

Available online at www.ujpronline.com Universal Journal of Pharmaceutical Research An International Peer Reviewed Journal ISSN: 2831-5235 (Print); 2456-8058 (Electronic)

Copyright©2023; The Author(s): This is an open-access article distributed under the terms of the CC BY-NC 4.0 which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited



RESEARCH ARTICLE

SEROPREVALENCE OF *HELICOBACTER PYLORI* AND HEPATITIS A VIRUS AMONG ORPHANAGE CHILDREN IN SANA'A, YEMEN

Wadhah Hassan Edrees^{1,2,3,4}, Nasser Mohammad Al-Aomary⁵, Lutf Mohammed Alrahabi⁶, Jameela Mohemmed Thabit³, Hamzah Muhammed Ali³, Aseel Qasim Saran³, Abdullah Abdul Karim Saleh³, Eyad Mohammed Al-shaouri³, Mohammed Taleb Al-Nahdi³,

Fadel Ahmed Dibwan³

¹Medical Microbiology Department, Faculty of Applied Sciences, Hajjah University, Yemen.
 ²Medical Laboratory Department, Faculty of Medical Sciences, Al-Razi University, Yemen.
 ³Medical Laboratory Department, Faculty of Medical Sciences, Queen Arwa University, Yemen.
 ⁴Yemen Foundation for Research and Health Development, Food and Drug Researches & Studies Center, Yemen.
 ⁵Medical Microbiology and Immunology Department, Faculty of Medical Sciences, Queen Arwa University, Yemen.
 ⁶Medical Laboratory Department, Faculty of Medical Sciences, Queen Arwa University, Yemen.

Article Info:



Article History: Received: 9 February 2023 Reviewed: 13 March 2023 Accepted: 27 April 2023 Published: 15 May 2023

Cite this article:

Edrees WH, Al-Aomary NM, Alrahabi LM, Thabit JM, Ali HM, Saran AQ, Saleh AAK, Alshaouri EM, Al-Nahdi MT, Dibwan FA. Seroprevalence of *Helicobacter pylori* and Hepatitis A virus among orphanage children in Sana'a, Yemen. Universal Journal of Pharmaceutical Research 2023; 8(2):53-60. https://doi.org/10.22270/ujpr.v8i2.927

*Address for Correspondence:

Dr. Wadhah Hassan Edrees, Medical Microbiology Department, Faculty of Applied Sciences, Hajjah University, Yemen. Medical Laboratory Department, Faculty of Medical Sciences, Al-Razi University, Yemen. Medical Laboratory Department, Faculty of Medical Sciences, Queen Arwa University, Yemen. Yemen Foundation for Research and Health Development, Food and Drug Researches & Studies Center, Yemen.Tel: +967-778555695; E-mail: wadah.edrees@alraziuni.edu.ye

Background: The epidemiological association between *Helicobacter pylori* (*H. pylori*) and hepatitis A virus (HAV) has been evaluated by different groups with conflicting conclusions.

Aims: The aim of this study was to determine the prevalence of hepatitis A virus and *H. pylori* infection among children of an orphanage in Sana'a, and to identify the socio-demographic factors associated with their prevalence, both individually and simultaneously.

Methods: This is a cross-sectional study carried out among 200 orphaned children who lived at Ahmed's orphanage in Sana'a City, Yemen during the period from October 2022 to February 2023. The stool and blood specimens were collected and the required data were filled in pretested questionnaire. By using the immunochromatographic assay technique, the seropositivity of the HAV antibody was screened in blood plasma while the *H. pylori* antigen was detected in stool. The obtained results were analyzed using SPSS software.

Results: Out of 200 participants; 2(1%) were positive for anti-HAV and 63(31.5%) for *H. pylori* antigen. The HAV and *H. pylori* infections, respectively, were significantly higher in the group aged 10-12 and 13-15 years, attending a primary and preparatory school, living with 3-5 individuals/room, their family infected with hepatitis A and *H. pylori*, and received a blood transfusion. The high risk of hepatitis A infection was in children who their family was infected by HAV and received a blood transfusion while *H. pylori* was in subjects who had a history of hospitalizations and whose family infected by *H. pylori*.

Conclusion: The high number of children suffering from both *H. pylori* and HAV antibodies among orphans in Sana'a indicates a high prevalence rate in the community. It is possible that this high prevalence reflects a prevalence specific to this group or age, and this connection is real.

Keywords: *Helicobacter pylori (H. pylori)*, Hepatitis A virus (HAV), Orphanage, Prevalence, Sana'a, Yemen.

INTRODUCTION

Helicobacter pylori (*H. pylori*) infection is currently recognized as a worldwide health problem and causes morbidity and mortality. Globally, *H. pylori* have infected about half of the total population (50%) and nearly one-third (32.3%) of all children, particularly in low-incoming nations¹⁻³. *H. pylori* is the main cause of

peptic ulcer disease which progress to develop into gastric cancer. The signs and symptoms associated with *H. pylori* infection are abdominal pain, nausea, belching, bloating, and sometimes vomiting. Also, the complication resulting from persistent infection can lead to iron deficiency anemia, decreasing blood platelet count, mental deficiency, birth defects, and fetal stunted growth in pregnant women⁴⁻⁶.

Hepatitis A virus (HAV) is considered one of the endemic infectious diseases worldwide and is usually asymptomatic in younger children (<6 years) and symptomatic in older children and adults. It infects the liver and can range in severity from a mild illness to a severe illness. Internationally, it was estimated by the World Health Organization (WHO) that approximately 1.4 million new cases of HAV infection and about 100 thousand people die resulting from acute HAV infection are reported each year7-9. Some studies suggested their association between H. pylori and HAV in epidemiology, transmission routes, agespecific seroprevalence, and acquisition of infection. The high incidence of both H. pylori and HAV are reported in populations with low economic status, high density of living, low levels of education, poor hygiene practices, one of a family infected, unsafe water or foods, lack of drinking water, and poor sewage system ¹⁰⁻¹³. The acquisition of *H. pylori* and HAV infections occurred during childhood suggesting another association between these types of infection¹⁴⁻¹⁶. Several reports documented the prevalence of HAV and H. pylori infections within the same study area in some countries¹⁵⁻¹⁸. Yemen is ranked as one of the poorest low-income countries in the world. According to recent reports, about 79% of the total population lives under the poverty line and approximately 65% of these individuals are classified as awfully poor ¹⁹.In addition, the majority of Yemenis are without clean drinking water, inadequate sanitation, absence of institutional stability, food insecurity, severe malnourishment, loss of livelihoods and income, inadequate hygienic practices, and inadequate access to healthcare services are factors contributing to increasing exposure to infectious diseases²⁰⁻²⁴.

