

TITLE:

**Hepatitis A seroprevalence in South Africa.
Are we in epidemiological transition?**

Annabel Enoch

University of Cape Town

MMED Medical Virology

University of Cape Town

The copyright of this thesis vests in the author. No quotation from it or information derived from it is to be published without full acknowledgement of the source. The thesis is to be used for private study or non-commercial research purposes only.

Published by the University of Cape Town (UCT) in terms of the non-exclusive license granted to UCT by the author.

Table of Contents

Declaration	3
Abstract	4
Acknowledgements	5
List of Tables	5
List of Figures	5
Abbreviations	6
Chapter 1: Literature review	7-12
References for Chapter 1	13-14
Chapter 2: Abstract	15
Introduction	16
Methods	16-19
Results	19-22
Discussion	23-24
Limitations	24
Conclusion	25
References for chapter 2	26-27
Appendices	28-34

Declaration

I, Annabel Enoch, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

I empower the university to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.


Signed

Signature:

Date: 08/03/2017

Abstract

Hepatitis A virus (HAV) is the most common cause of viral hepatitis worldwide. Infection with HAV is vaccine preventable, however, a vaccine against HAV is not included in the Expanded Programme on Immunization in South Africa (SA). South Africa was considered to be a high endemic country for hepatitis A in the past, hence there was no need for routine immunization against the virus. Our hypothesis is that SA is changing from high to intermediate endemic setting for hepatitis A. To test our hypothesis, we conducted a cross-sectional seroprevalence study in the 1-7 year age group in the Western Cape Province. Our samples for this study were from specimens, collected between August and October 2015, sent for routine diagnosis to referral hospitals in the Western Cape Province. We tested remaining serum of 482 samples sent for routine tests. A Siemens enzyme immunoassay was used to test for hepatitis A antibodies. We also analysed hepatitis A test results from the National Health Laboratory Services (NHLS) Disa*Lab database at Groote Schuur hospital from 2009–2014, as well as hepatitis A surveillance data from the National Institute for Communicable Diseases (NICD) from 2009-2014, to look at the past hepatitis A prevalence trend. Our cross-sectional study showed the seroprevalence to be 44.1% in the 1-7 year age group. The NHLS data showed a seroprevalence of <90% up to age 10 years, indicating intermediate endemicity. The NICD data showed that a substantial number of symptomatic hepatitis A infections occurred in the 7-40 year age group, suggesting an increasing proportion of a susceptible population to HAV infection. Taken together, these results indicate the need for further studies designed to aid the development of vaccination policies against HAV infection in South Africa.

Acknowledgements

I am grateful to God for this opportunity and for the ability to complete the required work.

I would like to thank Benjamin Kagina and Gregory Hussey for the conceptualization of the study, Monique Andersson for her expertise, especially with regards to the choice of the age of the study population. I would like to thank Diana Hardie for her advice regarding the laboratory work, supervision and support throughout the study. I would like to thank all supervisors for their suggestions and guidance in this study.

I acknowledge that this manuscript was written by myself under the supervision of all my supervisors.

List of Tables

Table 1: Participant gender, age groups and results of current study (anti-HAV total antibody).

List of Figures

Figure 1: Phylogenetic tree showing 12 Genera of Picornaviridae.

Figure 2: Clinical and immunological events during HAV infection.

Figure 3: Median age distribution of anti-HAV total antibody positive and negative individuals.

Figure 4: Heat map of the positive NHLS GSH hepatitis A IgG results 2009-2014.

Figure 5: Age distribution of the NICD Western Cape Hepatitis A surveillance data.

Figure 6: NICD hepatitis A IgM Western Cape Province surveillance data.

Abbreviations

HAV: Hepatitis A virus

SA: South Africa

NHLS: National Health Laboratory Services

NICD: National Institute for Communicable Diseases

RNA: Ribonucleic acid

SES: Socioeconomic status

OLT: Orthotopic liver transplant

WHO: World Health Organization

MSM: Men who have sex with men

EPI: Expanded Programme on Immunization

GSH: Groote Schuur Hospital

MENA: Middle East and North Africa region

Chapter one: Literature review

Introduction

Hepatitis A infection is caused by hepatitis A virus, a member of the *Hepatovirus* genus of the family *Picornaviridae*, one of 12 genera (**Figure 1**). Hepatitis A virus (HAV) is a non-enveloped single-stranded positive sense RNA virus (1, 2). HAV infects humans and nonhuman primates like African green monkeys. However the disease is usually milder in nonhuman primates (3).

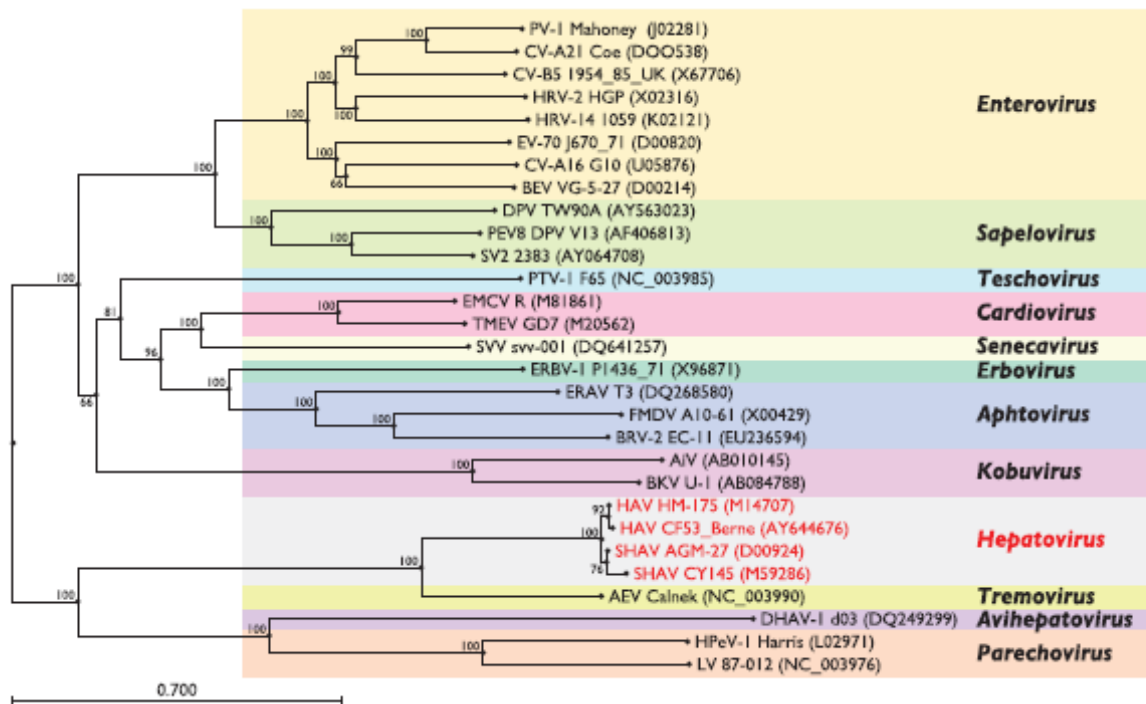


Figure 1. Phylogenetic tree showing 12 Genera of *Picornaviridae*. The figure is from Field's Virology, 6th edition, volume 1, chapter 19, page 551 (Figure 19.1), Permission for use of the figure in the dissertation was granted by Wolters Kluwer Health (Publishers).

Hepatitis A virus is very stable at low pH and is highly thermostable (3). Infectivity can be destroyed at temperatures above 85°C, however HAV infectivity is highly resistant to drying, detergents and organic solvents. In addition HAV can survive for several days to months in contaminated water sources and food (3). There are reports suggesting that even steaming might not be able to destroy HAV, as outbreaks of hepatitis A have been reported following ingestion of partially cooked bivalve molluscs (3).

Hepatitis A virus is the most common cause of acute viral hepatitis worldwide (2). The virus is responsible for approximately 1.4 million new infections each year (4-6). There are many factors influencing the epidemiology of HAV. For example, as countries improve their levels of sanitation and hygiene, the rates of HAV infection decline and the geographic distribution of HAV infection changes (3). Additionally, age, gender, and the illicit use of drugs may play a role in hepatitis A disease severity (1, 3, 7).

Transmission of HAV is via the faecal-oral route, either through an infectious person, or by ingestion of contaminated food or water (1, 8). Sexual contact has also been implicated (3).

Transmission of the virus has also been reported to occur during administration of high purity, solvent-detergent-treated clotting factor concentrates (3). For example, an outbreak of hepatitis A occurred between June 1993 and February 1994 in haemophiliacs in South Africa who received infusions of factor VIII concentrate (9). The factor VIII concentrate was treated with intermediate-purity solvent/detergent method, which does not inactivate non-enveloped viruses (9).

There are several risk factors for HAV infection (1). The risk factors include low socioeconomic status, a larger household size and overcrowding in rural areas, limited access to clean water sources and poor sanitation facilities (4, 7). High risk population groups include those who travel or emigrate from a HAV non-endemic region to an endemic region (1), patients with chronic liver diseases, men who have sex with men (MSM), military recruits, illicit drug users (injecting or noninjecting) and persons with clotting factor disorders (3).

