The Seroprevalence of *Helicobacter pylori* Infection in Tripoli (Libya)

Ibrahim Ali Altyar[1], Alsadig Mohammed Abdalla [2], Saif Sameer Abdalwahab[1] and Sara Sameer Abdalwahab[1]

[1.] Department of Medical Laboratory Sciences, Faculty of Engineering and Technology, Sebha University, Libya. [2.] Department of Microbiology, Faculty of Medicine, Sebha University, Sebha, Libya. E-mail: alsadigabdalla32@yahoo.com

Abstract: *Helicobacter pylori* (*H. pylori*) is one of the most common chronic bacterial infections in developing countries. H. pylori infection has been established firmly with the development of gastric neoplasia, including gastric adenocarcinoma and gastric mucosaassociated lymphoid tissue lymphomas. The aim of this study was to investigate the seroprevalence of Helicobacter pylori infection in symptomatic patients and to determine the relationship between H. pylori and ABO/Rhesus blood groups, age, gender, socio-economic status and smoking in Tripoli, Libya, using three different commercial serological test methods. One hundred patients from Alhanan Specialized Medical Clinic in Tripoli were included in the study. A total of 100 samples were collected from these patients undergo serologic testing for the presence of anti–*H pylori* antibodies in serum or whole blood. Results revealed that by using (ABON test method) antibodies were seen in 36% of the patients and 29% by using ADVANCE QUALTY test method and 35% in **(**Helicobacter pylori IgG Enzyme immunoassay test method) which was nearly similar to the result obtained by ABON test. We found that the seroprevalence of H. pylori infection was not significantly associated with ABO blood groups, age, sex, occupation, socioeconomic condition and smoking (P-value > 0.05).

[Ibrahim Ali Altyar, Alsadig Mohammed Abdalla, Saif Sameer Abdalwahab and Sara Sameer Abdalwahab. **The Seroprevalence of *Helicobacter pylori* Infection in Tripoli (Libya).** *Nat Sci* 2015;13(1):81-86]. (ISSN: 1545-0740). http://www.sciencepub.net/nature. 12

Keywords: *Helicobacter pylori*; seroprevalence; infection rate

1. Introduction

Helicobacter pylori represent one of the most common and medically important infections worldwide. *H. pylori* is Gram-negative, microaerophilic, spiral, motile bacterium that resides in the gastric pits and the overlying mucus blanked. (Versalovic, 2003) (Blaser, 1990). It is recognized as the major cause of gastritis, gastric and duodenal ulcers, gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma. The prevalence of *H pylori* infection is 70%-90% in developing countries and 25%-50% in developed countries (Demiray et al., 2006). Person-to-person spread is the most probable mode of transmission. Faecal-oral and oral-oral transmissions have been reported (Allaker et al, 2002).

H. pylori infection is usually acquired during the early years of life and persists for several years. The prevalence of *H. pylori* infection has been reported to increase with age. It seems to be an association between the prevalence of *H. pylori* in adults and the risk of acquisition of *H. pylori* infection during childhood (Ghanaei et al., 2009). The prevalence of *H pylori* infection varies among countries and within a country it is dependent on socio-economic conditions especially poverty, overcrowding, poor sanitation and hygiene. Also, within countries, there may be considerable variation in prevalence by ethnic group (Whittle et al., 2010). It was also shown that the frequency of O blood group and non-secretor phenotype of ABO antigens are higher among patients with peptic ulcers. However, several studies reported absence of association between H. pylori infection and ABO blood groups (Tadesse et al., 2014).

H pylori and its association with multiple gastroduodenal diseases have emphasized the importance of accurate and prompt diagnosis of symptomatic individuals. The “test and treat” approach may be justified on the basis of a significantly increased lifetime risk of chronic peptic ulcer disease and gastric cancer (Graham and Rabeneck, 1996) (O’Connor et al, 1996). It is well known that a successful eradication of H. pylori dramatically reduces the rate of recurrence of gastric and duodenal ulcers in affected children. An accurate *H. pylori* test is crucial for initiation of appropriate treatment. Numerous diagnostic tests are available to detect *H. pylori* infection and are divided into either invasive (histology, rapid urease test (RUT) and bacterial culture) or noninvasive tests (serology, 13C-urea breath test (13C-UBT), and stool antigen test) (Guarner et al., 2010). Effective antimicrobial treatment depends on sensitive and accurate diagnostic approaches (Warren and Marshall, 1983). However, there has been no single test available that can be used as a gold standard to detect *H. pylori* infection reliably and accurately (Frenck et al., 2006)

