症例２

代々木公園で感染したと考えられた国内デング熱の症例

[1]国立国際医療研究センター　国際感染症センター
[2]国立国際医療研究センター　総合診療科
忽那　賢志[1]　山元　佳[1]　藤谷　好弘[1]　馬渡　桃子[1]
早川佳代子[1]　竹下　望[1]　加藤　康幸[1]　金川　修造[1]
大曲　貴夫[1]　佐藤　達哉[2]　國松　淳和[2]

症例：10 代男性

主訴：発熱，全身倦怠感

現病歴：当院来院の 13 日前より咽頭痛，咳，痰が出現した．8 日前より 39℃の発熱が出現したため 7 日前に近医 A を受診したところ感冒と診断され感冒薬を処方された．4 日前に B 病院を受診し，血液検査で白血球減少（2600/μL）および血小板減少（9.2×10[4]/μL）が認められたため B 病院に入院となった．当院来院の 2 日目に四肢・体幹に皮疹が出現しツツガムシ病が疑われたためミノマイシン 100mg 1 日 2 回の投与が開始された．その後も血小板減少が進行し血球貪食症候群が疑われたため精査加療目的で国立国際医療研究センター 総合診療科に転院となった．

既往歴：特記事項なし

アレルギー：タラで蕁麻疹が出たことがある

職業：高校生で，部活は陸上部に所属している．

渡航歴：なし

sick contact：部活の友人が風疹と診断された

野外活動：発症約 4 週間前に山形県蔵王で陸上部合宿を行った

発症約 1 週間前に高尾山での部活の練習に参加した

来院時身体所見：

バイタルサイン：意識清明，体温 38.0℃，血圧 107/71mmHg，脈あり 58/min，SpO2　98%（室内気），呼吸数 16/min

頭頸部：眼瞼結膜充血なし，球結膜黄染なし，咽頭に軽度の発赤あり，硬口蓋に点状出血あり

胸部：呼吸音清，心音整・明らかな雑音なし，左腋窩リンパ腫大あり

腹部：平坦，軟，圧痛なし，腸蠕動音正常，両側鼠径リンパ節腫大あり

皮膚：四肢にびまん性の紅斑あり（Fig. 1），右上腕（血圧計カフ下）に皮下出血あり（Fig. 2）

血液検査：WBC 4080/μL (50.0% Lym 7.0% Mono 4.0% Eos 0.8% Baso 0.0% Aty-lym 4.0%)，RBC 5.45 ×106/μL，Hb 15.5g/dL，Ht 32.7%，Plt 4.0×10[4]/μL，Alb 3.2g/dL，T-Bil 0.8mg/dL，GOT 229IU/L，GPT 129IU/L，LDH 1267IU/L，ALP 226IU/L，BS 132mg/dL，CRP 0.34mg/dL，BUN 7.4mg/dL，Cre 0.68mg/dL，Na　134mEq/L，K　4.0mEq/L，Cl 101mEq/L，フェリチン 15570ng/mL，PT-INR 1.09，

Fig. 1　来院時にみられた下肢の紅斑

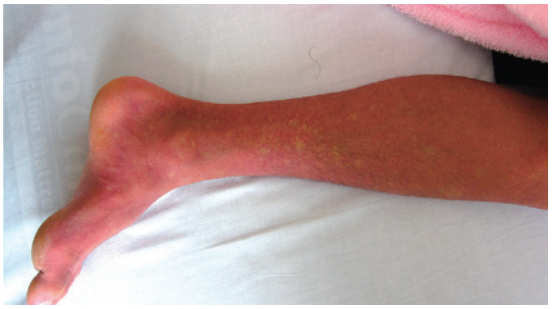
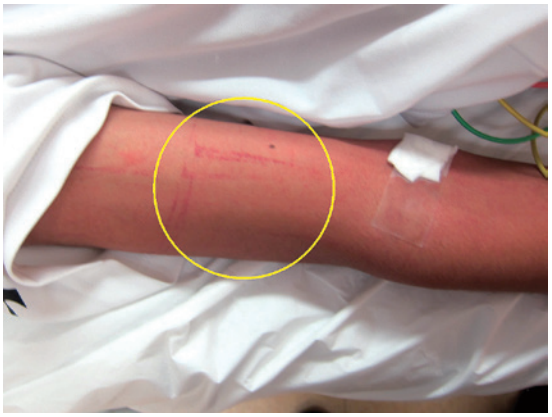


