Clinical and Laboratory Characteristics of Pediatric *Campylobacter* spp. Acute Gastroenteritis

Mojgan Mazaheri,[1,2] Mostafa Haji Rezaei,[1] Marzieh Aalinezhad,[3,4] Mohammad Reza Sharif,[1,*] and Tabassom Akhavan[1]

[1]Infectious Diseases Research Center, Kashan University of Medical Sciences, Kashan, Iran
[2]Department of Pediatrics, Semnan University of Medical Sciences, Semnan, Iran
[3]Department of Radiology, Isfahan University of Medical Sciences, Isfahan, Iran
[4]Medical Students' Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

[*]*Corresponding author*: Mohammad Reza Sharif, Infectious Diseases Research Center, Kashan University of Medical Sciences, Kashan, Iran. Tel: +98-3155540021, Fax: +98-3155540111, E-mail: Sharif-md@outlook.com

Abstract

Background: Although *Campylobacter* strains are one cause of acute bacterial gastroenteritis, their clinical and laboratory findings have only been examined in a few studies.
Objectives: This study was performed to evaluate the frequency level as well as the clinical and laboratory findings of patients with acute gastroenteritis caused by *Campylobacter*.
Patients and Methods: In this cross-sectional study, 419 Iranian children in Semnan city with acute gastroenteritis were assessed for their clinical and laboratory findings, including fever, abdominal pain, vomiting, dehydration, the presence of red blood cells and white blood cells (WBCs) in the stool, and leukocytosis. After being prepared for testing, a sample of the patient's stool was also examined for the presence of *Campylobacter* strains through microscopic examination, culture, and chemical reactions.
Results: There were 36 positive cultures (8.6%) for *Campylobacter*, with frequencies of 6.4% and 10.3% for boys and girls, respectively (P = 0.16). The highest frequency of positive culture belonged to the age group over six years (P = 0.02). The most common findings associated with *Campylobacter* diarrhea included abdominal pain (77.8% vs.1 8.8%, P < 0.001), fever (80.6% vs. 20.4, P < 0.001), leukocytosis (72.2% vs. 45.7%, P = 0.002), and WBCs in the stool (63.9% vs. 23.8%, P < 0.001).
Conclusions: This study showed that abdominal pain, fever, leukocytosis, and WBCs in the stool were associated with gastroenteritis infection caused by *Campylobacter*.

Keywords: Gastroenteritis, Pediatrics, Fever, Abdominal Pain, *Campylobacter*

1. Background

Acute gastroenteritis (AGE) refers to the inflammation of the mucous membranes lining the digestive tract that causes diarrhea and vomiting and is transmitted through person-to-person contact and the consumption of contaminated food (1). AGE is one of the most common human infectious diseases that affects more than 2.5 billion children annually (2, 3). AGE occurs in both developing and developed countries and is the second leading cause of child mortality worldwide; approximately 1.5 million children under the age of five die annually due to the incidence of the disease (4, 5). Viruses, bacteria, and parasites are the three main groups of agents that produce AGE in children, with viruses accounting for over 80% in this population (2). The most common bacteria responsible for AGE in children are *Escherichia coli*, *Shigella*, *Salmonella*, and *Campy-*

lobacter, and they can cause serious gastrointestinal and non-gastrointestinal complications despite their lower incidence than viral factors (6, 7).

The *Campylobacter* genus is a Gram-negative, curved, motile, thermophilic, and microaerophilic bacillus that does not produce spores (8) and is comprised of 26 species, the most important of which are *Campylobacter jejuni* and *Campylobacter coli* (9). Gastroenteritis, meningitis, and septicemia are considered as the most important infectious diseases caused by *Campylobacter* (9). *Campylobacter* is one bacterial cause of AGE in developed countries (10). In developed countries, *Campylobacter* accounts for about 5% - 20% of all cases of AGE in children younger than 5 years of age; in developing countries, this incidence ranges from 5% - 35% (11, 12). *Campylobacter* is transmitted through food and water and appears more often during the warm seasons (13). Although diarrhea caused by *Campylobacter* is

self-limiting in nature, antimicrobial therapy can reduce the severity of symptoms and shorten the course of the disease ([14]).

The quality of treatment for patients can be improved with the proper differentiation of diarrhea caused by *Campylobacter* from that precipitated by other factors. Cultivation of this organism, however, entails a period of about 48 - 72 hours, which can lead to a delay in the initiation of treatment.