(Koletzko et al., 2011). Seroepidemiological investigations represent the most rapid and convenient way of obtaining a picture of the prevalence of *H. pylori* infection in a population, but the assays used need to be validated in the population studied (Hoang et al., 2004**).** Because assays validated in one region may yield variable diagnostic performances in others. These variations may be attributed to many factors, including the source of antigen used, the prevalence of infection in each population studied, and the reference method used to determine true *H. pylori* infection status. Therefore, reevaluation is needed before implementing a test in different populations (Orrawadee et al., 2004).

The aim of this study was to investigate the seroprevalence of Helicobacter pylori infection in symptomatic patients and to determine the relationship between H. pylori and ABO/Rhesus blood groups, age, gender, socio-economic status and smoking in Tripoli, Libya. We therefore evaluated the performance of three different commercial diagnostic serological test methods used for detecting of immunoglobulin G (IgG) antibody to *H. pylori* and evaluated the possible risk factors for the *H. pylori* infection.

2. Material and methods

This study was carried out at the Specialized Alhanan Medical Clinic in Tripoli Libya. A total of 100 symptomatic patients selected randomly (39 males and 61 females; age range, 9 to 89 years) were studied. Written informed consents were obtained from all patients before the study.

2.1 Data Collection

On the enrollment date, questionnaire forms were collected back and dates of birth and other data of each patient were noted. All subjects were interviewed and personal, socioeconomic status, profession' educational level, environmental, and geographical data were obtained.

2.2 Serological tests:

Three ml (of clotted) venous blood was drawn from each patient and serum was stored refrigerated at (4–8 °C) for up to 48 hours. For a longer storage they were kept at -20 °C until analyzed for anti-*Helicobacter pylori* antibodies - IgG detection. Other 3ml of blood was drawn and put in tubes with anticoagulant (EDTA) to detect the blood groups (ABO). We used three different commercial test methods kits for detection of immunoglobulin G (IgG) antibody to *H. pylori*:

(1) (Helicobacter pylori IgG Enzyme immunoassay (EIA) test KIT: Enzyme Immunoassay for the detection of IgG Antibodies to Helicobacter pylori in human serum) (Biocheck, Inc. 323 Vintage Park Dr. Foster City, CA 94404). **(2)** (ABON: A rapid one step test for the qualitative detection of antibodies to Helicobacter pylori in human serum or plasma) (Abon Biopharm (Hangzhou) Co., Ltd. Hangzhou, 310018, P. R. China)-Wellkang Ltd SuiteB, 29 Harley Street, London, WIG 9QR, UK. **(3**) Advanced quality: Rapid Anti-H. pylori Test (Whole blood/ serum / plasma) (InTec products, INC.). All the tests were performed according to the manufacturer's instructions and without the knowledge of the status of the patient's infection.

2.3 Data analysis

Data analysis was made by using SPSS version 14 software. Chi-square test was used to test for presence of association between prevalence of *H. pylori* and age, sex and standard of living. P-values <0.05 were considered to show significant difference.

3. Results

A total of 100 patients (39 Males and 61 Females) attending the Specialized Elhanan Medical Clinic in Tripoli Libya included in this study. Helicobacter pylori infection was detected by three serological test methods.

Results revealed that antibodies were detected in 36% of the patients by using (ABON test method), in 29% by using (ADVANCE QUALTY test method) and in 35% by using (Helicobacter pylori IgG Enzyme immunoassay test method) which nearly similar to the result obtained by ABON test (table 1). The infection rate was most similar between males and females which was 35.9% and 36% respectively and there was no statistically significant difference in the prevalence of *H. pylori* with respect to gender (p=0.991) (table 2). The infection rate in patients with blood group AB was 40%, O was 38% and those with blood group B was 35% and A was 32.4%.