Fig. 2　来院時の血圧測定後にみられた皮下出血



APTT 36.3，Fib 259.3

入院後経過

2系統の血球減少と異型リンパ球の存在からパルボウイルスB19感染症などのウイルス性疾患（± 血球貪食症候群）を第一に考えた．潜伏期や皮疹の性状からはリケッチア症は否定的と考えられたためミノマイシンは中止した．骨髄穿刺も考慮したが，前医での血液検査と比較して白血球および血小板は増加しており，白血球減少・血小板減少は回復傾向と判断し経過観察可能と考えた．入院日翌日の血液検査ではWBC 6050/μL，Plt 9.8×10^4/μLと上昇した．

入院翌日の午後，厚生労働省より日本国内で感染したと考えられるデング熱の症例について発表された[1]．臨床像から本症例もデング熱の可能性があると考えられたため，追加で問診を行ったところ代々木公園で毎日ランニングをしていることが判明した．デング熱の診断について国立国際医療研究センター国際感染症センターについてコンサルテーションを行い，デング熱迅速診断検査が行われNS1抗原，デング熱IgM，デング熱IgGが陽性となった．症例の解析目的に国立感染症研究所　ウイルス第一部に検査を依頼したところデングウイルス1型が検出された．

最終診断：国内発症デング熱

症例の疑問点から研究的考察へ

本症例は2014年に東京を中心に流行し約160名もの感染者が出た[2]国内発症デング熱の1例であった．

2015年以降もデング熱の流行がみられる可能性があれば，今後国内でもデング熱に複数回罹患し重症化する症例が増えてくることが懸念される．またデング熱の流行を予防する方法としてワクチンの開発やベクターコントロールが重要と考えられる．したがって，下記の4点を疑問点として挙げ研究的考察を依頼した．

・なぜデング熱に2回目に罹患すると重症化しやすくなるのか？
・来年以降も日本で流行する可能性は？蚊の卵にデング熱ウイルスは残り，来年まで生き延び得るのか？
・デング熱のワクチンの開発状況は？
・媒介蚊のコントロールの具体的な方法は？

文献

1）厚生労働省報道発表資料．デング熱の国内感染症例について（第一報）（平成26年8月27日）http://www.mhlw.go.jp/stf/houdou/0000055605.html
2）Kutsuna S, Kato Y, Moi ML, Kotaki A, Ota M, Shinohara K, *et al.*：Autochthonous dengue fever, Tokyo, Japan, 2014. Emerg Infect Dis. 2015；21(3)：517―20.

利益相反自己申告：なし

“本症例の疑問点”から“研究的考察”へ

デング熱の病態解明，ワクチン開発，媒介蚊対策

国立感染症研究所ウイルス第一部
高崎　智彦

2014年，夏に69年ぶりに日本国内で流行したデング熱は，決して新しい感染症ではない．実に100年以上前から研究されていた，蚊が媒介するウイルス感染症である．その感染環は，サルと蚊の間で成立する報告もあるが，現在の流行はヒト–蚊–ヒトであると考えてよい．しかも，その媒介蚊はネッタイシマカとヒトスジシマカという人の住環境に生息するヤブカ属の蚊である．デング熱流行地の熱帯・亜熱帯地域の経済力発展に伴い，都市への人口集中が，デング熱流行の規模と頻度を大きくしている．このことは海外への渡航者の増加や海外からの来日者の増加とともに，日本へのデング熱輸入症例の増加の大きな要因となっている．