Knowledge of the epidemiology as well as the clinical and laboratory symptoms of AGE caused by *Campylobacter* will thus significantly contribute to improving the diagnosis and treatment process and thereby also reduce the incidence of complications from the infection.

Despite numerous studies on the frequency of this pathogen in different areas, clinical and laboratory symptoms of children suffering from *Campylobacter* infection have received little attention.

2. Objectives

This study was designed and implemented to evaluate the frequency level as well as the clinical and laboratory symptoms of diarrhea caused by *Campylobacter* in children with AGE in the city of Semnan, Iran.

3. Patients and Methods

3.1. Study Design and Participants

In this cross-sectional study, 419 children aged 1–12 years were examined during the period from July 2014 to January 2015.

Given the results of Sorokin et al.'s study, in which the frequency of diarrhea caused by *Campylobacter* spp. was reported to be 10.5%, the sample size was calculated as 419 by assuming $Z_{I-[U+0251]} / 2 = 1.96$ and $d = 0.03$ using the Kish Leslie formula ([15]).

All children referred to the pediatric emergency department of Semnan Imam Ali hospital with the complaint of acute diarrhea were selected for this study using a convenience sampling method until the required sample size was reached. In addition, children treated with fever-reducing drugs or methods were excluded, as were children who had received antibiotics within the previous two weeks or who suffered from anomalies of the gastrointestinal tract. After the provision of information on how to perform the study, written informed consent to participate in the study was obtained from the parents of the children, whose demographic and clinical information (including abdominal pain, vomiting, and dehydration) were then recorded in a questionnaire. The tympanic temperature

in the children was measured and recorded using a Braun Thermo Scan 5 IRT4520 digital thermometer that had been previously calibrated by the provider. Tympanic temperatures above 38°C were considered to be a fever.

3.2. Specimen Sampling and Laboratory Procedures

Five mL of venous blood were taken from all children by a qualified nurse. All samples were sent to the lab with a previously recorded identification (ID) number for blood cell count tests using the Sysmex KX-21N cell counter; the number of white blood cells (WBCs) was then recorded in the questionnaire. Leukocytosis was defined as a WBC count higher than the age-dependent upper reference value ([16]).

Before starting treatment with antibiotics, two samples were collected from the stools of patients: part of the sample was collected in sterile containers for the preparation of smears and to determine the red blood cell (RBC) and WBC count of the patients' stool (HiMedia, India). For bacteriological examination under sterile conditions, some of the patients' stool was transferred with a loop to Cary-Blair medium (MP202, HiMedia, India), where the sample was stored at 4°C before being sent to the laboratory. More than 5 RBCs and WBCs per high-power field [HPF] were considered positive for RBCs or WBCs.

The culture method was used to evaluate *Campylobacter* strains in the sample. The sample was moved using a sterile loop from the transport medium to the Skirrow's medium (MP994, HiMedia, India), where it was cultured using the Strick method. The prepared plates were microaerophilically incubated at 42°C for 48 - 72 hours using gas packs. Smears were taken from the colonies that appeared on the Skirrow's medium, and Gram staining was performed to assess the morphology of the organism. In addition, oxidase and catalase tests were carried out on all positive cultures, and any samples containing curved Gram-negative organisms (which were positive for oxidase and catalase production) were considered positive for the presence of *Campylobacter*.

3.3. Statistical Analysis

Data were analyzed using the statistical package for the social sciences (SPSS) version 18. Descriptive results were reported using absolute and relative abundance. After the data distribution was assessed by the Kolmogorov-Smirnov test, the average age between genders was compared using the Mann-Whitney U test, given the abnormal distribution of data on the patients' ages.

The demographic, clinical, and laboratory characteristics of patients were compared using the chi-square test.

The odds ratios and 95% confidence intervals were then reported. P values less than 0.05 were considered statistically significant.

4. Results

In total, 419 children were examined; they were mostly female (n = 233, 55.6%) with a mean age of 6.3 ± 3.36 years. The highest frequency age group included participants from 6 - 12 years (203 patients, 48.4%). During the visit, 23.9% of participants reported abdominal pain, 49.2% had vomiting, 25.5% reported a fever, and 71.6% experienced symptoms of dehydration. The characteristics of the study population are shown in Table 1.