The prevalence of H. pylori infection according to age, ranging from (6.3%) in the 9-19 year old group, to (55%) in the 40-49 year old and (0%) in the 80-89 year old and there was no statistically significant difference in the prevalence of *H. pylori* among age groups (p=0.243). (table 3). The seroprevalence according to occupation or profession was shown in table (4). Also we found that patients with low standard of living were associated with a higher prevalence of *H. pylori* (47%), Moderate 34% and those with high standard of living was 20% and there was no statistically significant difference (p=0.199) (Tables 5). As for Predisposing factors which lead to infection we found that 29.4% of the smokers were infected with H. pylori compared with 37.3% of non smokers infected with H. pylori and 34.7% of the patients who always drink coffee were infected and 40% of the patients who do not drink coffee were infected (Table 6).

(Table 1) Seroprevalence by using three different test methods

Test Method	ADVANCE QUALITY	ABON	EIA
Percentage	29%	36%	35%

(Table 2) Seroprevalence of H. pylori infection according to gender

Sex	No. Tested	No. Infected	(%)
Males	39	14	35.9%
Females	61	22	36 %
P-value	0.991		

(Table 3) Seroprevalence according to age

Age	No.Tested	No.Infected	(%)
9-19	16	1	6.3%
20 – 29	31	10	32.6 %
30 – 39	17	8	47%
40 – 49	20	11	55 %
50 – 59	8	4	50%
60 – 69	2	0	0%
70 – 79	4	2	50 %
80 – 89	2	0	0%
P-value	0.243		

(Table 4) Seroprevalence according to occupation

Profession	No. tested	No. infected	(%)
Employee	31	15	48.4%
Students	17	2	17.6%
Workers	20	5	25%
Housekeepers	32	14	43.8%
P-value	0.213		

Table (5) Prevalence of infection according to standard of living

Standard of living	No. Tested	No. infected	Percentage of infection
Low	17	8	47%
Moderate	78	27	34%
High	5	1	20%
P-value	0.199		

Table (6) percentage of infection according to smoking and drinking coffee

Predisposing factor	No. Tested	No. infected	Infection rate	*P-value*
Smokers	17	5	29%	
Non smokers	83	30	36%	0.708
Coffee drinker	63	27	42.9%	
Non coffee drinkers	37	9	24.3%	0.192

4. Discussions

Non-invasive tests are easier to accomplish but need appropriate equipment and validation of methods for each region, population and age. (Feldman and Evans, 1995).

Serologic testing represents a primary screening approach for evaluation of *H pylori* status in patients not immediately requiring Esophagogastroduodenal (EGD) endoscopic studies. (Kimmel et al., 2000). Overall, the medians of the sensitivity and specificity for *H pylori* serology kits have been reported as 92% and 83%, respectively (Laheij et al., 1998).

In this study we used three different commercial serological test methods for the detection of immunoglobulin G (IgG) antibody to *H. pylori* and evaluated the performance of these three commercial test kits. A total of 100 patients enroled in the study. Results revealed that antibodies were detected in 36% of the patients by using (ABON test method), in 29% by using (ADVANCE QUALTY test method) and in 35% by using (Helicobacter pylori IgG Enzyme immunoassay test method) which nearly similar to the result obtained by ABON test. We observed that 7 out of 36 seropositive samples (tested by ABON test method) gave negative results when tested by (ADVANCE QUALTY test method). We found that 6 of these 7 samples were positive when tested by using **(**Helicobacter pylori IgG Enzyme immunoassay test method). Rates of positive test results between (ABON test 36%) and **(**Helicobacter pylori IgG Enzyme immunoassay test 35%) were most similar 35% this mean that they were most sensitive than ADVANCE QUALTY test method). Performance varies significantly for commercial serologic kits, with top performers exceeding 90% in sensitivity and specificity and bottom performers having less than 90% in sensitivity and less than 80% in specificity (Laheij et al., 1998). Additional studies reported

variable sensitivities (86%-100%) and specificities (76%-98%) of assays measuring anti–*H pylori* serum IgG (Meijer et al., 1997).