Hepatitis A infection is vaccine preventable. The vaccine against HAV, when administered at least 2 weeks before travel is effective against infection for most travellers. However, among individuals whose immune response to the vaccine may not be adequate, like the immunocompromised, persons with chronic liver disease, adults older than 40 years of age, and who are planning travel to an endemic area, the simultaneous administration of the vaccine with immunoglobulin M (0.02-0.06ml/kg) should provide optimal protection. It is important that separate syringes and different injection sites be used (3).

Infection with HAV occurs at an early age, and close to 100% of children are infected in the first decade of life, in populations with poor or no sanitation and overcrowded living conditions as thought to be in majority of African countries (3). On the other hand, a decrease in hepatitis A seroprevalence has been noted in developing nations that have improved sanitation and hygiene. In settings of high HAV endemicity, improved sanitation and hygiene, coupled with overall economic developments can give rise to a susceptible population and epidemics may occur if the susceptible population has accidental exposure to HAV (3).

Currently, HAV circulates worldwide as a total of 6 genotypes (3, 10). Three genotypes (I, II, III) are divided into subtypes A and B (3, 11, 12). Genotypes I, II, III are human strains (12) with subgenotype IA (10, 11) and genotype III being the most prevalent worldwide (3, 10). Infection with any of the genotypes usually results in lifelong immunity against all strains of hepatitis A. A study conducted in SA between 1982 and 1996 looked at the molecular epidemiology of HAV strains and concluded that SA had predominantly genotype I, with most clustering in subgenotype IB, and the rest in subgenotype IA (13).

In children less than 6 years old, infection with HAV often presents asymptotically and results in lifelong immunity (1, 14). However, in children older than 6 years as well as adolescents and adults, hepatitis A infection can present with jaundice and other potentially severe symptoms (1, 5, 8). A typical course of acute infection has 4 clinical phases: (a) incubation or preclinical period, (b) prodromal or pre-icteric stage, (c) icteric phase, and (d) convalescent period (3). Atypical manifestations of hepatitis A include cholestasis, induction of type 1 autoimmune hepatitis, extrahepatic manifestations and relapsing hepatitis (3). Extrahepatic manifestations are unusual and include arthralgia, arthritis, haemolysis, leukocytoclastic vasculitis, membranoproliferative glomerulonephritis, and pancreatitis. Fulminant hepatitis, caused by severe liver necrosis during acute hepatitis A infection, occurs occasionally, and is characterized by sudden onset high fever, abdominal pain, vomiting, jaundice and the development of encephalopathy. The associated mortality rate is high .

Anti-HAV IgM antibodies are detectable in serum soon after acute infection, and can remain detectable for 6 months or longer in some instances, as indicated in **Figure 2**. IgM reflects

current or recent infection. In contrast, the presence of anti-HAV IgG antibodies in serum confirm past infection or vaccination, and usually indicates lifelong immunity. Serological testing for anti-hepatitis A total antibodies (vaccination, past infection or recent infection), anti-hepatitis A IgM antibodies (recent or acute infection), and anti-hepatitis A IgG antibodies (past infection or vaccination), are available for laboratory diagnosis.

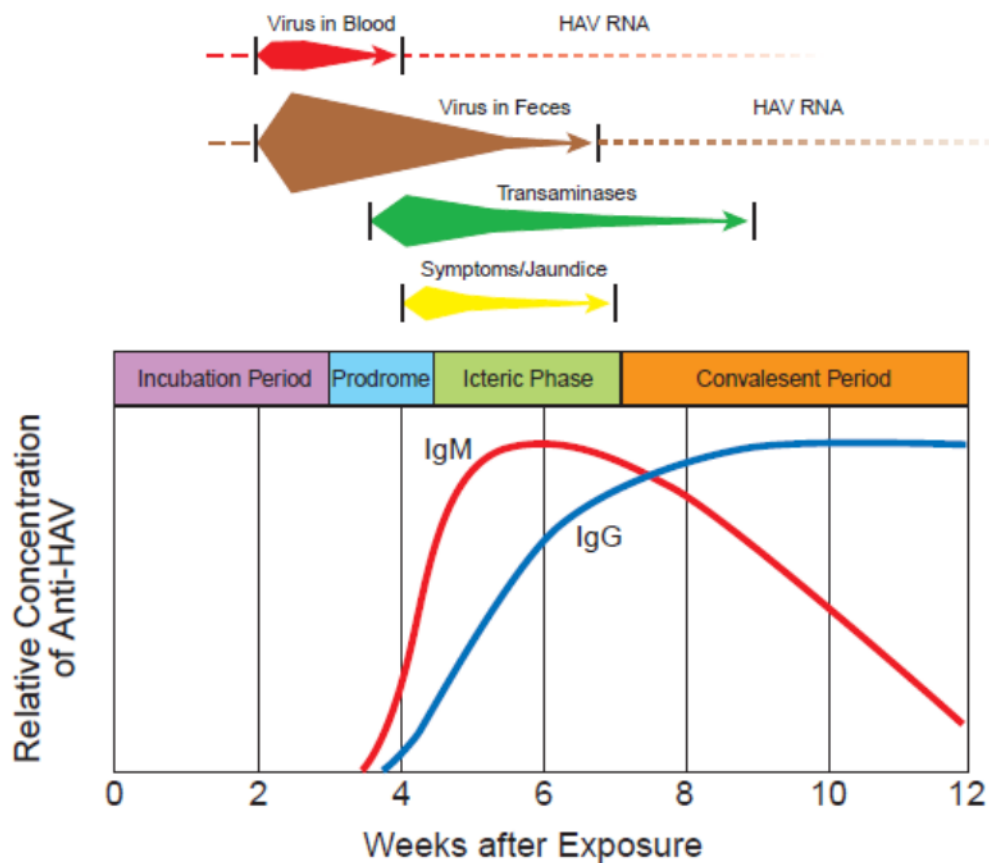


Figure 2. Clinical and immunological events during HAV infection. The figure is adapted from Field's Virology 6th edition, volume 1, chapter 19, and page 563 (figure 19.10). Permission was granted by Wolters Kluwer Health (Publishers).

There is no specific treatment for HAV infection and management usually remains supportive. The most important control measure is proper handwashing to prevent faecal contamination by infected individuals. Management of fulminant hepatitis includes immediate transfer of the patient to a liver transplant facility and aggressive management of complications. This improves the survival rate of the patient. Orthotopic liver transplantation (OLT) or auxiliary partial OLT in severely affected patients with fulminant hepatitis, leads to 1- year survival rates of 80% or more (15).

Acute HAV infection is vaccine preventable (16). There are two types of hepatitis A vaccines currently being used worldwide, formaldehyde inactivated vaccines and live attenuated vaccines (17, 18). The World Health Organization (WHO) 2012 position paper on hepatitis A vaccines states that both the inactivated and live attenuated vaccines are safe and highly immunogenic, and that in most cases these vaccines will generate long-lasting, and possibly

lifelong protection against HAV in both healthy children and adults (18). Childhood vaccination that provides effective long-lasting immunity into adulthood, is recommended in endemic areas, for protection and elimination of HAV in these areas (19). A more than 95% decrease of reported hepatitis A cases were observed in Israel from 1993-2004, and maintained through 2012. This was thought to be due to the universal use of hepatitis A vaccines, and enhancements in childhood immunization schedules (19).

Inactivated hepatitis A vaccines were developed in the early 1990s, and thereafter licensed for use in the mid-1990s (19). There are alum–or influenza virosome-adjuvanted, formaldehyde-inactivated vaccines (Havrix 720, Vaqta, Avaxim, and Epaxal). Both these vaccines are available worldwide. There are also freeze-died, live attenuated vaccines that are used primarily in China, and have recently been introduced into India. Most of the vaccines are produced by growing HAV strains in human diploid fibroblast cell cultures. Purification of the cell extracts is then done, followed by inactivation using formaldehyde, and finally adsorption onto aluminium hydroxide adjuvant (19). HAV-specific antigen has also been combined with other vaccines like hepatitis B vaccine (Twinrix, Twinrix Junior, Ambirix), or typhoid vaccines (ViATIM, Vivaxim, Hepatyrix). The HAV vaccines do not induce antigenic competition with the other vaccines, and the frequency of adverse events is not increased (3).

Two intramuscular injections of the inactivated vaccines are required 6 to 12 months apart (interval can be extended to 36 months), and all the inactivated vaccines display similar immunogenicity profile. However, the WHO suggest that in healthy individuals, comparable effectiveness has been achieved with a single dose. The minimum age of HAV vaccination is one year. According to estimates using kinetic models, protective levels of neutralizing antibodies could persist for 20 years or longer. At present the vaccine is only recommended for persons 1 year of age and older. The bivalent hepatitis A and B vaccine, Twinrix, is recommended for susceptible persons 18 years and older. The live attenuated hepatitis A vaccines used in China over the last decade, have be shown to be safe and immunogenic. Seroprotective levels for more than 10 years have been achieved from a single-dose immunization schedule, and decreased infection rates was observed in the unvaccinated population, indicating herd immunity (3).