Up-to-date, most of the studies conducted in Yemen interested in the prevalence of hepatitis A virus or *H. pylori* independently²⁵⁻²⁷, and only one study has determined hepatitis A virus and *H. pylori* infections among schoolchildren in Yemen²⁷. So, this study was intended to find out the seroprevalence and association of *H. pylori* and HAV infections among orphanage children in Sana'a-Yemen.

MATERIALS AND METHODS

Study design and period

This is a cross-sectional study conducted at Sana'a Orphanage which is situated on Taiz Street belonging to Al-Sabeen District, Sana'a City, Yemen between October 2022 to February 2023. The experimental analysis was done at the Medical lab. at Queen Arwa University (QAU).

Sample size

The sample size consisted of 200 samples collected randomly of orphaned children aged between 7-15 years who lived in the Sana'a orphanage.

Data collection

The questionnaire was intended to gather the required data from each study subject enrolled in this study. The questionnaire inquired about the age, educational level (either primary or preparatory school), number of children in the room, contact with flood water, infected before by hepatitis A/H. pylori, family history of hepatitis A virus/H. pylorus, hospitalizations, surgical operation, blood transfusion, drinking water source, eating vegetables/fruits after washing, washing hand after defecation, and vaccinated for hepatitis A. Moreover, signs and symptoms such as jaundice, fever, headache, weakness, heartburn, regurgitation, heartburn, and regurgitation were gathered through face-face interviews. Questionnaires were filled out by the orphan children or investigators teams.

Inclusion and exclusion criteria

The participants who lived in Ahmed's orphanage, signed the declaration of agreement, and bring blood and stool samples were included in this study. On the contrary, the participants who didn't reside in Sana'a orphan, refuse to sign conscious consent, and did not correctly collect the required specimens were completely excluded.

Blood and stool specimen collection

The blood specimens (3 mL) were collected from each subject and transported into an anticoagulant tube. In addition, the collection of stool specimens was achieved by giving each participant a clean, dry, codded, and screw-cap container and instructing them on how to collect and set aside the specimens properly. The collected specimens were transported immediately to the medical laboratories at QAU for examination.

Laboratory examination

The immunochromatographic assay technique (rapid test) was used to assess the anti-HAV and *H. pylori* antigen. The screening of HAV antibody in prepared plasma was performed by a commercially available Cassette (Healgen Scientific Limit., US) according to the manufacturer's instructions. Also, the seropositivity of *H. pylori* antigen in stool specimens was performed by using the *H. pylori* Ag Test Cassette (Safecare Bio-Tech Hangzhou Co., UK) based on the manufacturer's instructions.

Ethical declaration

The ethical declaration of this project was permitted by the QAU Ethical Review Committee and approval for sample collection was also obtained from the orphanage administration based on the university's letter. Further, the purpose and objectives of this work were explained briefly to all orphans' children and administrative staff working in the orphanage before specimen collection.

Statistical analysis

All variables were categorical and presented as counts and percentages in tables. The SPSS program (version 26) was used to determine the Odds ratio (OR), Chisquare test (χ^2), and 95% Confidence interval (CI). Pearson's chi-square test was used to evaluate the relationship between *H. pylori* antigen and HAV seropositivity with risk factors. The comparison between the seroprevalence of *H. pylori* antigen and HAV was analyzed by Logistic regression. Also, the relationship between *H. pylori* and HAV infection with age was calculated by linear association. A *p*-value less than 0.05 was regarded as significant.

RESULTS

Socio-demographic characterization

A total of 200 orphan children participated in this study aged between 7-15 years, with a mean age of 12.7 years. Most of the study subjects were aged 13-15 years (65%), attended a preparatory school (58%), lived in a room with children size between 9-12 individuals (27%), always contacted with flood water (97%), didn't have a history of hepatitis A (92%) and *H. pylori* (92.5%), no cases in their family hepatitis A (94%) and *H. pylori* (83%), didn't hospitalize 10(90%), surgical operation (83.5%), didn't receive a blood transfusion (93%), and vaccinated against hepatitis A (91%) Table (1).

| Variables Categories Examined Rate (%) | | | | | | | | | | |
|--|--|--|--|--|--|--|--|--|--|--|
| Categories | Examined | Rate (%) | | | | | | | | |
| 7-9 | 8 | 4 | | | | | | | | |
| 10-12 | 62 | 31 | | | | | | | | |
| 13-15 | 130 | 65 | | | | | | | | |
| Primary | 84 | 42 | | | | | | | | |
| Preparatory | 116 | 58 | | | | | | | | |
| 3-5 | 62 | 31 | | | | | | | | |
| 6-8 | 44 | 22 | | | | | | | | |
| 9-12 | 94 | 27 | | | | | | | | |
| Always | 194 | 97 | | | | | | | | |
| Sometimes | 6 | 3 | | | | | | | | |
| Yes | 16 | 8 | | | | | | | | |
| No | 184 | 92 | | | | | | | | |
| Yes | 15 | 7.5 | | | | | | | | |
| No | 185 | 92.5 | | | | | | | | |
| Yes | 12 | 6 | | | | | | | | |
| No | 188 | 94 | | | | | | | | |
| Yes | 34 | 17 | | | | | | | | |
| No | 166 | 83 | | | | | | | | |
| Yes | 20 | 10 | | | | | | | | |
| No | 180 | 90 | | | | | | | | |
| Yes | 33 | 16.5 | | | | | | | | |
| No | 167 | 83.5 | | | | | | | | |
| Yes | 14 | 7 | | | | | | | | |
| No | 186 | 93 | | | | | | | | |
| Yes | 182 | 91 | | | | | | | | |
| No | 18 | 9 | | | | | | | | |
| | Categories 7-9 10-12 13-15 Primary Preparatory 3-5 6-8 9-12 Always Sometimes Yes No | Categories Examined 7-9 8 10-12 62 13-15 130 Primary 84 Preparatory 116 3-5 62 6-8 44 9-12 94 Always 194 Sometimes 6 Yes 16 No 184 Yes 15 No 185 Yes 12 No 188 Yes 34 No 186 Yes 33 No 167 Yes 33 No 167 Yes 14 No 186 | | | | | | | | |

| Table 1. Socio-demographic characterization of stud | y participating in a study. |
|---|-----------------------------|
|---|-----------------------------|

Prevalence of Hepatitis A antibody and *H. pylori* antigen

This result reported that the seropositivity rate of HAV antibody and *H. pylori* antigen, respectively, were 2(1.0%) and 63(31.5%) recorded among the orphan children (Figure 1).