Compared to rates of infections found in other countries, the prevalence of Helicobacter pylori infection in this study was lower than that obtained by other workers. Alo et al., (2013) in their study in Ethiopie found that the prevalence of Helicobacter pylori infection was 38.3%. Our results are also much lower than those found in other studies conducted in some developing countries. A prevalence of 62% was found among Chinese populations (Shi et al., 2008), 63%-70% was found in Bahir Dar, Ethiopia (Tadege et al., 2005), while a prevalence of 64% was found in a study conducted in Ibadan, Nigeria (Jemilohun et al., 2011). Hoang et al., (2005) in their study, using a validated ELISA assay, reported a high overall seroprevalence of *H. pylori* infection of 74.6% in a Vietnamese population sample of 971 individuals aged 1 to 88 years. The variability of *H. pylori* seropositivity in different populations is likely partly explained by technical factors. Many of the studies used commercial kits that have been validated in developed countries but not in the populations investigated. These kits are usually based on strains from developed countries, while recent studies by us and others have shown that using local strains led to a significantly improved sensitivity and specificity (Romero-Gallo, et al**.**, 2002). In addition, even if validated with a local population, some of the assays used in seroepidemiological studies had been validated with patients undergoing endoscopy for gastrointestinal diseases. This approach allows determination of the sensitivity of the assay against the "gold standard," i.e., culture of *H. pylori*. It entails, however, the risk of setting an erroneous cutoff level if antibody concentrations differ significantly between symptomatic and asymptomatic individuals (Hoang et al., 2004).

As to the relation between ABO blood groups and H. pylori infection we observed that ABO was not significantly associated with infection in our study population, many epidemiologic studies had found that nonsecretors of ABO blood group antigens and individuals of blood group O were overrepresented among patients with peptic ulcers. These studies encouraged many researchers to investigate the relation between ABO blood groups and their secretor status with peptic ulcer. Many authors report an association between blood group O and *H. pylori* infection, while others failed to find such an association (Jaff, 2011).

In this study the prevalence of *Helicobacter pylori* infection in males 35.9% and females 36% and there was no statistically significant difference in the prevalence of *H. pylori* with respect to gender. Previous investigations have shown the importance of sex and age in the acquisition of *H. pylori* infection. (Goodman and Correa, 1995). Some studies have shown no sex difference in the prevalence of *H. pylori*. (Altuglu et al., 2001). but in a study in Iran, *H. pylori* prevalence was higher in males than in females. (Jafarzadeh et al., 2007). Also Kanbay et al., (2005) in their study reported that H. pylori infection can be related to ABO blood group, age, gender, and smoking.

In our study we found that the seroprevalence of H. pylori infection progressively increased with age, ranging from (6.3%) in the 9-19 year old group, to (55%) in the 40-49 year old, then it drop ranging from 50% in the age groups (50-59 and 70-79) to 0% in the age groups (60-69 and 80-89) There was no statistically significant difference in the prevalence of *H. pylori* among all age groups (p=0.243). This is in agreement with the results obtained by Tadesse et al., (2014) and in contrast to the results obtained by Moujaber et al., (2008).

Low socioeconomic conditions and occupation were not associated with the prevalence of *H. pylori* infection in our studied population. This is in contrast to the results obtained by other workers who found that low socioeconomic status is associated with increase in prevalence of *H. pylori* infection (Banatvala et al., 1993) and (Goldman et al., 2006**)**. Analysis of the relationship between smoking and H. pylori infection in recent studies has provided conflicting results. While some results have reported significantly elevated risks of H. pylori infection with smoking and one reported significantly reduced risk, most, similar to ours, found no significant association with current smoking (David and Hoda, 2002).

Conclusion

H. pylori infection was detected using serological test, which is acceptable for epidemiological studies. Serological detection of IgG antibody against H. pylori provides a reliable assessment of current or past H. pylori infection. H. pylori infection is usually lifelong therefore a positive test usually denotes active infection unless the patient has received eradication therapy. The results of this study demonstrate that the prevalence of infection with H. pylori in Libia was lower than rates reported in other developing countries, at 29-36.%. H. pylori infection positivity rate was not related to ABO blood groups, gender, age, low socioeconomic conditions and smoking. Further studies to determine subgroups at higher risk of infection may help target the more susceptible populations to establish the prevalence pattern in recent times and to determine if there is a change in the prevalence of Helicobacter pylori infection. Accurate information about the incidence

and prevalence of *H.* pylori infection will help in the planning of community-wide H. pylori eradication programs.

Acknowledgements

The authors would like to thank the administration of the Specialized Alhanan Medical Clinic in Tripoli Libya for their help and cooperation and giving the facilities to do this study and the patients for their cooperation.