A phase 2 follow-up study was recently conducted in Israel to assess the long-term immunogenicity of Epaxal Junior which is a paediatric dose of an aluminium-free inactivated hepatitis A vaccine, in children receiving routine childhood vaccination (19). The formalin-inactivated HAV is associated with immunopotentiating reconstituted influenza virosomes in Epaxal, and is therefore based on a different adjuvant system. The standard doses of Epaxal offers long-term seroprotection which persists for about 30 years. Epaxal Junior is also reported to have satisfactory immune responses and safety profiles in children 1-16 years of age, and in toddlers (19).

For post exposure prophylaxis, the Advisory Committee on Immunization Practices (ACIP) in the US recommends the use of the vaccine and/or immunoglobulin, in the following persons and situations: (a) close personal contact including household and sexual (consider vaccine alone, or vaccine and immunoglobulin if the contact shared illicit drugs with the case), (b) outbreaks in childcare facilities, (c) common-source exposure (food handlers of index worker and patrons if identified and treated within 2 weeks of exposure), (d) outbreaks confirmed in schools, hospitals and work settings (3).

In 1988 in Shanghai, China, an explosive outbreak of hepatitis A infection was reported with over 300,000 cases and 47 deaths. The outbreak was associated with the consumption of raw clams, and again, represents an example of the magnitude of hepatitis A infection in a susceptible population (17).

Infection with HAV late in childhood or in adulthood, may also require hospitalisation, and may cause absenteeism from school or work for prolonged periods of time. There is a rising risk of fulminant hepatic failure, and the case-fatality ratio can reach 2.1% among adults above the age of 40 years (19). In terms of both direct medical costs and loss of productivity, infection at an older age can therefore be expensive (1, 7). A study conducted in Brazil in 2012 used an age and time dependant dynamic model, and concluded that a universal immunization programme for hepatitis A would result in a 64% decrease in the number icteric hepatitis A cases, a 59% reduction in deaths caused by the disease, and a 62% decrease in life years lost, in a national perspective (20).

Countries are classified according to the following levels of hepatitis A endemicity: (21, 22)

1. high ($\geq 90\%$ by age 10 years)
2. intermediate ($\geq 50\%$ by age 15 years, with $< 90\%$ by age 10 years)
3. low ($\geq 50\%$ by age 30 years, with $< 50\%$ by age 15)
4. very low ($< 50\%$ by age 30 years)).

South Africa is classified as a high HAV endemic country. The WHO co-ordinated a systematic review in 2010 (1) and reported the distribution of hepatitis A seroprevalence in SA, classifying the country as a highly endemic one. The WHO systematic review included studies done in South Africa from 1981 – 1996, including those by Taylor *et al.* in 1995 (23), as well as by Abdool Karim in 1993 (24). The WHO systematic review reported differences in hepatitis A seroprevalence between the different population groups in SA. It is very likely the difference in socioeconomic status between these ethnic groups could partly explain the observed variability of hepatitis A seroprevalence.

To the best of our knowledge, the most recent study on Hepatitis A in the Western Cape was published by Solomons *et al.* in 2008 (14). This study was conducted from 2001-2004 at Tygerberg Children's Hospital, and indicated that the median population incidence of serologically proven HAV infection was 45.4/100 000/year (14). This study included only serum IgM positive results. This reported incidence is higher than that recommended as a threshold (20/100 000/year), for introducing universal or targeted vaccination into the immunization schedule (14, 25).

The 2010 WHO review also indicated that sub-Saharan Africa had one of the highest hepatitis A seroprevalence rates in the world. This classified the whole continent as a high hepatitis A endemic region, where nearly all older children and adults tested, showed acquisition of natural immunity against the virus (26).

Currently none of the countries in the WHO Afro region have included HAV vaccine in the routine Expanded Programme on Immunization (EPI) because it is assumed that exposure to, and infection with the virus, is widespread at an earlier stage in life (http://apps.who.int/immunization_monitoring/globalsummary/schedules). However, there are studies reporting a decrease, in exposure and infection at an early age, in some countries within Africa (1, 4, 7, 26).

There are several factors that may contribute to a lower infection rate at an earlier age. Among these factors are: improved socioeconomic status (SES) (7), higher education levels, increased access to clean water and better sanitation (27). For example a study was done to describe hepatitis A outbreak in Roma in north eastern Greece, looking at 124 cases of hepatitis A infection, occurring in three prefectures, between 2 July and 30 November 2007. The study conclusions were that it is probable that Roma communities experienced a relative

improvement in sanitary conditions, and a switch from high to intermediate level of HAV endemicity was made. This could be an explanation for the occurrence of the outbreak that affected mainly the children and adolescents. Following the findings of this study in Greece, vaccination against HAV as a preventative measure was suggested (28).

South Africa (SA), in the last two decades, has made tremendous progress in improving water sources and sanitation (29), although studies conducted in rivers and source water dams in Gauteng from 1997-2000 and the Eastern Cape Province's Buffalo river and three water source dams on its route, revealed a high potential risk of HAV infection to persons using the surface dam and river water for domestic and recreational purposes (30, 31). It is presumed that hepatitis A infection occurs early in life, and therefore a vaccine against hepatitis A infection is not included in the EPI of the country. Thus, hepatitis A associated morbidity and mortality may increase as the age of HAV infection increases (2, 4, 7, 26, 27).

In resource-poor regions, socioeconomic development and improvement in sanitation may lead to a change from high to intermediate HAV endemicity. This is known as "epidemiologic transition" (32). Such a transition paradoxically increases the morbidity and mortality associated with HAV infection. Recently, Melhem *et al* showed that the Middle East and North African countries, similar to many other areas in the world, demonstrate an epidemiological shift in the age of hepatitis A infection, from that of a younger age group to that of an older age group (33). This may be associated with increased outbreaks among the younger more susceptible age groups.

Our hypothesis is that SA is changing from high to intermediate endemic setting for hepatitis A. We tested the hypothesis of our study by conducting a pilot cross-sectional study in the Western Cape Province aimed at assessing the current hepatitis A seroprevalence. We also assessed the trend in hepatitis A seroprevalence in the Western Cape, by analysing Disa*Lab data from the Groote Schuur hospital database, looking at hepatitis A anti-IgG antibody test results from 2009 to 2014. Disa*Lab is a laboratory information management system that was used by the NHLS in the past, based on distributed computer networks. In addition, we analysed surveillance data from the National Institute for Communicable Diseases (NICD), looking at HAV anti-IgM antibody tests from 2009 to 2014.

References

1. The World Health Organization. the Global Prevalence of Hepatitis A Virus Infection and Susceptibility: A systematic review (WHO/IVB/10.01), <http://www.who.int/ivb/documents>; (accessed 17.02.2017)
2. Harrison TJ, Dusheiko GM, Zuckerman AJ. Hepatitis viruses. In: Zuckerman AJ, Banatvala JE, Schoub BD, Griffiths PD, Mortimer P, editors. Principles and Practice of Clinical Virology, 6th ed. Chichester: Wiley Blackwell. p.273-320.
3. Hollinger FB, Emerson SU. Hepatitis A virus. In Knipe DM, Howley PM, Cohen JI, Griffin DE, Lamb RA, Martin MA, Racaniello VR, Roizman B, editors. Fields Virology, 6th ed. Philadelphia: Wolters Kluwer/Lippincott Williams and Wilkins; 2013. Vol 1: p.550-581.
4. Jacobsen KH, Koopman JS. The effects of socioeconomic development on worldwide hepatitis A virus seroprevalence patterns. International journal of epidemiology. 2005;34(3):600-9.
5. Saha S-K. Community-based cross-sectional seroprevalence study of hepatitis A in Bangladesh. World Journal of Gastroenterology. 2009;15(39):4932.
6. Kanyenda TJ, Abdullahi LH, Hussey GD, Kagina BM. Epidemiology of hepatitis A virus in Africa among persons aged 1-10 years: a systematic review protocol. Systematic Reviews. 2015;4:129.
7. Jacobsen KH. Hepatitis A virus in West Africa: Is an epidemiological transition beginning? Nigerian medical journal : Journal of the Nigeria Medical Association. 2014;55(4):279-84.
8. Savage RD, Rosella LC, Brown KA, Khan K, Crowcroft NS. Underreporting of hepatitis A in non-endemic countries: a systematic review and meta-analysis. BioMed Central Infectious Diseases. 2016;16(1):281.
9. Kedda M-A, et al. An outbreak Of Hepatitis A Among South African Patients With Haemophilia: Evidence Implicating Contaminated Factor VIII Concentrate as the Source. Hepatology.1995;22(5):1363-67.
10. Costa-Mattioli M, Di Napoli A, Ferre V, Billaudel S, Perez-Bercoff R, Cristina J. Genetic variability of hepatitis A virus. Journal of General Virology. 2003;84:3191-201.
11. Lu L, Ching KZ, de Paula VS, Nakano T, Siegl G, Weitz M, et al. Characterization of the complete genomic sequence of genotype II hepatitis A virus (CF53/Berne isolate). Journal of General Virology. 2004;85(Pt 10):2943-52.
12. Robertson BH JR, Khanna B, Totsuka A, Nainan OV, Siegl G, et al. Genetic relatedness of hepatitis A strains recovered from different geographical regions. Journal of General Virology. 1992;73(Pt 6):1365-77.
13. Taylor MB. Molecular Epidemiology of South African Strains of Hepatitis A Virus:1982-1996. Journal of Medical Virology. 1997;51:273-79.
14. Solomons RS, Rabie H, Nel ED, Cotton MF. An overview of hepatitis A at Tygerberg Children's Hospital. South African Journal of Child Health. 2008;2(2):43-5.
15. Polson J, Lee WM, American Association for the Study of Liver D. AASLD position paper: the management of acute liver failure. Hepatology. 2005;41(5):1179-97.
16. Lazcano-Ponce E, Conde-Gonzalez C, Rojas R, DeAntonio R, Romano-Mazzotti L, Cervantes Y, et al. Seroprevalence of hepatitis A virus in a cross-sectional study in Mexico: Implications for hepatitis A vaccination. Human vaccines & immunotherapeutics. 2013;9(2):375-81.
17. Verma R, Khanna P. Hepatitis A vaccine should receive priority in National Immunization Schedule in India. Human vaccines & immunotherapeutics. 2014;8(8):1132-4.

