@$200	**$1400**	**$1000**	**$800**	**$600**	**0**
Syringes and needles 1@$0.2@3	**$300**	**$285**	**$255**	**$210**	**0**
Testing procedure supplies	**$1200**	**$1200**	**$1200**	**$1200**	**0**
Total procurement	**$2900**	**$2485**	**$2255**	**$2010**	**0**
Surveys-2(n=250 for 1 survey)					
Interview and filling questioners 1@$10/q.-re	**$5000**	**$4750**	**$4250**	**$3500**	**0**
Incentives 1@$15	**$7500**	**$7125**	**$6375**	**$5250**	**0**
Cost	**$49182**	**$45342**	**$43362**	**$40492**	**$5931**
Indirect cost 12%	**$5902**	**$5441**	**$5203**	**$4859**	**$711**
Overall cost	**$55804**	**$50783**	**$48565**	**$45351**	**$6642**

Appendix #9

Glossary of Terms

A

Acute: of short and sharp duration

Antibodies: substances produces by the body in response to a foreign substance, like disease viruses and bacteria

C

Capsid - [L. *capsa* a box] the shell of protein that protects nucleic acid of virus

Chronic: of long duration

F

Fulminant: occurring with lightning-like speed

H

Hepatic: related to the liver

Hepatocellular: relating to liver cells

I

Immunoglobulin-any of the structurally related glycoproteins that function as antibodies, divided into five classes (IgG, IgA, IgD, and IgE) on the basis of structure and biologic activity

Incubation-the development5 of an infectious disease from the entrance of the pathogen to the appearance of clinical symptoms

J

Jaundice: yellow-staining of some tissues, such as the whites of eyes; symptom of hepatitis

V

Viremia- the presence of viruses in the blood, usually characterized by malaise, fever, and aching of the back and extremities

Virus: an agent of infectious disease capable of growth only by entering and reproducing within living cells

S

Serum: a clear, watery fluid, such as that excluded from inflamed tissues

Serologic test-any laboratory test involving serologic reactions (precipitin reaction, agglutination, complement fixation, etc.),especially any such test measuring serum antibody titer.