Figure 1: Prevalence of HAV antibody and *H. pylori* antigen.

Risk factors associated with anti-HAV and *H. pylori* antigen

The current result revealed that the prevalence rate of HAV antibody and *H. pylori* antigen, respectively, was detected in the group aged 10-12 years (3.2%; p=0.125) and 13-15 years (40%; p=0.000), attending a

primary (2.4%; p=0.096) and preparatory school (41.4%; p=0.000). Also, the higher rate of anti-HAV and *H. pylori* antigen was found among children who lived in a room containing between 3-5 individuals (1.6% and 35.5%, respectively) and always contact with flooding water (1% and 31.9%, respectively) (Table 2).

However, the hepatitis A antibody was observed among children who had and their families a history of HAV at 12.5%, and 16.67%, respectively, (p=0.000), and had (6.7%; p=0.022) and their families didn't have a history of *H. pylori* (5.9%; *p*=0.522), nonhospitalized (1.1%; p=0.638), and had a surgical operation (6.1%; p=0.001). While H. pylori was among subjects didn't have (33.7%; p=0.023) and their families a history of HAV infection (33%; p=0.075), didn't have a history of H. pylori (31.9%; p=0.677), H. pylori cases in their family (58.8%; p=0.000), hospitalized (60% p=0.004), and didn't subject to surgical operation (32.9%; p=0.328) as listed in the Table 2. Furthermore, a higher prevalence of HAV antibody and H. pylori antigen, respectively, was observed in participating respondents who received blood transfusion (14.28%; *p*=0.000) and 30.6%; p=0.345), drunk treated water (1.4%) and (34%; p=0.204). The majority of anti-HAV seropositivity was detected among individuals who eat unwashed

vegetables (1.6%) and washed fruits (2.4%), washed their hands after defection (1.1%), and non-vaccinated for hepatitis A (1.11%; p=0.657). Whereas *H. pylori* antigen was found among participants who eat washed vegetables (37.3%) and fruits (37.4%), didn't wash their hands after defection (41.2%), and vaccinated for hepatitis A (33.5%; p=0.051) as summarized in Table 2. This data showed that the detection of anti-HAV was only reported among participants who suffer from jaundice (25%; p=0.000), fever (6.45%; p=0.001), headache (1.26%; p=0.466), and weakness (2.6%; p=0.070). While most of the study subjects who didn't suffer from eye jaundice (32.8%) and headache (33.3%) were positive for *H. pylori* antigen, as well as it, was detected among subjects who had a fever (38.7%) and weakness (34.2%) signs with no significant difference (p>0.05) as summarized in Table 3.The anti-HAV was only noticed among participants having signs and symptoms of heartburn (3.3%), regurgitation 2(3.2%), and heartburn and regurgitation 2(5.4%) with statistically significant (p<0.05).

|--|

| Variables | Categories | Enomined | Нера | atitis A | <i>P</i> - | H. pylor | <i>i</i> antigen | Р- | |
|------------------------|-------------|------------|---------|-----------|------------|----------|------------------|-------|--|
| | ., | Examined | +ve | -ve | value | +ve | -ve | value | |
| | | No. (%) | n (%) | n (%) | | n (%) | n (%) | | |
| | 7-9 | 8 (4) | 0(0) | 8(100) | | 0(0) | 8(100) | | |
| Age (in years) | 10-12 | 62 (31) | 2(3.2) | 60(96.8) | 0.125 | 11(17.7) | 51(82.3) | 0.000 | |
| | 13-15 | 130 (65) | 0(0) | 130(100) | | 52(40) | 78(60) | | |
| Educational level | Primary | 84 (42) | 2(2.4) | 82(97.6) | 0.096 | 15(17.9) | 69(82.1) | 0.000 | |
| Educational level | Preparatory | 116 (58) | 0(0) | 116(100) | 0.090 | 48(41.4) | 68(58.6) | 0.000 | |
| Number of | 3-5 | 62 (31) | 1(1.6) | 61(98.4) | | 22(35.5) | 40(64.5) | | |
| children in the | 6-8 | 44 (22) | 0(0) | 44(100) | 0.795 | 13(29.5) | 31(70.5) | 0.477 | |
| room | 9-12 | 94 (27) | 1(1.1) | 93(98.9) | | 28(29.9) | 66(70.1) | | |
| Contact with | Always | 194 (97) | 2(1) | 192(99) | 0.804 | 62(31.9) | 132(68.1) | 0.430 | |
| flooding water | Sometimes | 6 (3) | 0(0) | 6(100) | 0.804 | 1(16.7) | 5(83.3) | 0.450 | |
| History of | Yes | 16(8) | 2(12.5) | 14(87.5) | 0.000 | 1(6.3) | 15(93.7) | 0.022 | |
| hepatitis A | No | 184 (92) | 0(0) | 184(100) | 0.000 | 62(33.7) | 122(66.3) | 0.023 | |
| Hepatitis A cases | Yes | 12(6) | 2(16.7) | 10(83.3) | 0.000 | 1(8.3) | 11(92.7) | 0.075 | |
| in the family | No | 188 (94) | 0(0) | 188(100) | 0.000 | 62(33) | 126(67) | 0.075 | |
| History of H. | Yes | 15 (7.5) | 1(6.7) | 14(93.3) | 0.022 | 4(26.7) | 11(73.3) | 0.677 | |
| pylori | No | 185 (92.5) | 1(0.5) | 184(99.5) | 0.022 | 59(31.9) | 126(68.1) | | |
| H. pylori cases in | Yes | 34 (17) | 2(5.9) | 32(94.1) | 0.522 | 20(58.8) | 14(41.2) | 0.000 | |
| the family | No | 166 (83) | 0(0) | 6(100) | 0.322 | 43(25.9) | 123(74.1) | | |
| Hospitalizations | Yes | 20 (10) | 0(0) | 20(100) | 0.638 | 12(60) | 8(40) | 0.004 | |
| Hospitalizations | No | 180 (90) | 2(1.1) | 178(98.9) | 0.038 | 51(28.3) | 129(71.7) | | |
| Surgical operation | Yes | 33 (16.5) | 2(6.1) | 31(93.9) | 0.001 | 8(24.3) | 25(75.7) | 0.328 | |
| Surgical operation | No | 167 (83.5) | 0(0) | 167(100) | 0.001 | 55(32.9) | 112(67.1) | 0.328 | |
| Blood transfusion | Yes | 14 (7) | 2(14.3) | 12(85.7) | 0.000 | 6(42.9) | 8(57.1) | 0.345 | |
| Blood transfusion | No | 186 (93) | 0(0) | 186(100) | 0.000 | 57(30.6) | 129(69.4) | 0.345 | |
| Source of | Treated | 147 (73.5) | 2(1.4) | 145(98.6) | 0.396 | 50(34) | 97(66) | 0.204 | |
| drinking water | Untreated | 53 (26.5) | 0(0) | 53(100) | 0.390 | 13(24.5) | 40(75.5) | 0.204 | |
| Eating vegetables | Yes | 125 (62.5) | 0(0) | 75(100) | 0.273 | 28(37.3) | 47(62.7) | 0.171 | |
| after washing | No | 75 (37.5) | 2(1.6) | 123(98.4) | 0.275 | 35(28) | 90(72) | 0.171 | |
| Eating fruits after | Yes | 117 (58.5) | 2(2.4) | 81(97.6) | 0.092 | 31(37.4) | 52(62.6) | 0.135 | |
| washing | No | 83 (41.5) | 0(0) | 117(100) | 0.072 | 32(27.4) | 85(72.6) | 0.155 | |
| Washing hand | Yes | 183 (91.5) | 2(1.1) | 181(98.9) | 0.667 | 56(30.6) | 127(69.4) | 0.372 | |
| after defecation | No | 17 (8.5) | 0(0) | 17(100) | 0.007 | 7(41.2) | 10(58.8) | 0.572 | |
| Hamatitia A | Yes | 182 (91) | 0(0) | 182(100) | | 61(33.5) | 121(66.5) | | |
| Hepatitis A vaccinated | No | 18 (9) | 2(1.1) | 16(98.9) | 0.657 | 2(11.1) | 16(88.9) | 0.051 | |
| , accinated | | | | | | | | | |