18. The World Health Organization. Hepatitis A vaccines-July 2012. WHO position paper. Summary, http://www.who.int/immunization/position_papers/PP_hep_Ajuly2012_summary.pdf ; 2012 (accessed 02.01.2017).
19. Dagan R, Ashkenazi S, Livni G, Go O, Bagchi P, Sarnecki M. Long-term Serologic Follow-up of Children Vaccinated with a Pediatric Formulation of Virosomal Hepatitis A Vaccine Administered With Routine Childhood Vaccines at 12-15 Months of Age. *The Pediatric Infectious Disease Journal*. 2016;35(7):e220-8.
20. Sartori AM, et al. Cost-effectiveness analysis of universal childhood hepatitis A vaccination in Brazil: Regional analyses according to the endemic context. *Vaccine*. 2012;30(2012):7489– 97.
21. Lazcano-Ponce E, Conde-Gonzalez C, Rojas R, DeAntonio R, Romano-Mazzotti L, Cervantes Y, et al. Seroprevalence of hepatitis A virus in a cross-sectional study in Mexico. *Human vaccines & immunotherapeutics*. 2014;9(2):375-81.
22. The World Health Organization. STEPS Sample Size calculator and Sampling Spreadsheet ,<http://www.who.int/chp/steps/resources/sampling/en/> ;2017 (accessed 17.02.2017).
23. Taylor MB, Becker PJ, Van Rensburg EJ, Harris BN, Baily IW, Grabouw WO. A serosurvey of waterborne pathogens amongst canoeists in South Africa. *Epidemiology & Infection*. 1995;115(2):299-307.
24. Abdool Karim SS CA. Sero-epidemiology of hepatitis A in black South African children. *South African Medical Journal*. 1993(83):748-9.
25. Centres for Disease Control and Prevention. Prevention of hepatitis A through active or passive immunization. *Morbidity and Mortality Weekly Report*. 2006;55(RR07):1-23.
26. Jacobsen KH, Wiersma ST. Hepatitis A virus seroprevalence by age and world region, 1990 and 2005. *Vaccine*. 2010;28(41):6653-7.
27. Afegbua SL, Bugaje MA, Ahmad AA. Seroprevalence of hepatitis A virus infection among schoolchildren and adolescents in Kaduna, Nigeria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 2013;107(10):627-30.
28. Vantarakis A et al. An outbreak of hepatitis A in Roma populations living in three prefectures in Greece. *Epidemiology & Infection*. 2010(138(2010)):1025-31.
29. The World Bank Databank. Improved sanitation facilities (% of population with access) for South Africa, <http://www.data.worldbank.org/> ; 2016 (accessed 17.02.2017).
30. Venter JME, van Heerden J, Vivier JC, Grabouw OK, Taylor MB. Hepatitis A virus in surface water in South Africa: what are the risks? *Journal of Water and Health*. 2007(05.2):229-40.
31. Chigor VN, Sibanda T, Okoh AI. Assessment of the risks for human health of adenoviruses, hepatitis A virus, rotaviruses and enteroviruses in the Buffalo River and three source water dams in the Eastern Cape. *Food Environ Virol*. 2014;6(2):87-98.
32. Aggarwal R, Goel A. Hepatitis A: epidemiology in resource-poor countries. *Current Opinion in Infectious Diseases*. 2015;28(5):488-96.
33. Melhem NM, Talhouk R, Rachidi H, Ramia S. Hepatitis A virus in the Middle East and North Africa region: a new challenge. *Journal of Viral Hepatitis*. 2014;21(9):605-15.

Hepatitis A seroprevalence in South Africa. Are we in epidemiological transition?

Annabel Enoch^{a*}, Diana R Hardie^a, Monique I Andersson^b, Gregory D Hussey^c, Benjamin M Kagina^c

^a*University of Cape Town (UCT), Division of Medical Virology, Department of Pathology, Anzio Road, Observatory, Cape Town, South Africa*

^b*Infectious Diseases, Oxford University Hospitals NHS Foundation Trust, Oxford, Oxfordshire, UK*

^c*Vaccine for Africa Initiative (VACFA) and School of Public Health & Family Medicine, University of Cape Town, Anzio Road, Observatory, Cape Town, South Africa*

*Corresponding author.

E-mail address: annabelenoch@gmail.com (A Enoch)

Abstract

Hepatitis A virus (HAV) is the most common cause of viral hepatitis worldwide. Infection with HAV is vaccine preventable, however, a vaccine against HAV is not included in the Expanded Programme on Immunization in South Africa (SA). South Africa was considered to be a high endemic country for hepatitis A in the past, hence there was no need for routine immunization against the virus. Our hypothesis is that SA is changing from high to intermediate endemic setting for hepatitis A. To test our hypothesis, we conducted a cross-sectional seroprevalence study in the 1-7 year age group in the Western Cape Province. Our samples for this study were from specimens, collected between August and October 2015, sent for routine diagnosis to referral hospitals in the Western Cape Province. We tested remaining serum of 482 samples sent for routine tests. A Siemens enzyme immunoassay was used to test for hepatitis A antibodies. We also analysed hepatitis A test results from the National Health Laboratory Services (NHLS) Disa*Lab database at Groote Schuur hospital from 2009–2014, as well as hepatitis A surveillance data from the National Institute for Communicable Diseases (NICD) from 2009-2014, to look at the past hepatitis A prevalence trend. Our cross-sectional study showed the seroprevalence to be 44.1% in the 1-7 year age group. The NHLS data showed a seroprevalence of <90% up to age 10 years, indicating intermediate endemicity. The NICD data showed that a substantial number of symptomatic hepatitis A infections occurred in the 7-40 year age group, suggesting an increasing proportion of a susceptible population to HAV infection. Taken together, these results indicate the need for further studies designed to aid the development of vaccination policies against HAV infection in South Africa.

Keywords: Hepatitis A seroprevalence, incidence ,epidemiological transition, faecal-oral route, liver failure

1. Introduction

Hepatitis A virus (HAV) is responsible for approximately 1.4 million new infections each year (1-3). The virus is a member of the *Hepatitis A virus* genus of the family *Picornaviridae*, and is a non-enveloped single-stranded positive sense RNA virus (4, 5).

Transmission of HAV is via the faecal-oral route, either through an infectious person, or by ingestion of contaminated food or water (4, 6). The risk factors for infection with HAV include low socioeconomic status, a larger household size and overcrowding in rural areas, limited access to clean water sources and sanitation facilities (1, 7).

The geographic distribution of HAV infection is uneven worldwide, and the epidemiology is changing in some regions due to improved sanitation and living standards (8, 9). In modern urban populations a decrease in HAV prevalence has been noted, whereas in populations with poor or no sanitation and overcrowded living conditions infection occurs at an early age. In this context, close to 100% of children acquire infection in the first decade of life (8). In the past it was thought that South Africa was highly endemic for HAV infection, but currently the epidemiology in South Africa is unknown.

Hepatitis A virus infection is vaccine preventable (10). The World Health Organization (WHO) 2012 position paper on hepatitis A vaccines states that both the inactivated and live attenuated vaccines are safe and highly immunogenic, and that in most cases these vaccines will generate long-lasting, and possibly lifelong protection against HAV in both children and adults (10). The WHO recommends vaccination for children ≥ 1 year of age in countries of intermediate or low endemicity. Understanding the epidemiology of the HAV in South Africa is a prerequisite for the development of a vaccination policy for the disease.

South Africa has shown marked improvement in water sources and sanitation facilities (11). We therefore propose that South Africa could be in an epidemiological transition. To investigate this, we performed a pilot cross-sectional seroprevalence study in young children. This study is part of the first author's minor dissertation for a masters' degree in Medical Virology at the University of Cape Town. We chose the Western Cape Province as the study site for convenience.

2. Methods

2.1 Study design

We used a two pronged methodological approach:

- A. We conducted an observational cross-sectional study to examine the seroprevalence of hepatitis A in children between 1 and 7 years of age in the Western Cape Province of South Africa. Serum samples sent for routine diagnostic testing were collected from 3 Western Cape referral laboratories, and were tested for antibodies against HAV. The study population was stratified into 3 age groups: 1-2 years, 3-4 years and 5-7 years.
- B. We also conducted a retrospective review of hepatitis A laboratory results from 2 sources: hepatitis A IgG age specific prevalence from routine diagnostic samples submitted to Groote Schuur Hospital (GSH) National Health Laboratory Service (NHLS) Virology laboratory from 2009-2014, and surveillance data on acute hepatitis A virus infections in the Western Cape Province between 2009 and 2014 collected by the National Institute for Communicable Diseases (NICD).