1. デング熱流行と研究の歴史

デング熱は，そのカタカナ3文字の名前から新しい病気のように誤解されることもあるが，その歴史は意外に古く，1942～1945年の流行以前に我が国においても戦前には台湾や沖縄で流行があり[1]，大正時代から研究されていた感染症である．デング熱患者の血液をいろいろな動物に接種して同様の症状が発生しないかといった病原体および病態解析に関する研究がなされていた．マウス，ラット，モルモット，ウサギ，イヌ，ヤギなどの哺乳類をはじめハトやトカゲにいたるまで試されたがいずれも明確な症状を呈することはなかった[2]．そして，太平洋戦争中に西日本を中心にデング熱の国内流行で，1943年に堀田進博士らが長崎でデング熱患者からデングウイルス1型を分離した[3]．この時の分離ウイルスはデングウイルス1型望月株（Dengue virus type 1, Mochizuki strain）と名付けられ，世界で最初のデングウイルス分離株として認められている．当時の流行は1942年8月に長崎市で突然デング熱流行が発生し，佐世保や大阪・神戸でも発生したが，11月には終息した．しかし翌年の夏になると再び流行し1945年まで夏季にはデング熱流行が発生した．4シーズン合わせたデング熱患者発生数は，少なくとも20万人と推計されている[4]．当時の流行について，今となっては検証の方法はないが，デングウイルス感染ヒトスジシマカの経卵巣伝播の報告[5]もあることから，デングウイルスが経卵越冬した可能性も否定はできない．終戦後，南方戦線でデング熱に履患した帰還兵の減少，焼夷弾に備えた防火水槽などの減少，日本脳炎を恐れた米軍による蚊対策によりヒトスジシマカの絶対数が減少したことなどを要因として，デング熱流行は終息した．