Corresponding Author:
Dr. Alsadig Mohammed Abdalla
Department of Microbiology
Faculty of Medicine, Sebha University
Sebha, Libya
E-mail: alsadigabdalla32@yahoo.com

References:

1. Versalovic J. *Helicobacter pylori*: Pathology and Diagnostic Strategies. Am. J. Clin Pathol. 2003; 119: 403-412.
2. Blaser MJ. *Helicobactor pylori* and the pathogenesis of gastroduodenal inflammation. The Journal of infectious Diseases. 1990; 161: 626- 633.
3. Demiray E, Yılmaz Ö, Şarkış C, Soytürk M, Şimşek İ. Comparison of invasive methods and two different stool antigen tests for diagnosis of *H pylori* infection in patients with gastric bleeding. World J Gastroenterol. 2006; 12(26): 4206-4210.
4. Allaker RP, Young KA, Hardie JM, Domizio P, Meadows NJ. Prevalence of helicobacter pylori at oral and gastrointestinal sites in children: evidence for possible oral-to-oral transmission. J Med Microbiol 2002; 51: 312-317.
5. Ghanaei M, Mashhour YM, Joukar F, Sedigh M, Bagher AH -Zadeh, Jafarshad R. Prevalence of *Helicobacter Pylori* Infection among Children in Rasht, Northern Iran. Middle East Journal of Digestive Diseases. 2009; 1 (2): 152-158.
6. Whittle DO, Ewing R, Lee MG. The Prevalence of *Helicobacter pylori* Infection in Patients Undergoing Upper Gastrointestinal Endoscopy in the Turks and Caicos Islands. West Indian Med J. 2010; 59 (3): 309.
7. Tadesse E, Deresse Daka, Demo Yemane and Techalew Shimelis. Seroprevalence of *Helicobacter pylori* infection and its related risk factors in symptomatic patients in southern Ethiopia. BMC Research Notes. 2014, 7:834 http://www.biomedcentral.com/1756-0500/7/834.
8. Graham DY, Rabeneck L. Patients, payers, and paradigm shifts: what to do about *Helicobacter pylori* [editorial]. Am J Gastroenterol. 1996; 91:188-190.
9. O'Connor F, Buckley M, O'Morain C. Helicobacter pylori: the cancer link. J R Soc Med. 1996; 89: 674-678.
10. Guarner J, Kalach N, Elitsur Y, Koletzko S: *Helicobacter pylori* diagnostic tests in children: review of the literature from 1999 to 2009. Eur J Pediatr. 2010; 169:15–25.
11. Warren JR, Marshall BJ. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet. 1983;1: 1273-1275.
12. Frenck RW, Fathy HM, Sherif M, Mohran Z, El Mohammedy H, Francis W, Rockabrand D, Mounir BI, Rozmajzl P, Frierson HF. Sensitivity and specificity of various tests for the diagnosis of *Helicobacter pylori* in Egyptian children. Pediatrics. 2006; 118:1195–1202.
13. Koletzko S, Jones NL, Goodman KJ, Gold B, Rowland M, Cadranel S, Chong S, Colletti RB, Casswall T, Elitsur Y, Guarner J, Kalach N, Madrazo A, Megraud F, Oderda G: H pylori Working Groups of ESPGHAN and NASPGHAN: evidence based guidelines from ESPGHAN and NASPGHAN for *Helicobacter pylori* infection in children. J Pediatr Gastroenterol Nutr. 2011; 53: 230–243.
14. Hoang TT, Bengtsson C, Phung DC, Sorberg M, and Granstrom M. Seroprevalence of *Helicobacter pyl*ori Infection in Urban and Rural Vietnam. Clinical and Diagnostic Laboratory Immunology. 2005; 12 (1): 81–85.
15. Orrawadee H, Pongpanich A, Thong-Ngam D, Thammacharoenrach N, and Kullavanijaya P. Evaluation of Commercial Immunoassays for Detection of Antibody against *Helicobacter pylori* in Thai Dyspeptic Patients. Clinical and Diagnostic Laboratory Immunology. 2004; 11 (3): 618–620.
16. Feldman RA, Evans SJW. Accuracy of diagnostic methods used for epidemiological studies of *Helicobacter pylori*. Aliment Pharmacol Ther. 1995; 9: 21-31.
17. Kimmel B, Bosserhoff A, Frank R. Identification of immunodominant antigens from *Helicobacter pylori* and evaluation of their reactivities with sera from patients with different gastroduodenal pathologies. Infect Immun. 2000; 68: 915-920.
18. Laheij RJ, Straatman H, Jansen JB. Evaluation of commercially available *Helicobacter pylori* serology kits: a review. J Clin Microbiol. 1998; 36: 2803-2809.