A. Seroprevalence study:

2.1.1 Sample size

Our hypothesis is that the Hepatitis A infection rates have dropped markedly in young children in the Western Cape due to improvements in hygiene standards and access to clean water. This would be reflected as a reduction in the seroprevalence particularly among the 1-7 year olds. To determine an appropriate sample size that would enable an accurate assessment of hepatitis A seroprevalence in the study population, the following formula was used:

$$Z_{1-\alpha/2}^2 P(1-P) / d^2 \quad (12, 13)$$

$Z_{1-\alpha/2}^2$: is a measure of the level of confidence and describes the level of uncertainty in the sample prevalence as an estimate of the population prevalence. A value of **1.96** which reflects a 95% confidence Interval is recommended.

P: The estimated prevalence within the target population. We hypothesized that an estimated **50%** of our study population would be seropositive for hepatitis A. Previously it was thought that the seroprevalence in South Africa was 90% or more.

d: is the margin of error, which is the half width of the confidence interval. The larger the sample size, the smaller the margin. We used the recommended value of **0.05**.

$$\text{Sample size} = 1.96 \times 1.96 \times 0.5 / 0.05 \times 0.05 = 384 \quad (13)$$

Based on an expected prevalence of about 50% in the study population and a margin of error of 5% (0.05), a sample size of 384 was deemed sufficient.

2.1.2 Sample selection

A total of 508 “left over” serum samples that were sent for routine diagnostic testing, from patients aged 1-7 years old presenting for routine or emergency medical treatment, was collected. Samples from 3 laboratories, collected between August 2015 and October 2015 were tested. The blood samples were drawn at primary and tertiary level health care facilities and then sent to the referral laboratories for the routine tests. Convenient sampling method was used for sample collection from the laboratories. Samples from patients with liver disease were excluded, and the HIV status of the patients as well as ethnicity, was not known.

From these samples, 482 met our study inclusion criteria. We decided to use the 482 samples instead of the calculated 384 to accommodate for any potential equivocal results, as well as to maximise the use of the ELISA plates which could accommodate 92 samples per plate. The sources of the serum samples that were included are as follows: 41 from the Groote Schuur NHLS Virology laboratory, 265 from Red Cross Children’s Hospital and NHLS Chemistry and Haematology laboratories, and 176 samples from Tygerberg Hospital.

On collection, the samples were numbered and stored in a -80°C freezer at the NHLS Virology laboratory in Groote Schuur Hospital. The following demographic details of the patients’ serum samples were also collected: age, gender and residential location or referring clinics. Informed consent was not necessary to obtain as there was no direct contact with the patients, the results of the study did not impact patient treatment and ethics approval was obtained from the University of Cape Town Human Research Ethics and University of Stellenbosch Ethics Committees.

2.1.3 Inclusion criteria

1. Residents of the Western Cape Province (according to the demographic records)
2. 1 – 7 years old
3. Sera with a minimum volume of 100 μ l

2.1.4 Exclusion criteria

1. Samples from patients with documented acute hepatitis infection

2.1.5 Research location

Hepatitis A total antibody testing was performed at the NHLS diagnostic Virology laboratory in Groote Schuur Hospital.

2.1.6 Procedure

Samples were retrieved from the -80°C freezer and thawed at room temperature. Testing was performed according to manufacturer's instructions (14). Serum samples were tested for anti-HAV total antibodies (IgG and IgM), using a manual enzyme immunoassay (Siemens Enzygnost anti-HAV), as per Siemens protocol (14).

This is a competitive assay. The ELISA plate is coated with anti-hepatitis A antibody. 25 μ l of test sample and controls were used. There were 2 incubation steps, and the assay was read within an hour of stopping the reaction. Bio-Tek ELx800 ELISA reader was used and the assay was read at a wavelength of 450nm.

Test samples with equivocal results were retested in duplicate, and remaining samples were stored at -80°C . Anti-HAV total antibody results were entered on a Microsoft Excel spreadsheet, and stored on an access-controlled computer located at the NHLS Virology laboratory in Groote Schuur Hospital.

B. Retrospective review

1. Routinely collected hepatitis A anti-IgG antibody test results were extracted from the Groote Schuur Hospital National Health Laboratory Service (NHLS) database, from January 2009 to December 2014. These results were analysed to provide a retrospective review of the hepatitis A seroprevalence during that period.
2. Also, acute hepatitis A surveillance data (anti-HAV IgM antibody results) routinely collected from public sector hospitals and clinics in the Western Cape Province by the National Institute for Communicable Diseases (NICD) between 2009 and 2014, was analysed.

No patient identifiers were available from the routinely reported data that we used for our retrospective review analysis. Therefore, only basic demographic data for these samples is reported.

2.2. Data analysis

Data from the GSH NHLS laboratory database (anti-HAV IgG results, January 2009 – December 2014), as well as the hepatitis A surveillance data provided by the NICD (anti-HAV IgM results, 2009 – 2014) were analysed using SPSS version 23. SPSS version 23 was also used for the analysis of the current seroprevalence study. Equivocal test results were excluded from analysis.

For both the NHLS and NICD data sets we evaluated the hepatitis A trends from 2009 to 2014. All data was entered on a Microsoft Excel spreadsheet, and stored on an access-controlled computer at Groote Schuur Hospital (NHLS server). No patient identifiers were present, and no direct contact was made with the patients.

2.3. Ethics

Ethics approval was obtained from the University of Cape Town Human Research Ethics and University of Stellenbosch Ethics Committees (for samples collected from Tygerberg Hospital). Permission to conduct a study using samples from Red Cross Children’s Hospital was also obtained.

3. Results

3.1. Seroprevalence study

Our first aim was to establish the seroprevalence of HAV among 1-7 year old children. To address this aim, we tested 482 serum samples that met our inclusion criteria. Of the total samples tested, 231 (47.9%) were females (**Table 1**).

Seroprevalence, defined by the proportion of the serum samples that tested positive for total anti-HAV antibodies, was 44.1% (212 out of 482). As expected, seroprevalence increased with age: 22.2% (45/203) for the 1-2 year old children, 51.7% (31/60) for the 3-4 year old children and 62.4% (136/218) in the 5-7 year old children. One serum sample test was equivocal (male, age 1), and even on repeat testing in duplicate, remained equivocal. This sample was excluded from analysis.

Table 1

Participant gender, age groups and results of current study (anti-HAV total antibody).

Gender and results	1-2 years	3-4 years	5-7 years	Total
Gender				
Female n (%)	91 (44.8)	36 (60)	104 (47.7)	231 (48.0)
Male n (%)	112 (55.2)	24 (40)	114 (52.3)	250 (52.0)
Total samples, n (%)	203 (42.2)	60 (12.5)	218 (45.3)	481 (100)
Results				
Negative, n (%)	158 (77.8)	29 (48.3)	82 (37.6)	269 (55.9)
Positive, n (%)	45 (22.2)	31 (51.7)	136(62.4)	212 (44.1)

Abbreviations: n, number. Fisher’s Exact test showed no significant statistical difference between males and females.

The results obtained from the current study were plotted to show the median age distribution of children who were hepatitis A antibody positive and antibody negative (**Figure 3**). The median age of antibody positive children was 4 years and that of antibody negative children was around 2 years of age.

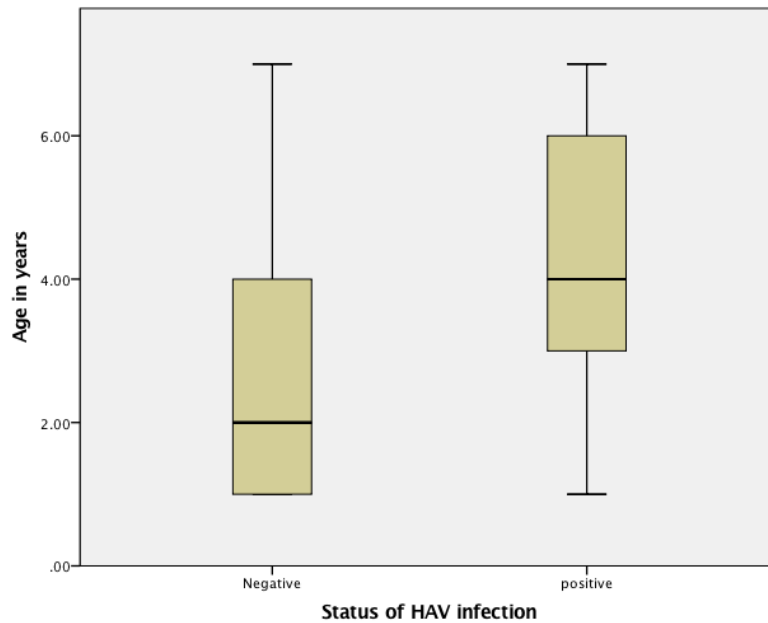


Figure 3: Median age distribution of anti-HAV total antibody positive and negative individuals. The box plot demonstrating the negative and positive results, as well as the median age distribution of the 482 tested samples. The median age of positivity in the sample population is around 4 years of age, and no statistical difference is noted between the positive and negative samples at this age.