Table 1. Demographic and Clinical Characteristics of the Study Population

Variable	Gender		P Value
	Male (n = 186)	Female (n = 233)	
Age[a]	6.48 ± 3.36	6.16 ± 3.37	0.36
Age group[b]			0.39
< 2	27 (14.5)	45 (19.3)	
2 - 6	68 (36.6)	76 (32.6)	
< 6	91 (48.9)	112 (48.1)	
Abdominal Pain[b]			0.43
No	145 (78.0)	174 (74.7)	
Yes	41 (22.0)	59 (25.3)	
Vomiting[b]			0.14
No	87 (46.8)	126 (54.1)	
Yes	99 (53.2)	107 (45.9)	
Dehydration[b]			0.36
No	57 (30.6)	62 (26.6)	
Yes	129 (69.4)	171 (73.4)	
Fever[b]			0.21
No	144 (77.4)	168 (72.1)	
Yes	42 (22.6)	65 (27.9)	

[a]Values are expressed as mean ± SD.
[b]The values are presented as No. (%).

Microscopic examination of the stool samples showed the presence of RBCs in 29 patients (6.9%) and WBCs in 114 patients (27.2%). A complete blood count indicated the presence of leukocytosis in 201 patients (48.0%). Thirty-six participants (8.6%) had positive bacteriological examinations for infection with *Campylobacter* strains, and the frequency of diarrhea caused by *Campylobacter* was 6.4% and 10.3% in boys and girls, respectively. Table 2 shows the de-

mographic, clinical, and laboratory characteristics of children with acute gastroenteritis by etiologic agent.

5. Discussion

In this study, 419 children with acute gastroenteritis were examined for infection with *Campylobacter* strains, and *Campylobacter* was the cause of diarrhea in 8.6% of the participants. Several Iranian studies have obtained different values than those reported in our study. Feizabadi et al. ([17]) investigated 500 Tehrani children with acute diarrhea, and the frequency of *Campylobacter* strains in their stool cultures was reported to be 8%; over 85% of these were *Campylobacter* jejuni. According to Alborzi et al.'s study ([18]) of 719 Iranian children to investigate the etiology of acute gastroenteritis in Shiraz, 2% of the cases of acute diarrhea in children were caused by *Campylobacter* strains. Dallal identified a frequency of 0.9% for *Campylobacter* strains in acute diarrhea among children younger than 5 years of age in south Tehran ([19]). Various studies have reported *Campylobacter* frequencies, such as 3.0% in Senegal, 8.4% in Uruguay, 9.6% in Egypt, 9.7% in Tanzania, 9.3% in Uganda, 10.5% in Romania, 11.0% in Sudan, 12.7% in Ethiopia, and 22.6% in Ghana ([11], [12], [15], [20]-[25]). Therefore, the average contribution of *Campylobacter* strains to AGE in children was about 9% in most studies. The significant differences observed in some studies necessitate epidemiological examinations in different regions to better understand the etiological status of acute diarrhea in children.

This study found that 47.5% of cases of diarrhea caused by *Campylobacter* occurred in children aged 6 - 12 years and there is a relationship between age group and infection with AGE caused by *Campylobacter*. Relevant studies have shown inconsistent results. According to a study conducted in Shiraz, children over the age of 11 years had the highest incidence of *Campylobacter* ([26]). In contrast, Rathaur et al. ([27]) found that the age group from 1 - 3 years made up 52.9% of patients with *Campylobacter* diarrhea. Children from 2 - 5 years of age had the highest risk of diarrhea caused by *Campylobacter* in a Ghanaian study (26). In addition, a Polish study of all cases of *Campylobacter*iosis diagnosed in 2012 revealed that over 79% of patients were younger than 4 years ([28]), while German children aged 1 - 4 years constituted the majority of patients in a 10-year study of *Campylobacter*iosis ([29]). A review of the studies that included a wide (statistical) population indicated that most *Campylobacter* infections occur from the ages of 1 - 5 years. A meta-analysis should make the situation clearer.