In similar, the *H. pylori* antigen was significantly more detected among participants suffering from heartburn (34.4%; p=0.557), regurgitation (38.7%; p=0.143), and heartburn and regurgitation (56.7%; p=0.000) as recorded in the Table 4.

Associations of risk factors with seropositivity of HAV antibody *H. pylori* antigen

Table 5 reveals the association between some risk factors and seropositivity of the HAV antibody *H. pylori* antigen. This finding found that the high risk of HAV infection was in children whose family had a history of HAV cases (OR=19.800; 95% CI=10.824–

36.220), followed by receiving blood transfusion (OR=16.500; 95% CI=9.535–28.552), and had a history of surgical operation (OR=6.387; 95% CI=4.623–8.825). In addition, the high risk of H. *pylori* infection was noticed among participant study who were hospitalized (OR=3.262; 95% CI=1.403–7.581). Moreover, the result of logistic regression showed that age was the only factor affecting the prevalence of H. *pylori* significantly. Also, no significant correlation was detected between the seropositivity of anti-HAV and H. *pylori* antigens in this study (Table 6).

| Table 3: Hepatitis A and <i>H. pylori</i> infection concerning signs and symptoms of Hepatitis A. | | 1 6 | • • • | 4 PTT 414 A |
|---|------------------------------|------------------|----------------------|----------------------------|
| Table 5. Repairing 11 and 11. pytott infection concerning signs and symptoms of repairing 11. | Table 3. Henstitis A and H | nvlori infection | concerning signs and | symptoms of Henglifis A |
| | Table 5. Hepatius 11 and 11. | pyion miccuon | concerning signs and | symptoms of fitepaties m |

| Variables | | Examined | Examined Hepatitis A | | <i>P</i> - | H.] | <i>P</i> - | | |
|---|-----|------------|----------------------|-----------|------------|----------|------------|-------|--|
| | | No. (%) | +ve | -ve | value | +ve | -ve | value | |
| | | | n (%) | n (%) | | n (%) | n (%) | | |
| Jaundice | Yes | 8(4) | 2(25) | 6(75) | 0.000 | 0(0) | 8(100) | 0.051 | |
| Jaundice | No | 192 (96) | 0(0) | 192(100) | 0.000 | 63(32.8) | 129(67.2) | 0.031 | |
| Fever | Yes | 31 (15.5) | 2(6.5) | 29(93.5) | 0.001 | 12(38.7) | 19(61.3) | 0.350 | |
| revei | No | 169 (84.5) | 0(0) | 169(100) | 0.001 | 51(30.2) | 118(69.8) | 0.350 | |
| Headache | Yes | 158 (97) | 2(1.3) | 156(98.7) | 0.466 | 49(31.) | 109(67) | 0.775 | |
| neadache | No | 42 (21) | 0(0) | 42(100) | 0.400 | 14(33.3) | 28(66.7) | 0.775 | |
| Weakness | Yes | 76 (38) | 2(2.6) | 74(97.4) | 0.070 | 26(34.2) | 50(65.8) | 0.521 | |
| vv eakness | No | 124 (62) | 0(0) | 124(100) | 0.070 | 37(29.8) | 87(68.2) | 0.321 | |
| *Significant statistics at <i>P</i> -value <0.05. | | | | | | | | | |

| Variables | | Examined | Hepatitis A | | <i>P</i> - | H. pylori | | P- | |
|---------------|-----|------------|-------------|----------|------------|-----------|-----------|-------|--|
| | | No. (%) | +ve | -ve | value | +ve | -ve | value | |
| | | | n (%) | n (%) | | n (%) | n (%) | | |
| Heartburn | Yes | 61 (30.5) | 2(3.3) | 59(96.7) | 0.032 | 21(34.4) | 40(65.6) | 0.557 | |
| Heartburn | No | 139 (69.5) | 0(0) | 139(100) | 0.032 | 42(30.2) | 97(69.8) | 0.557 | |
| Heartburn and | Yes | 62 (31) | 2(3.2) | 60(96.8) | 0.034 | 24(38.7) | 38(61.3) | 0.143 | |
| regurgitation | No | 138 (69) | 0(0) | 138(100) | 0.054 | 39(28.3) | 99(71.7) | 0.145 | |
| Pagurgitation | Yes | 37 (18.5) | 2(5.4) | 35(94.6) | 0.003 | 21(56.7) | 16(43.3) | 0.000 | |
| Regurgitation | No | 163 (81.5) | 0(0) | 163(100) | 0.005 | 42(25.8) | 121(74.3) | 0.000 | |