3.2. Retrospective study

3.2.1 Hepatitis A seroprevalence from the NHLS GSH laboratory database from 2009-2014

We displayed the NHLS data from GSH as a heat map (**Figure 4**) showing the seroprevalence in small age increments, namely 1-2 years, 3-4 years, 5-7 years, 8-10 years, 11-15 years, 16-20 years, 21-25 years, 26-30 years, 31-35 years, 36-40 years, and >40 years. A 3-colour scale was used: purple-to-white-to-green to indicate increasing seroprevalence. The minimum (lowest value): purple; midpoint (50th percentile): white; maximum (highest value): green. Seropositive percentages < 50% are displayed in white font, as well as percentages $\geq 90\%$. A seroprevalence of $\geq 50\%$ by age 15 years, but < 90% by age 10 years, is noted in all years (intermediate endemicity as defined by the WHO). Of note the seroprevalence was consistently < 90% under the age of 10 for all calendar years. This suggests that hepatitis A is not highly endemic in this population.

Age in years	2009	2010	2011	2012	2013	2014
1-2	56	46	25	47	46	56
3-4	65	56	79	62	56	65
5-7	66	73	78	82	77	73
8-10	72	77	85	74	77	78
11-15	79	81	84	72	80	75
16-20	83	93	84	78	89	75
21-25	77	71	79	85	88	85
26-30	76	81	88	88	83	83
31-35	82	84	83	90	83	90
36-40	85	89	90	91	95	87
> 40	89	90	92	95	95	93

Figure 4. Heat map of the positive NHLS GSH hepatitis A IgG results 2009-2014. The total number of samples tested from 2009 – 2014 was 2456, 2207, 2182, 2287, 2244, 2318 respectively. Pearson Chi Square test showed no statistical difference between samples tested per year. The positive hepatitis A IgG results that were extracted from GSH NHLS database (Disa*Lab) from 2009-2014, are reflected as a percentage and are stratified into the following groups: 1-2 years; 3-4 years; 5-7 years; 8-10 years; 11-15 years, 16-20 years; 21-25 years; 26-30 years, 31-35 years; 36-40 years, and >40 years. A 3 colour scale was used: purple-to-white-to-green. Minimum (lowest value): purple; midpoint (50th percentile): white; maximum (highest value): green. Seropositive percentages < 50% are displayed in white font, as well as percentages ≥ 90%. Of note is the seroprevalence is < 90% by age 10 from 2009-2014.

3.2.2. Review of acute hepatitis A surveillance data from the Western Cape 2009-2014

The results analysed and displayed in **Figure 5** are a summary of the surveillance data provided by the NICD, and reflects the hepatitis A IgM positive tests from 2009–2014, showing a peak increase in positivity of around age 6.

The positive hepatitis A IgM results that were recorded from hospitals in the Western Cape from 2009-2014, provided by the NICD, are also displayed in **Figure 6**. We grouped the results from the patients into 3 categories: 1-6 years, 7-40 years and > 40 years and plotted them according to each calendar year. Across all years, the highest number of clinical hepatitis A cases was in the 7-40 year age group. Cases in the >40 year age group were very few accounting for only 4.2% of the total positives in 2009, 2.5% in 2010, 3% in 2011, 6.5% in 2012, 3.4% in 2013 and 4.7% in 2014.

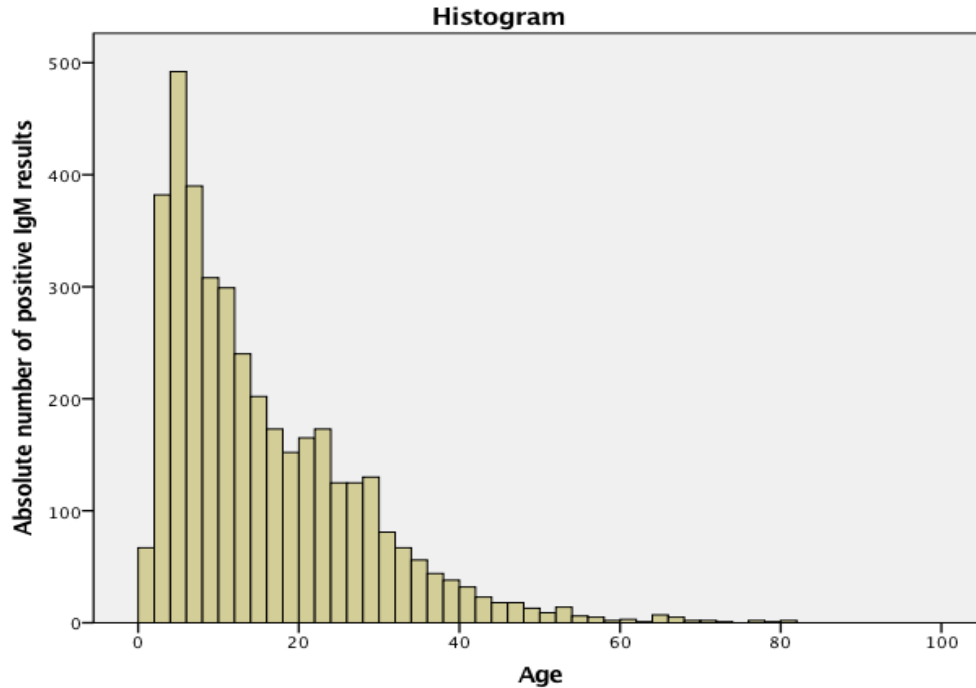


Figure 5. Age distribution of the NICD Western Cape hepatitis A surveillance data. The positive hepatitis A IgM results were provided by the NICD collected from Western Cape clinics and hospitals, and shows an increase in positivity at age 6.

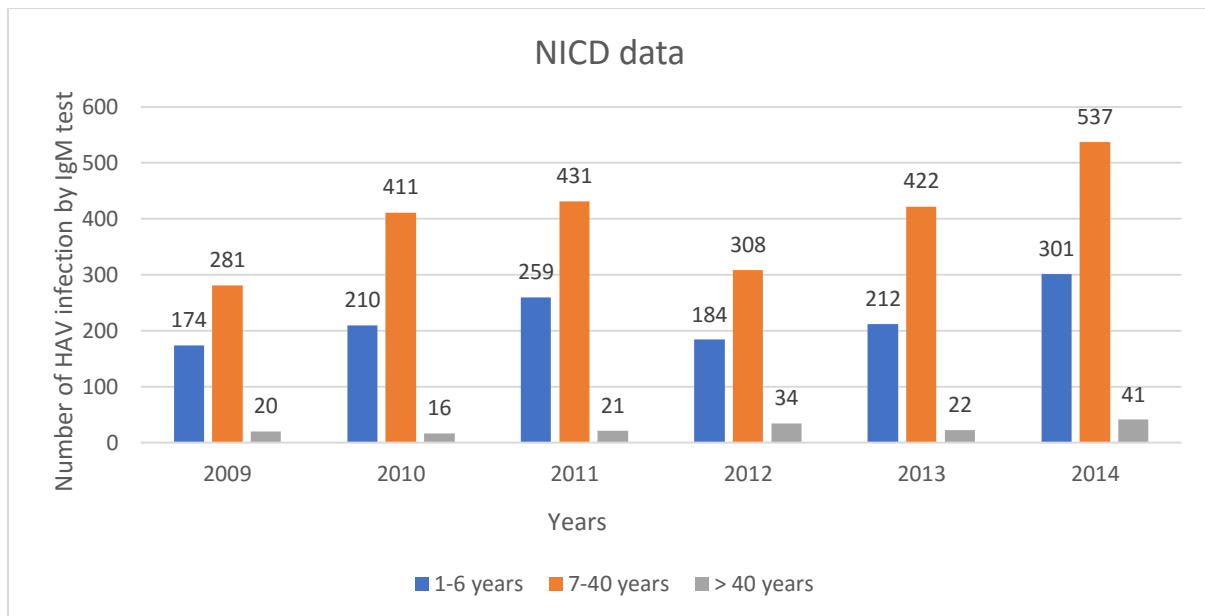


Figure 6. NICD hepatitis A IgM Western Cape Province surveillance data. These are results collected by the NICD from various clinics and hospitals in the Western Cape Province. The results are displayed are absolute numbers of positive tests and not percentages as the total number of tested samples per age group (positive and negative) was not made available. We grouped the results from the patients into 3 groups: 1-6 years, 7-40 years and > 40 years. Cases in the >40 year age group across all years were very few. The highest number of clinical hepatitis A cases was in the 7- 40 year age group from 2009-2014.

3. Discussion

Our cross-sectional study, the samples of which were collected between August and October 2015, indicate a seroprevalence of 44.1% in the 1-6 age group, suggesting that South Africa may be undergoing an epidemiological transition of HAV infection. The retrospective study using the NHLS GSH data also reflects intermediate endemicity, while the analysis of the NICD data displays an increase of symptomatic of hepatitis A infection in the 7-40 year age group. These results indicate a possible epidemiological change of HAV infection. We propose that better designed studies be prioritized in the future to guide the development of vaccination policies against HAV infection in the country.