In this study, fever, abdominal pain, leukocytosis, and WBCs in the stool were associated with diarrhea caused by *Campylobacter*. Different results have been found in the few existing relevant studies. One report on children under 5

Table 2. Demographic, Clinical, and Laboratory Characteristics of Children Both With and Without *Campylobacter* Infection

Variable[a]	*Campylobacter*		P Value	OR (95% CI)
	Positive	**Negative**		
Gender			0.16	1.66 (0.81 - 3.43)
Male	12 (33.3)	174 (45.4)		
Female	24 (66.7)	209 (54.6)		
Age group			0.02	-
< 2	10 (27.8)	62 (16.2)		
2 - 6	5 (13.9)	139 (36.3)		
< 6	21 (58.3)	182 (47.5)		
Abdominal pain			< 0.001	15.12 (6.61 - 34.55)
No	8 (22.2)	311 (81.2)		
Yes	28 (77.8)	72 (18.8)		
Vomiting			0.13	1.7 (0.84 - 3.42)
No	14 (38.9)	199 (52.0)		
Yes	22 (61.1)	184 (48.0)		
Dehydration			0.39	1.43 (0.63 - 3.23)
No	8 (22.2)	111 (29.0)		
Yes	28 (77.8)	272 (71.0)		
Dehydration severity			0.38	-
Mild	150 (54.3)	19 (67.9)		
Moderate	80 (29.0)	6 (21.4)		
Severe	46 (16.7)	3 (10.7)		
Fever				16.2 (6.84 - 38.36)
No	7 (19.4)	305 (79.6)		
Yes	29 (80.6)	78 (20.4)	< 0.001	
Leukocytosis			0.002	3.09 (1.45 - 6.58)
No	10 (27.8)	208 (54.3)		
Yes	26 (72.2)	175 (45.7)		
Stool WBC			< 0.001	5.68 (2.76 - 11.66)
No	13 (36.1)	292 (76.2)		
Yes	23 (63.9)	91 (23.8)		
Stool RBC			0.3	1.79 (0.59 - 5.46)
No	32 (88.9)	358 (93.5)		
Yes	4 (11.1)	25 (6.5)		

[a]Values are expressed as No. (%).

years merely noted a relationship between the frequency of *Campylobacter* and abdominal pain (30); the frequency of fever, vomiting, and dehydration in another study was similar in several different types of diarrhea caused by bacterial pathogens (27). A comparison between children with *Campylobacter* diarrhea and those with diarrhea caused by *Salmonella* showed a relationship between *Campylobacter* and abdominal pain and leukocytosis; four criteria have been recommended for the differentiation of *Campylobacter* diarrhea: age over 5 years, leukocytosis, abdominal pain, and diarrhea (31). Based on the evidence and results of this study, abdominal pain is a common clinical

symptom in patients with *Campylobacter*, but other clinical and laboratory findings are not reliable enough; the epidemiological situation and clinical and laboratory symptoms must therefore be considered for the diagnosis of AGE caused by *Campylobacter*. Further studies will be required to develop reliable diagnostic criteria.

This study is one of the few that has included a wide population; it also attempted to use the most precise equipment to measure the clinical, bacteriological, and laboratory variables to evaluate the clinical and laboratory symptoms of AGE caused by *Campylobacter* in children. There were some limitations to the study as follows. *Campylobacter* strains cannot be determined exactly, and differences in the development of symptoms in various strains may disrupt the careful examination of the study variables. Therefore, future studies should consider the role of different strains in developing clinical and laboratory symptoms.

Acknowledgments

Footnotes

Authors' Contribution: Mojgan Mazaheri contributed to the study design and supervision; Mostafa Haji Rezaei aided in the data collection, performed the statistical analysis, and drafted the manuscript; Marzieh Aalinezhad contributed to the data collection and critical revision of the manuscript; Mohammad Reza Sharif assisted with the study design and drafted and approved the final manuscript; Tabassom Akhavan contributed to the revision of the manuscript.

References

1. Noguera T, Wotring R, Melville CR, Hargraves K, Kumm J, Morton JM. Resolution of acute gastroenteritis symptoms in children and adults treated with a novel polyphenol-based prebiotic. *World J Gastroenterol.* 2014;**20**(34):12301–7. doi: 10.3748/wjg.v20.i34.12301. [PubMed: 25232265].

2. Osborne CM, Montano AC, Robinson CC, Schultz-Cherry S, Dominguez SR. Viral gastroenteritis in children in Colorado 2006-2009. *J Med Virol.* 2015;**87**(6):931–9. doi: 10.1002/jmv.24022. [PubMed: 25776578].