DISCUSSION

The acquiring infections of HAV and *H. pylori* are commonly occurring early in the life of childhood and most of them become infected when they reach late adolescence^{28,29}. The overall rate of HAV antibody and *H. pylori* antigen were recorded among the orphan children at 1% and 31.5%, respectively. This result is lower than that of the rate of HAV and *H. pylori*, respectively, reported among children at 63% and 87% in Italy³⁰, 20.5% and 7% in South Korea³¹, 31% and

*Significant statistics at *P*-value <0.05.

5% in Japan¹⁷, 71.3% and 61.6% in Lebanon¹⁸, 21.1% and 26% in Turkey³², and higher than reported in Taiwan (6% and 0.8%)¹⁵, and similar to that reported in Yemen before (2.7% and 12.3%) in Sana'a²⁷.

The difference in prevalence rate may be referred to by some factors such as geographical distribution, size of the sample, study population, hygienic practices, environmental conditions, socioeconomic status, food consumption, and diagnostic techniques employed by the participants.

| Table | 5: | The asso | ciation | between | risk | factors | with | seropositivity | v of | anti-HAV | and H. | pvlori Ag. |
|-------|----|----------|---------|---------|------|---------|------|---------------------|------|----------|--------|------------|
| | | | | | | | | ~~- ~ F ~~~ · · · · | , ~- | | | ry |

| Variables | | Hepatitis A virus | H. pylori Ag | | | |
|---------------------------------|--------|-------------------|--------------|--------|---------------|----------|
| | OR | CI 95% | χ^2 | OR | CI 95% | χ^2 |
| Educational level | 1.193 | 0.295-4.816 | 0.053 | 1.407 | 1.021-1.939 | 0.143 |
| Number of children in the room | N.A | N.A | 0.076 | N.A | N.A | 0.038 |
| Contact with flooding water | 1.031 | 1.002 - 10.57 | 0.018 | 1.021 | 0.976-1.069 | 0.056 |
| History of hepatitis A | 15.231 | N.A | 0.341 | 0.145 | 0.071-1.016 | -0.160 |
| Hepatitis A cases in the family | 19.800 | 10.824-36.220 | 0.398 | 0.198 | 0.023 - 1.498 | -0.126 |
| History of H. pylori | N.A | N.A | 0.353 | | 1.059-1.191 | 0.015 |
| H. pylori cases in the family | 1.207 | 1.133-1.286 | -0.045 | 3.107 | 1.681-5.742 | -0.193 |
| Hospitalizations | 1.112 | 1.062-1.166 | -0.034 | 3.262 | 1.403-7.581 | 0.205 |
| Surgical operation | 6.387 | 4.623-8.825 | 0.226 | 0.696 | 0.333-1.456 | -0.069 |
| Blood transfusion | 16.500 | 9.535-28.552 | 0.366 | 1.631 | 0.591-4.503 | 0.067 |
| Source of drinking water | 1.366 | 1.255-1.485 | 0.060 | 1.121 | 0.950-1.323 | 0.090 |
| Eating vegetables after washing | 1.610 | 1.444-1.795 | 0.078 | 0.846 | 0.657 - 1.088 | -0.097 |
| Eating fruits after washing | 2.444 | 2.068-2.890 | -0.119 | -0.106 | 0.621-1.079 | -0.106 |
| Washing hand after defecation | 1.026 | 1.048-1.142 | 0.031 | 0.052 | 0.951-1.125 | 0.052 |
| Hepatitis A vaccinated | 1.100 | 1.053-1.150 | 0.032 | 0.138 | 1.017 - 1.182 | 0.138 |

 χ^2 = Chi-square test. OR=odds ratio, CI 95%=confidence interval, NA= not applicable

Also, the study conducted in Orphanage revealed the decline in hepatitis B virus among study subjects³³ and this may be due to the fact that the environment in which the orphans live, which separates them from the external environment, has contributed significantly to reducing the spread of pathogenic viruses. The high rate of communicable diseases in some parts of Yemen is well-reported^{21,34-37}. These data showed that the

prevalence rate of anti-HAV was observed in the age group of 10-12 years (3.2%) while *H. pylori* antigen was in the group aged 13-15 years (40%; p=0.000). These data are in accordance with the results observed in the preceding reports^{27,31}. Several previous reports that had documented a relationship between the HAV and *H. pylori* in transmission routes revealed the seroprevalence of HAV and *H. pylori* increasing simultaneously when increasing age^{31,32,38}.

H. pylori and HAV, as indicated by a similar pattern of increase in seropositivity with age, may share a common mode of transmission, but changes in environmental conditions make this very difficult if not impossible to prove with seroepidemiological data³².

The seropositivity rate of HAV was in this result only found among students who attended primary schools (2.4%). Whereas, the *H. pylori* antigen was highly detected in children who attended a preparatory school (41.4%) with a significant difference (p=0.000).

Similarly, the low-education individuals in some parts of Yemen were found to be more infected compared to high-educated persons^{24,39,40}. Education is a significant social determinant of health. Also, strength of association between educational status and health has been recognized. The effect of education is affecting better general self-awareness of individual health and the creation of healthcare more accessible. Well-educated persons have better health as revealed in the low levels of mortality, morbidity, and disability⁴¹.

| Variables | H. pylori Ag | | | | | | | |
|-------------|--------------|----------------|----------|--------------|--|--|--|--|
| variables | Beta | Standard error | P- value | CI 95% | | | | |
| Age | 0.612 | 0.058 | 0.027 | 0.015-0.244 | | | | |
| Hepatitis A | -0.267 | 0.329 | 0.417 | -0.912-0.381 | | | | |
| | | Hepatitis A | | | | | | |
| | Beta | Standard error | P- value | CI 95% | | | | |
| Age | -0.011 | 0.013 | 0.404 | 0.036-0.014 | | | | |
| H. pylori | -0.013 | 0.015 | 0.417 | 0.043-0.018 | | | | |
| | | r=0.338** | | | | | | |

**Regression tests, 95% CI=confidence interval, p-value <0.05

However, a higher percentage of HAV and *H. pylori* infections were found in this result in children living in room content between 3-5 individuals (1.6% and 35.5%, respectively). This finding is in agreement with published studies documented that HAV and *H. pylori* prevalence were significantly augmented among individuals when person size per room is increasing^{32, 42,43}. Moreover, a study by Bizri *et al.*,¹⁸ indicated that family size is a significant factor in increasing the prevalence rate of *H. pylori* but not to HAV. On the other hand, our result was in disagreement with the results of Kury *et al.*,⁴⁴ and Edrees *et al.*,²⁷.