According to a WHO position paper on hepatitis A vaccines, levels of endemicity are classified as follows: high ($\geq 90\%$ by age 10 years), intermediate ($\geq 50\%$ by age 15 years, with $< 90\%$ by age 10 years), low ($\geq 50\%$ by age 30 years, with $< 50\%$ by age 15) and very low ($< 50\%$ by age 30 years) (15). In the past South Africa was considered to be highly endemic for hepatitis A, as it was thought that most children were exposed to the virus by the age of 6 (4, 16). Our cross-sectional data suggest otherwise as we showed a seroprevalence of 44% among 1-7 years old.

Our results indicating intermediate HAV endemicity could be explained by a few factors. First, South Africa has shown marked improvement in water sources and sanitation facilities in the last two and a half decades (11). Second, there has been socioeconomic development in resource-poor regions, and improved hygiene. Together, these factors, may lead to change from a high HAV endemicity pattern to an intermediate endemicity pattern, and this is known as “epidemiologic transition” (17). This is because hepatitis A is enterically transmitted and as hygiene improves, children can be expected to be exposed less frequently, and acquire infection at a later age. Thus the seroprevalence, which reflects past infection, declines in young children.

For our cross-sectional study we tested children attending public health facilities in the Cape Town area. We found the seroprevalence to be 44.1% in the total study population, ages 1-7. This shows a shift from the previously high seroprevalence (almost 100%) reflected in the studies done (4, 16) in the past, however these study populations may not be directly comparable. The 1993 study conducted by Abdool Karim looked at seroprevalence in Durban, limited to the black urban population from newborn to 13 years of age and the results showed that the seroprevalence in this age group in SA was almost 100% (by age 6).

More than 50% were already infected by the age of 4 in our cross-sectional study, which indicates that early hepatitis A infection remains high, and is likely to be higher still in more rural environments. More studies need to be conducted in the more rural areas, and should be extended to the rest of the country to correctly describe the seroprevalence.

We were not privy to the socio-economic status of the subjects tested or their accessibility to clean water sources. However, our selected population may well be representative of an “average SA household”. According to the data available on the World Bank, in 1990 only 38% of the rural population in South Africa had access to sanitation facilities, and this improved to 61% by 2015. In contrast 64% of the urban population had access to sanitation facilities in 1990, and this increased to 70% by 2015 (11). Access to clean water also varies: in 1990, 66% of the rural population had access to water sources and this increased to 81% by 2015, as opposed to 98% of the urban population in 1990, and 100% by 2015 (11).

The NHLS data from GSH reflected a seroprevalence of $< 90\%$ by age 10, indicating intermediate endemicity from as early as 2009. There was minimal variation through all the

age groups from 2009-2014. Our analysis of the NICD data on acute hepatitis A virus infections revealed an increased number of symptomatic infections in the 7-40 year group, however the peak age of symptomatic infection is seen at age 6. This demonstrates that adolescents are particularly vulnerable to symptomatic hepatitis A in the Western Cape.

A similar trend was noted in a recent systematic review of hepatitis A seroprevalence in Middle East and North Africa region (MENA) (18). One of the studies mentioned in this review, was conducted in Saudi Arabia and demonstrated a decline in HAV prevalence reported among Saudi children and adolescents over a 20 year period, from 52% in 1989 to 25% in 1997 to 18.6% in 2008. In another study, Memish et al looked at trends in Saudi Arabia from 2000-2007, and reported 20-30% declining trends. HAV incidence decreased by 42% in the < 15 year olds and increased by 61% in the > 15 year olds (19). Another study mentioned in the systematic review looking at seroprevalence in MENA countries was conducted in Tunisia (2000-2007), where the prevalence was documented as follows: 91.9% and 80.6% in 2000 and 2007 among the 18-20 year olds, versus seroprevalence of 99% and 92% in adults > 26 years of age (20). This study highlighted the susceptibility of adolescents and young adults to HAV. Recent epidemiological studies in India have also shown a decline in hepatitis A seroprevalence among younger children and an increasing susceptibility in the older children and young adults (21), a trend we observed in our study.

A possible explanation for the higher seroprevalence displayed in the NHLS data from GSH as compared to our cross-sectional study could be that the NHLS data contained results from individuals that were being screened for hepatitis A immunization status, for example children who were vaccinated and awaiting liver transplants. This could have inflated the seroprevalence in this age group. Nonetheless the NHLS data also revealed that the seroprevalence was < 90% by age 10, and \geq 50% by age 15 from 2009-2014, which fits the definition of intermediate endemicity as defined by the WHO (15).

The observed increase in average age of infection is a concern because it is associated with increased morbidity and mortality due to hepatitis A (17, 18, 22). Because of this the WHO recommends integration of the hepatitis A vaccine into the national immunization schedule for children \geq 1 year of age in counties of intermediate or low endemicity. It is an expensive vaccine and it is not cost effective in areas of high endemicity (15). We propose that the younger susceptible population may benefit from the integration of the hepatitis A vaccine into the national vaccine schedule in South Africa.

5. Limitations

There were study limitations, particularly with the retrospective NHLS data as well as the NICD surveillance data. The retrospective data only included samples that were sent to the laboratory for testing, and therefore does not represent the population as a whole. Many of the patients from the retrospective data were also being actively screened for viral hepatitis. The results from the current study also only include samples sent to the laboratory for routine testing from an urban setting in the Western Cape Province, and therefore does not represent the population as a whole. The samples that were tested for hepatitis A total antibodies only represent the public sector in South Africa, as we did not obtain samples from the private laboratories. We did not have data regarding the socioeconomic conditions of the patients whose sera were used in the current study. More studies need to be conducted especially in the rural areas.

6. Conclusion

The results obtained from the current study reflect a hepatitis A seroprevalence of 44% in the 1-7 year old population. The NHLS data reflects intermediate endemicity from 2009-2014. The surveillance (NICD) data reflects that most of the symptomatic hepatitis A infections occurred in the 7-40 year age group in the Western Cape Province. This pilot study highlights the need for further studies to determine whether a change regards the hepatitis A vaccination policy is required. These studies should be extended to the rest of the country, including rural areas.

Conflict of interest

The authors have no financial or other interests regards the submitted manuscript.

Financial support

The project was funded by the Poliomyelitis Research Foundation (PRF) as well as the National Health Laboratory Services (NHLS) Research Trust. The sponsors of the project played no role in the study design, data collection, data analysis or writing of the manuscript.

Author's contributions

GDH and BMK conceptualised and designed the study. DRH supervised the laboratory work. AE wrote the study protocol, conducted the sample collection, laboratory assays, data analysis and drafted the manuscript under supervision from GDH, DRH, MIA and BMK. All authors provided comments to the draft manuscript, and approved the final version of the manuscript.

Acknowledgements

We thank the NICD for the surveillance data provided. We express gratitude to the laboratory managers of the NHLS Tygerberg Hospital chemistry and the NHLS Red Cross Children's Hospital chemistry and haematology laboratories. We are grateful to Red Cross Children's Hospital for the permission granted to conduct the study.

References

1. Jacobsen KH, Koopman JS. The effects of socioeconomic development on worldwide hepatitis A virus seroprevalence patterns. *International journal of epidemiology*. 2005;34(3):600-9.
2. Saha S-K. Community-based cross-sectional seroprevalence study of hepatitis A in Bangladesh. *World Journal of Gastroenterology*. 2009;15(39):4932.
3. Kanyenda TJ, Abdullahi LH, Hussey GD, Kagina BM. Epidemiology of hepatitis A virus in Africa among persons aged 1-10 years: a systematic review protocol. *Systematic Reviews*. 2015;4:129.
4. The World Health Organization. the Global Prevalence of Hepatitis A Virus Infection and Susceptibility: A systematic review (WHO/IVB/10.01), <http://www.who.int/ivb/documents>; (accessed 17.02.2017).
5. Harrison TJ, Dusheiko GM, Zuckerman AJ. Hepatitis viruses. In: Zuckerman AJ, Banatvala JE, Schoub BD, Griffiths PD, Mortimer P, editors. *Principles and Practice of Clinical Virology*, 6th ed. Chichester: Wiley Blackwell. p.273-320.
6. Savage RD, Rosella LC, Brown KA, Khan K, Crowcroft NS. Underreporting of hepatitis A in non-endemic countries: a systematic review and meta-analysis. *BioMed Central Infectious Diseases*. 2016;16(1):281.
7. Jacobsen KH. Hepatitis A virus in West Africa: Is an epidemiological transition beginning? *Nigerian medical journal : Journal of the Nigerian Medical Association*. 2014;55(4):279-84.
8. Hollinger FB, Emerson SU. Hepatitis A virus. In Knipe DM, Howley PM, Cohen JI, Griffin DE, Lamb RA, Martin MA, Racaniello VR, Roizman B, editors. *Fields Virology*, 6th ed. Philadelphia: Wolters Kluwer/Lippincott Williams and Wilkins; 2013. Vol 1: p.550-581.
9. Kim YJ, Lee HS. Increasing incidence of hepatitis A in Korean adults. *Intervirolgy*. 2010;53(1):10-4.
10. The World Health Organization. Hepatitis A vaccines-July 2012. WHO position paper. Summary. http://www.who.int/immunization/position_papers/PP_hep_A_July2012_summary.pdf ; 2012 (Accessed 02.01.2017).
11. The World Bank DataBank. Improved sanitation facilities (% of population with access) for South Africa, <http://www.data.worldbank.org/> ; 2016 (Accessed 17.02.2017).
12. The World Health Organization. STEPS Sample Size calculator and sampling Spreadsheet, <http://www.who.int/chp/steps/resources/sampling/en/>; 2017 (Accessed 17.0.2017).
13. Charan J, Biswas T. How to calculate sample size for different study designs in medical research? *Indian Journal of Psychological Medicine*. 2013;35(2):121-6.
14. Siemens. Enzygnost Anti-HAV package insert. 2013.
15. The World Health Organization. Weekly epidemiological record. WHO position paper on hepatitis A vaccines-June 2012, http://www.who.int/wer/2012/wer8728_29.pdf ; 2012 (Accessed 16.02.2017).
16. Abdool Karim SS CA. Sero-epidemiology of hepatitis A in black South African children. *South African Medical Journal*. 1993(83):748-9.
17. Aggarwal R, Goel A. Hepatitis A: epidemiology in resource-poor countries. *Current Opinion in Infectious Diseases*. 2015;28(5):488-96.
18. Melhem NM, Talhouk R, Rachidi H, Ramia S. Hepatitis A virus in the Middle East and North Africa region: a new challenge. *Journal of Viral Hepatitis*. 2014;21(9):605-15.
19. Memish ZA, Knawy BA, El-Saed A. Incidence trends of viral hepatitis A, B, and C seropositivity over eight years of surveillance in Saudi Arabia. *International Journal of Infectious Diseases*. 2010;14(2):e115-20.