19. Meijer BC, Thijs JC, Kleibeuker JH. Evaluation of eight enzyme immunoassays for detection of immunoglobulin G against *Helicobacter pylori*. J Clin Microbiol. 1997;35:292-294.
20. Alo MN. The Prevalence of Helicobacter Pylori Infection in Asymptomatic Persons in Ethiope East Local Government Area, Delta State, Nigeria. Int. J. Pub. Health& Pharm. 2013, 1 (1): 115-119.
21. Shi R, Xu S, Zhang H and Ding Y. Prevalence and risk factor for *Helicobacter pylori* Infection in Chinese populations. 2008; 13: 157-165.
22. Tadege T. Tesfahun, M. Yohannes, D. Kassu and Daniel, A.,Seroprevalence of *Helicobacter pylori* infection and itsrelationship with abo blood groups. Ethiopian Journal of Health Development. 2005; 19(1): 55-60.
23. Jemilohun AC, Otegbayo JA, Ola SO, Oluwasola OA and Akere A. Prevalence of *Helicobacter pylori* among Nigerian patients with dyspepsia in Ibadan. Pan African Medical Journal. 2011; 6: 18.
24. Romero-Gallo J, Perez-Perez GI, Novick RP, Kamath P, Norbu T, and Blaser MJ. Responses of endoscopy patient in Ladakh, India, to *Helicobacter pylori* whole-cell and CagA antigens. Clin. Diagn. Lab. Immunol. 2002; 9:1313–1317.
25. Hoang, T. T. H., T. U. Wheeldon, C. Bengtsson, D. C. Phung, M. So¨rberg, and M. Granstro¨M. Emzyme-linked immunosorbent assay for *Helicobacter pylori* needs adjustment for the population investigated. J. Clin. Microbiol. 2004; 42:627–630.
26. Jaff MS. Relation between ABO blood groups and *Helicobacter pylori* infection in symptomatic patients. Clinical and Experimental Gastroenterology. 2011:4, 221-226.
27. Goodman KJ, Correa P. The Transmission of *Helicobacter pylori*. A critical review of the evidence. Int J Epidemiol. 1995; 24(5): 875-87.
28. Altuglu I, Sayiner AA, Ozacar T, Egemen A, Bilgic A. Seroprevalence of Helicobacter pylori in a pediatric population. Turk J Pediatr. 2001; 43(2):125-7.
29. Jafarzadeh A, Ahmedi-Kahanali J, Bahrami M, Taghipour Z. Seroprevalence of anti-*Helicobacter pylori* and anti-CagA antibodies among healthy children according to age, sex, ABO blood groups and Rh status in south-east of Iran. Turk J Gastroenterol. 2007;18 (3):165-71.
30. K, Gür G, Arslan H, Yilmaz U, Boyacioglu S. The relationship of ABO blood group, age, gender, smoking, and *Helicobacter pylori* infection. Dig Dis Sci. 2005; 50 (7):1214-7.
31. Moujaber T, MacIntyre CR, Backhouse J, Gidding H, Quinn H, Gilbert GL. The seroepidemiology of *Helicobacter pylori* infection in Australia. International Journal of Infectious Diseases. 2008; 12, 500—504
32. Banatvala N, Mayo K, Megraud F, Jennings R, Deeks JJ, Feldman RA. The cohort effect and Helicobacter pylori. J Infect Dis. 1993; 168 (1):219-21.
33. Goldman C, Barrado A, Janjetic M, Balcarce N, Cueto Rua E, Oshiro M, et al. Factors associated with *H. pylori* epidemiology in symptomatic children in Buenos Aires, Argentina. World J Gastroenterol. 2006; 12 (33):5384-8.
34. David YG and Hoda MM. Commentary: what remains to be done regarding transmission of *H. pylori.* International Journal of Epidemiology. 2002; 36: 646-647.

1/13/2015