This report is in agreement with earlier reports^{27,43}. Also, the transmitting hepatitis A virus among hospitalized persons was established earlier⁴⁵. This result is in agreement with the observations reported by Pirinççioğlu *et al.*,⁴³ and Edrees *et al.*,²⁷. Mothers play an important role in the transmission of the *H. pylori* bacterium to their children¹¹.

The current result showed the respondents receiving blood transfusion had a higher rate of anti-HAV (p < 0.05). This result is in accordance with the result of Edrees et al.,²⁷ where the rate of HAV infection is significantly more among children receiving blood transfusion. The rate of HAV and H. pylori seropositivities were found in this project among subjects drunk from treated water. These findings are in apparent disagreement with the results of Edrees et al.,²⁷. Also, а report by Nassrolahei and Khalilian⁴⁶ revealed there was a non-significant relationship between H. pylori seropositivity and the source of drinking water. Moreover, transmission routes for H. pylori occur mainly oral-oral or faecaloral route is further most probable while the role of water as a transmission route for H. pylori remains unproven^{11,47}.

The seropositivity of HAV was detected among individuals who eat unwashed vegetables, washed

fruits, and washed their hands after defection. This result is concordant with the previous study²⁷. Infected persons are able to transmit hepatitis A infection through dirty hands during food preparation to family members9. Furthermore, the H. pylori antigen was observed in this result among subjects who consumed washed vegetables and fruits. In contrast, the previous results documented that a high rate of anti-H. pylori was reported among children who eat unwashed vegetables and fruits and washed their hands after defecation^{24,42,49}. The study participants non-vaccinated for hepatitis A showed positive for HAV antibodies at1.11% and this is comparable to outcomes by Wu et al.,¹⁵ and Obyyah *et al.*,³⁹. The detection of anti-HAV in this work was only observed in subjects suffering from jaundice and fever (P < 0.05) as well as headache and weakness. This finding is in conformity with Obyyah *et al.*, 39 .

In similar, the highest seropositivity of H. pylori was detected in this finding among children with heartburn, regurgitation, and heartburn and regurgitation. This result is in consonance with the recent finding²⁷. The infection of H. pylori in some children may be asymptomatic throughout life¹¹. However, the result of this work revealed that the high risk of HAV infection was among children whose families were infected before with HAV (OR=19.800; 95% CI=10.824-36.220), followed by receiving blood transfusion (OR=16.500; 95% CI=9.535-28.552), and had a history of surgical operation (OR=6.387; 95% CI=4.623-8.825). In addition, the high risk of H. pylori infection was noticed in participants who were hospitalized (OR=3.262; 95% CI=1.403-7.581) and H. pylori cases in the family (OR=3.107; 95% CI=1.681-5.742). Moreover, an insignificant correlation was detected between the seropositivity of anti-HAV and H. pylori antigen. This result is in agreement with previous studies^{31,32,50}.

Limitations of the study

The limitations of this work are including; the small sample size, serologic tests done by rapid tests, and the absence of advanced diagnostic techniques such as Enzyme-linked Immunosorbent assay (ELISA) that is highly accurate and reliable due to limited resources.

CONCLUSIONS

In conclusion, the high seroprevalence of H. pylori among study subjects remaining life-threatening to infected individuals when will become adults if not completely eradicated. Also, poor hygiene practices, inadequate awareness, living in overcrowded conditions, absence of institutional stability, and lack of access to safe water may contribute to spread of the HAV and *H*. pylori infections. So. effective preventative measures are important to reduce infections among orphaned children through increasing knowledge about disease transmission, hygiene practices, improving living conditions, and supply of safe water and foods.

ACKNOWLEDGEMENT

The authors would like to thank the general Manger of the Orphanage and all workers working in Sana'a orphanage for their invaluable help and coordination. Also, they would like to thank Dr. Gader and all members of staff of the medical laboratories at Queen Arwa University for their help in specimen analysis.

AUTHOR'S CONTRIBUTION

Edrees WH: conceived the project and designed the experiments. Al-Aomary NM: collected and analyzed the samples. Alrahabi LM: data collection and data analysis. Thabit JM: drafting of manuscript. Ali HM: Literature survey, analysis of data. Saran AQ: data interpretations. Saleh AAK: Literature survey. Alshaouri EM: lab work. Al-Nahdi MT: methodology, investigation. Dibwan FA: data analysis, report drafting. All authors reviewed, revised, and approved the manuscript for submission.

DATA AVAILABILITY

Data will be made available on reasonable request.

CONFLICT OF INTEREST

None to declare.

REFERENCES

- Park JS, Jun JS, Seo JH, Youn HS, Rhee KH. Changing prevalence of *Helicobacter pylori* infection in children and adolescents. Clin Exp Pediatr 2021; 64(1):21-25. https://doi.org/10.3345/cep.2019.01543
- 2. Lupu A, Miron IC, Cianga AL, *et al.* The relationship between anemia and *Helicobacter pylori* infection in Children. Children (Basel) 2022; 30; 9(9):1324. https://doi.org/10.3390/children9091324