3. Zou W, Cui D, Wang X, Guo H, Yao X, Jin M, et al. Clinical characteristics and molecular epidemiology of noroviruses in outpatient children with acute gastroenteritis in Huzhou of China. *PLoS One.* 2015;**10**(5):e0127596. doi: 10.1371/journal.pone.0127596. [PubMed: 26011043].

4. Whyte LA, Al-Araji RA, McLoughlin LM. Guidelines for the management of acute gastroenteritis in children in Europe. *Arch Dis Child Educ Pract Ed.* 2015;**100**(6):308–12. doi: 10.1136/archdischild-2014-307253. [PubMed: 25939578].

5. Anderson EJ. Prevention and treatment of viral diarrhea in pediatrics. *Expert Rev Anti Infect Ther.* 2010;**8**(2):205–17. doi: 10.1586/eri.10.1. [PubMed: 20109050].

6. Langendorf C, Le Hello S, Moumouni A, Gouali M, Mamaty AA, Grais RF, et al. Enteric bacterial pathogens in children with diarrhea in Niger: diversity and antimicrobial resistance. *PLoS One.* 2015;**10**(3):e0120275. doi: 10.1371/journal.pone.0120275. [PubMed: 25799400].

7. Laham NA, Elyazji M, Al-Haddad R, Ridwan F. Prevalence of enteric pathogen-associated community gastroenteritis among kindergarten children in Gaza. *J Biomed Res.* 2015;**29**(1):61–8. doi: 10.7555/JBR.29.20130108. [PubMed: 25745477].

8. Shyaka A, Kusumoto A, Chaisowwong W, Okouchi Y, Fukumoto S, Yoshimura A, et al. Virulence characterization of Campylobacter jejuni isolated from resident wild birds in Tokachi area, Japan. *J Vet Med Sci.* 2015;**77**(8):967–72. doi: 10.1292/jvms.15-0090. [PubMed: 25843040].

9. Fitzgerald C. Campylobacter. *Clin Lab Med.* 2015;**35**(2):289–98. doi: 10.1016/j.cll.2015.03.001. [PubMed: 26004643].

10. Mason J, Iturriza-Gomara M, O'Brien SJ, Ngwira BM, Dove W, Maiden MC, et al. Campylobacter infection in children in Malawi is common and is frequently associated with enteric virus co-infections. *PLoS One.* 2013;**8**(3):eee59663. doi: 10.1371/journal.pone.0059663. [PubMed: 23555739].

11. Mshana SE, Joloba M, Kakooza A, Kaddu-Mulindwa D. Campylobacter spp among children with acute diarrhea attending Mulago hospital in Kampala–Uganda. *Afr Health Sci.* 2009;**9**(3):201–5. [PubMed: 20589152].

12. Abd El-Baky RM, Sakhy M, Gad GF. Antibiotic susceptibility pattern and genotyping of campylobacter species isolated from children suffering from gastroenteritis. *Indian J Med Microbiol.* 2014;**32**(3):240–6. doi: 10.4103/0255-0857.136550. [PubMed: 25008814].

13. Salim SM, Mandal J, Parija SC. Isolation of Campylobacter from human stool samples. *Indian J Med Microbiol.* 2014;**32**(1):35–8. doi: 10.4103/0255-0857.124294. [PubMed: 24399385].

14. Humphries RM, Schuetz AN. Antimicrobial susceptibility testing of bacteria that cause gastroenteritis. *Clin Lab Med.* 2015;**35**(2):313–31. doi: 10.1016/j.cll.2015.02.005. [PubMed: 26004645].

15. Sorokin M, Usein CR, Irimia M, Damian M. A laboratory-based survey of Campylobacter infections in Prahova County. *Roum Arch Microbiol Immunol.* 2007;**66**(3-4):85–9. [PubMed: 18928068].

16. Engorn B, Flerlage J. A Manual for Pediatric House Officers. 20 ed. Philadelphia: Saunders; 2015. p. 333.

17. Feizabadi MM, Dolatabadi S, Zali MR. Isolation and drug-resistant patterns of Campylobacter strains cultured from diarrheic children in Tehran. *Jpn J Infect Dis.* 2007;**60**(4):217–9. [PubMed: 17642538].