20. Louati N, et al. Comparison of hepatitis A seroprevalence in Saudi Arabia. *International Journal of Infectious Diseases*. 2009;14:115-20.
21. Mathur P AN. Epidemiological transition of hepatitis A in India: issues for vaccination in developing countries. *Indian Journal of Medical Research*. 2008(128(6)):699-704.
22. Abraham P. Viral hepatitis in India. *Clinica in Laboratory Medicine*. 2012;32(2):159-74.

Appendices

Vaccine Author information pack

UCT Ethics letter

UCT protocol amendment

SUN ethics letter

Red Cross Children's Hospital permission letter

Wolters Kluwer letter of permission



VACCINE

The official journal of: [The Edward Jenner Society](#) and [The Japanese Society for Vaccinology](#).

AUTHOR INFORMATION PACK

TABLE OF CONTENTS

• Description	p.1
• Audience	p.1
• Impact Factor	p.1
• Abstracting and Indexing	p.2
• Editorial Board	p.2
• Guide for Authors	p.5



ISSN: 0264-410X

DESCRIPTION

Vaccine is the pre-eminent journal for those interested in **vaccines** and **vaccination**. **Submissions** to the following categories are welcomed: **Human Vaccines** - infectious diseases Human Vaccines - non-infectious diseases **Veterinary Vaccines Immunology** and Animal Models Vectors, Adjuvants and Delivery Systems Production, manufacturing Vaccine **Safety Regulatory, Societal and Legislation Aspects History of Vaccinology** For more specifics please go to [Article Type - Guidelines](#)

Benefits to authors

We also provide many author benefits, such as free PDFs, a liberal copyright policy, special discounts on Elsevier publications and much more. Please click here for more information on our [author services](#).

Please see our [Guide for Authors](#) for information on article submission. If you require any further information or help, please visit our [Support Center](#)

AUDIENCE

Research workers, product developers, clinicians and practitioners with interests in virology, bacteriology, parasitology, mycology, immunology, genetics, biotechnology and biochemistry in the medical and veterinary fields.

IMPACT FACTOR

2015: 3.413 © Thomson Reuters Journal Citation Reports 2016



UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee



Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492 • Facsimile [021] 406 6411
Email: Sumayah.ariefdien@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

04 June 2015

HREC/REF: 227/2015

Prof G Hussey
Vaccines for Africa Initiative (VACFA)
IDM
WBN Room N2.09A
FHS

Dear Prof Hussey

**Project Title: HEPATITIS A SEROPREVALENCE IN SOUTH AFRICA. ARE WE IN
EPIDEMIOLOGICAL TRANSITION? (Dr A Enoch)**

Thank you for your response letter dated 01 June 2015, addressing the issues raised by the Human Research Ethics Committee (HREC).

It is a pleasure to inform you that the HREC has **formally approved** the above mentioned study.

Approval is granted for one year until the 30 June 2016.

Please submit a progress form, using the standardised Annual Report Form, if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.

We acknowledge that the following student:-Dr Annabel Enoch is also involved in this project.

Please note that the on-going ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC REF in all your correspondence.

Yours sincerely

Signed

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS

Federal Wide Assurance Number: FWA00001637.

Hrec/ref: 227/2015



Form FHS006: Protocol Amendment

HREC office use only (FWA00001637; IRB00001938)		
<input checked="" type="checkbox"/> Approved	<input checked="" type="checkbox"/> Type of review: Expedited	<input type="checkbox"/> Full committee
This serves as notification that all changes and documentation described below are approved.		
Signature Chairperson of the HREC	Signed	Date 30/6/2015

Note: All major amendments should include a **PI Synopsis** justifying the changes for the amendment (please see notice dated 23 April 2012)

Comments to PI from the HREC

RESEARCH ETHICS COMMITTEE

2015 -06- 26
 HEALTH SCIENCES FACULTY
 UNIVERSITY OF CAPE TOWN

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	2015/06/26	
HREC REF Number	HREC/REF: 227/2015	
Protocol title	Hepatitis A Seroprevalence in South Africa. Are we in epidemiological transition?	
Protocol number (if applicable)		
Principal Investigator	Prof. Gregory Hussey	
Department / Office Internal Mail Address	VACFA IDM Room N2.09A FHS	
1.1 Is this a major or a minor amendment? (see FHS006hlp) Major (tick box) Minor (tick box)	<input type="checkbox"/> Major	<input checked="" type="checkbox"/> Minor
1.2 Does this protocol receive US Federal funding?	<input type="checkbox"/> Yes	<input checked="" type="checkbox"/> No
1.3 If the amendment is a major amendment <u>and</u> receives US Federal Funding, does the amendment require full committee approval?	<input type="checkbox"/> Yes	<input checked="" type="checkbox"/> No



UNIVERSITEIT • STELLENBOSCH • UNIVERSITY
jou kennisvenoot • your knowledge partner

Ethics Letter

26-Aug-2015

Ethics Reference #: S15/08/173

Clinical Trial Reference #:

Title: Hepatitis A seroprevalence in South Africa. Are we in epidemiological transition?

Dear Dr Annabel Enoch,

We acknowledge receipt of documents pertaining to the above study and the approval letter from the UCT Health Sciences REC for this project.

The approval of the UCT HREC is recognised by the Stellenbosch University HREC for this particular project. Review by the Stellenbosch University HREC is therefore not required.

However please continue to keep us informed of the progress of the project, by submitting annual progress reports.

If you have any queries or need further assistance, please contact the HREC Office 0219399657.

Sincerely,

REC Coordinator
Franklin Weber
Health Research Ethics Committee 1



Dr R Mistry
Manager: Medical Services
Email: Roshni.Mistry@westerncape.gov.za
Tel: +27 21 658 5788 fax: +27 21 658 5166

Dr A Enock
Red Cross War Memorial Children's Hospital

Dear Dr A Enock

APPROVAL OF RESEARCH

PROJECT TITLE: HEPATITIS A SEROPREVALENCE IN SOUTH AFRICA. ARE WE IN EPIDEMIOLOGICAL TRANSITION ?

We have the pleasure of informing you that approval is hereby granted to conduct the above-mentioned study at Red Cross War Memorial Children's Hospital.

Yours sincerely,

Signed

DR ROSHNI MISTRY
MANAGER: MEDICAL SERVICES
DATE: 03 AUGUST 2015

**WOLTERS KLUWER HEALTH LICENSE
TERMS AND CONDITIONS**

Jul 28, 2016

This Agreement between Annabel Enoch ("You") and Wolters Kluwer Health ("Wolters Kluwer Health") consists of your license details and the terms and conditions provided by Wolters Kluwer Health and Copyright Clearance Center.

License Number	3917601308091
License date	Jul 28, 2016
Licensed Content Publisher	Wolters Kluwer Health
Licensed Content Publication	WK Health Book
Licensed Content Title	Fields Virology
Licensed Content Author	David M. Knipe, Peter Howley,
Licensed Content Date	2013
Type of Use	Dissertation/Thesis
Requestor type	academic/educational
Format	print and electronic
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	2
The ID numbers of the figures/tables/illustrations are...	Figure 19.1. and Figure 19.10.
Will you be translating?	no
Reusing current or a previous edition	current edition
Circulation/distribution	50
Order reference number	
Title of your thesis / dissertation	Hepatitis A seroprevalence in South Africa. Are we in epidemiological transition?
Expected completion date	Sep 2016
Estimated size (number of pages)	20
Requestor Location	Annabel Enoch No 8 La Casa 10 Rosmead Avenue Claremont Cape Town, 7708 South Africa Attn: Annabel Enoch
Billing Type	Invoice
Billing Address	Annabel Enoch No 8 La Casa 10 Rosmead Avenue Claremont Cape Town, South Africa 7708 Attn: Annabel Enoch
Total	0.00 USD
Terms and Conditions	