- 3. Yuan C, Adeloye D, Luk TT, *et al.* Global health epidemiology research group. The global prevalence of and factors associated with *Helicobacter pylori* infection in children: A systematic review and meta-analysis. Lancet Child Adolesc Health 2022; 6(3):185-194. https://doi.org/10.1016/S2352-4642(21)00400-4
- Franceschi F, Annalisa T, Teresa DR, et al. Role of Helicobacter pylori infection on nutrition and metabolism. World J Gastroentero 2014; 20(36):12809–12817. https://dx.doi.org/10.3748/wjg.v20.i36.12809
- Wawro N, Amann U, Butt J, et al. Helicobacter pylori seropositivity: Prevalence, associations, and the impact on incident metabolic diseases/risk factors in the populationbased KORA study. Front. Pub Health 2019; 7:96. https://doi.org/10.3389/fpubh.2019.00096
- Borka BR, Meliţ LE, Mărginean CO. Worldwide prevalence and risk factors of *Helicobacter pylori* Infection in Children. Children 2022; 9(9):1359. https://doi.org/10.3390/children9091359
- Lesmanawati DA, Adam D, Hooshmand E, *et al.* The global epidemiology of hepatitis A outbreak 2016-2018 and the utility of EpiWATCH as a rapid epidemic intelligence service. Glob Biosecur 2021, 3. http://dx.doi.org/10.31646/gbio.100
- Iorio N, John S. Hepatitis A. 2022; [cited 2023 February 29] https://www.ncbi.nlm.nih.gov/books/NBK459290/
- 9. World Health Organization (WHO). Hepatitis A. World Health Organization. 2022. Geneva, Switzerland [cited 2023 Jun 22].
- https://www.who.int/news-room/fact-sheets/detail/hepatitis-a
 10. Tsongo L, Nakavuma J, Mugasa C, Kamalha E. Helicobacter pylori among patients with symptoms of gastro-duodenal ulcer disease in rural Uganda. Infect Ecol Epidemiol; 2015; 5(1). https://doi.org/10.3402/iee.v5.26785
- 11. Mitchell H, Katelaris P. Epidemiology, clinical impacts and current clinical management of *Helicobacter pylori* infection. Med J Aust, 2016; 204(10): 376-380. https://doi.org/10.5694/mja16.00104
- 12. 12. Jacobsen KH. Globalization and the changing epidemiology of hepatitis A virus. Cold Spring Harb Perspect Med 2018; 1;8(10): a031716. https://doi.org/10.1101/cshperspect.a031716
- 13. Bin-Hameed EA, Barajash HM. Screening for the prevalence of *Helicobacter pylori* infection among dyspeptic patients using simple fecal antigen and serum antibody diagnostic methods at Mukalla city Hospitals, Hadhramout, Yemen. Afr J Microbiol Res 2021; 15(6): 325-333. https://doi.org/10.5897/AJMR2020.9413
- Al-Shamahy HA. Seroprevalence of *Helicobacter pylori* among children in Sana'a, Yemen. Ann Saudi Med 2005; 25(4):299-303. https://doi.org/10.5144/0256-4947.2005.299
- 15. Wu CM, Sung CH, Chang CM, *et al.* Seroprevalence of *Helicobacter pylori* and hepatitis A virus among children in rural Central Taiwan. Jpn J Infect Dis 2015; 68: 494–503. *https://doi.org/10.7883/yoken.JJID.2014.321*
- 16. Pretolani S, Stroffolini T, Rapicetta M, et al. Seroprevalence of hepatitis A virus and Helicobacter pylori in the general population of a developed European country (the San Marino study): Evidence for similar pattern of spread. Eur J Gastroenterol Hepatol 1997; 9:1081–1084. https://doi.org/10.1097/00042737-199711000-00010
- Malaty HM, Tanaka E, Kumagai T, *et al.* Seroepidemiology of *Helicobacter pylori* and hepatitis A virus and the mode of transmission of infection: A 9-year cohort study in rural Japan. Clinical Infectious Diseases 2003; 37:1067–1072. https://doi.org/10.1086/378276
- 18. Bizri AN, Nuwayhid IA, Hamadeh GN, et al. Association between hepatitis A virus and Helicobacter pylori in a developing country: The saga continues. J Gastroenterol Hepatol 2006; 21(10):1615-21. https://doi.org/10.1111/j.1440-1746.2006.04268.x
- The United Nations Development Programme (UNDP). Prolonged conflict would make Yemen the poorest country in the world, UNDP study says. The United Nations

Development Programme. September 26, 2019. [cited 2023 March 15].

- 20. The European Commission (EC). International Partnerships. Yemen.https://internationalpartnerships.ec.europa.eu/countr ies/yemen_en
- 21. Abdullah QY, *et al.* Seroprevalence of dengue fever virus among suspected patients in Taiz Governorate-Yemen. Universal J Pharm Res 2020; 5(5):21-26. https://doi.org/10.22270/ujpr.v5i5.482
- Edrees WH, Mogalli NM, Alabdaly KW. Assessment of some clinical and laboratory profiles among dengue fever patients in Hajjah government, Yemen. Universal J Pharm Res 2021; 6(2):38-41. https://doi.org/10.22270/ujpr.v6i2.571
- Al-Haik MW, Al-Haddad MA, Al-Kaf GA, et al. Antimicrobial activities for hadhrami honey on growth of some pathogenic bacteria. Universal J Pharm Res 2017: 2(6), 7-12. http://dx.doi.org/10.22270/ujpr.v2i6.R2
- 24. Edrees WH. Seroprevalence and risk factors for *Helicobacter pylori* infection among school students in Sana'a City, Yemen. Universal J Pharm Res 2022; 7(2):67-73. https://doi.org/10.22270/ujpr.v7i2.747
- Bawazir AA, Hart CA, Sallam TA, Parry CM, Beeching NJ, Cuevas LE. Seroepidemiology of hepatitis A and hepatitis E viruses in Aden, Yemen. Trans R Soc Trop Med Hyg 2010; 104: 801–805.