18. Alborzi A, Aelami MH, Astaneh B, Pourabbas B, Farshad S, Kalani M, et al. Is Escherichia coli O157:H7 a common pathogen in children with bloody diarrhea in Shiraz, Iran?. *Turk J Pediatr.* 2008;**50**(4):349–53. [PubMed: 19014048].

19. Dallal MM, Khorramizadeh MR, MoezArdalan K. Occurrence of enteropathogenic bacteria in children under 5 years with diarrhoea in south Tehran. *East Mediterr Health J.* 2006;**12**(6):792–7. [PubMed: 17333824].

20. Sire JM, Garin B, Chartier L, Fall NK, Tall A, Seck A, et al. Community-acquired infectious diarrhoea in children under 5 years of age in Dakar, Senegal. *Paediatr Int Child Health.* 2013;**33**(3):139–44. doi: 10.1179/2046905512Y.0000000046. [PubMed: 23930725].

21. Varela G, Batthyany L, Bianco MN, Perez W, Pardo L, Algorta G, et al. Enteropathogens associated with acute diarrhea in children from households with high socioeconomic level in uruguay. *Int J Microbiol.* 2015;**2015**:592953. doi: 10.1155/2015/592953. [PubMed: 25861274].

22. Deogratias AP, Mushi MF, Paterno L, Tappe D, Seni J, Kabymera R, et al. Prevalence and determinants of Campylobacter infection among under five children with acute watery diarrhea in Mwanza, North Tanzania. *Arch Public Health.* 2014;**72**(1):17. doi: 10.1186/2049-3258-72-17. [PubMed: 24932408].

23. Saeed A, Abd H, Sandstrom G. Microbial aetiology of acute diarrhoea in children under five years of age in Khartoum, Sudan. *J Med Microbiol.* 2015;**64**(Pt 4):432–7. doi: 10.1099/jmm.0.000043. [PubMed: 25713206].

24. Mulatu G, Beyene G, Zeynudin A. Prevalence of Shigella, Salmonella and Campylobacter species and their susceptibility patters among under five children with diarrhea in Hawassa town, south Ethiopia. *Ethiop J Health Sci.* 2014;**24**(2):101–8. [PubMed: 24795510].

25. Krumkamp R, Sarpong N, Schwarz NG, Adlkofer J, Loag W, Eibach D, et al. Gastrointestinal infections and diarrheal disease in Ghanaian infants and children: an outpatient case-control study. *PLoS Negl Trop Dis.* 2015;**9**(3):e0003568. doi: 10.1371/journal.pntd.0003568. [PubMed: 25738935].

26. Hassanzadeh P, Motamedifar M. Occurrence of Campylobacter jejuni in Shiraz, Southwest Iran. *Med Princ Pract.* 2007;**16**(1):59–62. doi: 10.1159/000096142. [PubMed: 17159366].

27. Rathaur VK, Pathania M, Jayara A, Yadav N. Clinical study of acute childhood diarrhoea caused by bacterial enteropathogens. *J Clin Diagn Res.* 2014;**8**(5):PC01–5. doi: 10.7860/JCDR/2014/6677.4319. [PubMed: 24995223].

28. Sadkowska-Todys M, Kucharczyk B. Campylobacteriosis in Poland in 2012. *Przegl Epidemiol.* 2014;**68**(2):239–41. [PubMed: 25135507] 349-51.

29. Schielke A, Rosner BM, Stark K. Epidemiology of campylobacteriosis in Germany - insights from 10 years of surveillance. *BMC Infect Dis.* 2014;**14**:30. doi: 10.1186/1471-2334-14-30. [PubMed: 24422983].

30. Lengerh A, Moges F, Unakal C, Anagaw B. Prevalence, associated risk factors and antimicrobial susceptibility pattern of Campylobacter species among under five diarrheic children at Gondar University Hospital, Northwest Ethiopia. *BMC Pediatr.* 2013;**13**:82. doi: 10.1186/1471-2431-13-82. [PubMed: 23694714].

31. Lee CN, Chen CJ, Tang KS, Huang FC. Avoidance of antibiotic administration to Campylobacter enterocolitis mimicking severe salmonellosis by clinical and laboratory features. *J Trop Pediatr.* 2015;**61**(1):25–31. doi: 10.1093/tropej/fmu061. [PubMed: 25418893].