https://doi.org/10.1016/j.trstmh.2010.08.007

- 26. Al-Shami HZ, Al-Mutawakal ZAM, Al-Kholani AIM, et al. Prevalence of hepatitis A virus, hepatitis B virus, and hepatitis C virus, among patients with hepatic jaundice in Sana'a city, Yemen: A hospital based study. Universal J Pharm Res 2021; 6(6):12-17. https://doi.org/10.22270/ujpr.v6i6.693
- 27. Edrees WH, Banafa AM, Al-Awar MS, Al-Shehari WA. Hepatitis A virus and *Helicobacter pylori* among schoolchildren at Sana'a-Yemen: Seroprevalence and risk factors. Al-RaziUniv J Med Sci 2023; 7(1):34-45. https://doi.org/10.51610/rujms6.2.2022.135
- Diestag JL, Szmuness W, Stevens CE, et al. Hepatitis A virus infection: New insight from seroepidemiologic studies. J Infect Dis 1978; 137:328-340. https://doi.org/10.1093/infdis/137.3.328
- 29. Chung GE, Yim JY, Kim D, *et al.* Seroprevalence of hepatitis A and associated socioeconomic factors in young healthy Korean adults. Gut Liver 2011;5: 88-92. https://doi.org/10.5009/gnl.2011.5.1.88
- Luzza F, Imeneo M, Maletta M, et al. Seroepidemiology of *Helicobacter pylori* infection and hepatitis A in a rural area: Evidence against a common mode of transmission. Gut 1997; 41: 164–168. http://dx.doi.org/10.1136/gut.41.2.164
- 31. Kim SY, YO, Chung HY, Kim BJ, Ma JS. Concordance of seropositivity between *Helicobacter pylori* and hepatitis A virus IgG in children of Gwangju and Chonnam Area. Korean J Pediatr Gastroenterol Nutr 2001; 4(2):191-198. https://doi.org/10.5223/kjpgn.2001.4.2.191
- 32. Egemen A, Yilmaz O, Akil I, Altuglu I. Evaluation of association betweenhepatitis A and *Helicobacter pylori* infections and routes of transmission. Turk J Pediatr 2006; 48:135–139. PMID: 16848113
- 33. Banafa AM, Edrees WH, Al-Falahi GH, Al-Shehari WA. Prevalence of hepatitis B surface antigen among orphans children living in orphanage in Sana'a city, Yemen. PSM Microbiol 2022; 7(1): 19-26.
- 34. Al-Shamahy HA, Jaadan BM, Al-Madhaji AG, et al. Prevalence and potential risk factors of hepatitis B virus in a sample of children in two selected areas in Yemen. Universal J Pharm Res 2019; 4(3): 17-21. https://doi.org/10.22270/ujpr.v4i3.269
- 35. Edrees WH, Al-Ofairi BA, Alrahabi LM, et al. Seroprevalence of the viral markers of hepatitis B, hepatitis C, and HIV among medical waste handlers in some hospitals in Sana'a city- Yemen. Universal J Pharm Res 2022; 7(3):12-19. https://doi.org/10.22270/ujpr.v7i3.774

- Edrees WH, Banafa AM, Al-Awar MS. Risk factors and seroprevalence of hepatitis B virus antigen among university students in the Sana'a City, Yemen. Al-Razi Univ J Med Sci 2022; 6(1):8-16.
- Al-Moayad EE, Alghalibi SM, Al-Shamahy HA, Nasher AT, Al-hebshi NN. Normalized real-time PCR for diagnosis of *H. pylori* infection. Qatar Med J 2014: 19. http://dx.doi.org/10.5339/qmj.2014.19
- 38. BinSaeed AA. Is there a link between seropositivity to Helicobacter pylori and hepatitis A virus? A systematic review. Int J Infect Dis 2010; 14: e567–e571. https://doi.org/10.1016/j.ijid.2009.09.003
- 39. Obyyah A, SharafAddeen A, Rafeeq A, et al. Hepatitis A virus and Helicobacter pylori infections: Prevalence, knowledge, and preventative practices among school children in Sana'a capital of Yemen. Bachelor Thesis, Medical Laboratory Dep., Al-Razi University 2023; 22-55.
- 40. Alshafli A, Al-Zain A, Faydi I, *et al.* Seroprevalence and risk factors of *Helicobacter pylori* Infection among school children presenting at schools in Sana'a city, Yemen. Bachelor Thesis, Medical Laboratory Dep., Yemen and Gulf University for Science and Technology 2022; 27-49.
- Raghupathi, V., Raghupathi, W. The influence of education on health: An empirical assessment of OECD countries for the period 1995–2015. Arch Public Health 78, 20 (2020). https://doi.org/10.1186/s13690-020-00402-5
- 42. Abebaw W, Kibret M, Abera B. Prevalence and risk factors of H. pylori from dyspeptic patients in Northwest Ethiopia: A hospital based cross-sectional study. Asian Pac J Cancer Prev. 2014; 15(11): 4459–4463. https://doi.org/10.7314/apjcp.2014.15.11.4459
- 43. Pirinççioğlu AG, Adıgüzel S, Özekinci T. Seropositivity of hepatitis A in children aged 7–14 years in Diyarbakir province center. Med Sci Monit 2018; 24: 936-943. https://doi.org/10.12659/MSM.906861
- 44. Kury CM, Pinto MA, Cruz OG, *et al.* Hepatitis A seroprevalence in public school children in Campos dos Goytacazes, Rio de Janeiro State, Brazil, prior to the introduction of the hepatitis A universal childhood vaccination. Cad. Saúde Pública 2016; 32(11):e00175614. https://doi.org/10.1590/0102-311X00175614
- 45. Hofmeister MG, Yin S, Aslam MV, Teshale EH, Spradling PR. Hepatitis A hospitalization costs, United States, 2017. Emerg Infect Dis. 2020; 26(5):1040-1041. https://doi.org/10.3201/eid2605.191224
- 46. Nassrolahei M and Khalilian A. Seropositivity of antibodies against *Helicobacter pylori* and hepatitis A virus in Iran. Annals Saudi Med 2004; 24(1):61-64. https://doi.org/10.5144/0256-4947.2004.61b
- 47. Sierra MS, Hastings EV, Fagan-Garcia K, et al. Epidemiology, transmission and public health implications of *Helicobacter pylori* infection in Western countries. In: Buzas GM, editor. *Helicobacter pylori*-A worldwide perspective. Bentham Science Publishers 2014: 25-79.
- 48. Mynepalli SM, Maureen O, Mumuni A. Prevalence of *Helicobacter pylori* and hygiene practices among public secondary school students in Ikeja local government area, Lagos, Nigeria. Health 2014; 6: 250-258. http://dx.doi.org/10.4236/health.2014.64037
- 49. Moreno-Mesonero L, Hortelano I, Moreno Y, Ferrus MA. Evidence of viable *Helicobacter pylori* and other bacteria of public health interest associated with free-living amoebae in lettuce samples by next generation sequencing and other molecular techniques. Int J Food Microbiol 2020; 318: 108477. https://doi.org/10.1016/j.ijfoodmicro.2019.108477
- Chen LK, Hwang SJ, Wu TC, *et al. Helicobacter pylori* and hepatitis A virus infection in school-aged children on two isolated neighborhood islands in Taiwan. Helicobacter. 2003; 8:168-172.

https://doi.org/10.1046/j.1523-5378.2003.00140.x