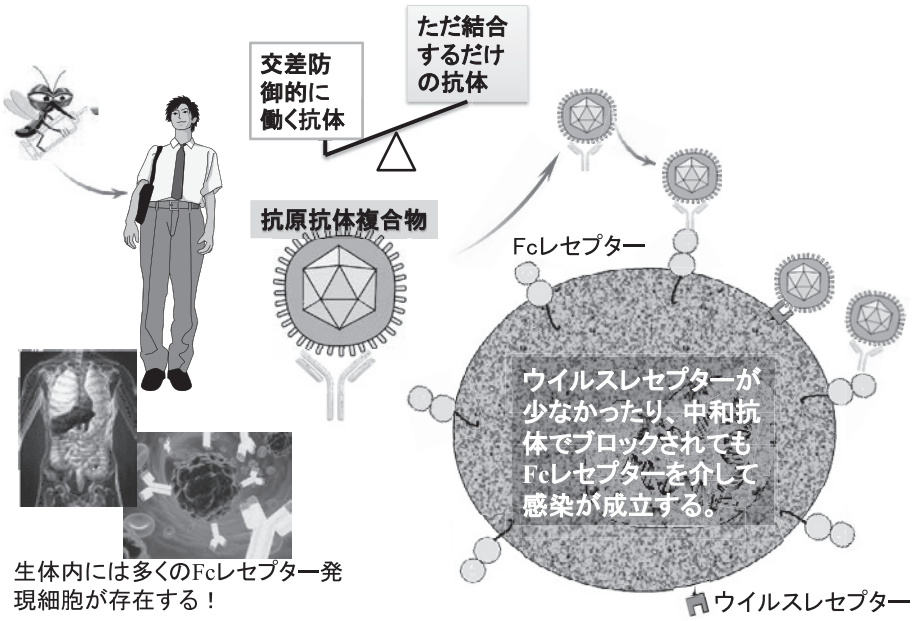
2. デングワクチン開発

日本脳炎ウイルスと近縁のデングウイルスの大きな違いは，日本脳炎ウイルスは単一血清型であるが，デングウイルスは4つの血清型があることである．同じ血清型のウイルスに対しては終生免疫とされるが，他の血清型ウイルスに対する防御能は感染後数か月で失われる．そして，異なる血清型ウイルスに感染した場合，抗体依存性感染増強現象（ADE：Antibody-dependent enhancement）が生じ，ウイルス増殖が増し重症化する場合がある．したがって，ワクチン開発では4つの血清型すべてに対して，防御できる免疫を誘導する必要がある．これが，ワクチン開発の困難さの一つの要因である．デングワクチンの開発の困難さの他の要因として，ワクチンの有効性を評価できる霊長類以外の動物モデルが存在しないことである．デングウイルスはサルには感染する．しかし，カニクイザルやアカゲザルなどの旧世界ザルにおけるウイルス血症は，ウイルス量は少なく期間も1～2日と短い[6]．しかし，我々が見出した新世界ザルであるマーモセットは，ウイルス接種後，高いウイルス血症が確認され，接種後2～7日目以上持続する[7]．もちろん，遺伝子改変マウスなどを用いれば，デングウイルス感染は成立するが，病態解析には有用でもワクチンや抗ウイルス剤の評価には適さない．

3. 重症化のメカニズム

デング熱は熱帯・亜熱帯の流行地では，子供の病気である．デング熱が重症化する場合は，その多くはデング出血熱（DHF），デングショック症候群（DSS）の病態を呈する．その重症化は，デング熱の症状を呈した後，解熱傾向を示すころに血小板減少，出血傾向，血管透過性亢進が認められるため，病初

Fig. 1　異なる血清型の「デングウイルス―ヘテロ抗体複合物」は中和されず結合するだけで感染性を有しており，抗体のレセプターである Fc レセプターを介して細胞内に侵入し感染が成立する．つまり，ウイルスレスプターを介する経路以外にも感染経路が生じ，体内にあるウイルスレセプターを持たない Fc レセプターを有する細胞も感染の標的となる．ヘテロの抗体の中にも防御的に働く抗体（交差防御抗体）も存在し，その交差防御抗体の割合が ADE を起こす抗体よりも高ければ再感染でも不顕性感染や軽症で済むことになる．3 度目，4 度目の感染では交差防御抗体の割合が高いことが多いと考えられる．



期に重症化を予測することは困難である．デング熱の流行地においては，小児期にデング熱に 2〜3 回感染することにより，成人においては 1〜4 型すべてに対して防御免疫を獲得する人が多い．したがってデングウイルスに感染する高齢者は比較的少なく，アジアでは 15 歳以下の小児で DHF，DSS の発症が多い．

デング熱の重症化の機序は完全に解明されているわけではない．ウイルスの病原性そのものにより重症化，あるは宿主側の要因が関与する場合もある．しかし，1960 年代にタイで行われた臨床研究によると，重症化例の 85% が再感染例で，既感染と異なるウイルス感染時に発症したこと[8]，さらに乳児における母親由来の移行抗体の関与も報告されている[9]ことから，抗体依存性感染増強（antibody-dependent enhancement；ADE）の関与が，有力な説と考えられている．たとえば初感染がデングウイルス 1 型であった人が，後に 3 型に感染した場合に，1 型に対する抗体のなかで 3 型ウイルスを中和するものは少ないが，ウイルス粒子と結合はする．この「3 型ウイルス―抗体複合物」は感染性を有しており，抗体のレセプターである Fc レセプターを介して細胞内に侵入し感染が成立する（Fig. 1）．つまり，ウイルスレスプターを介する経路以外にも感染経路が生じるわけである．しかし，ヘテロの抗体の中にも防御的に働く抗体もあり，その交差防御抗体の割合が ADE を起こす抗体よりも高ければ再感染でも不顕性感染や軽症で済むことになる（Fig. 1）．この ADE という現象は，*in vitro* で他のウイルスでも抗体を希釈していくとある希釈領域で生じることがある．しかし，デングウイルス 1〜4 型の抗原性の近似の程度が，血清を希釈することなく生じることがある離れ具合であると考えると理解しやすい．

4. 媒介蚊対策

デングウイルスの媒介蚊は，ネッタイシマカとヒトスジシマカである．ネッタイシマカは黄熱ウイルスの媒介蚊として有名であるが，デングウイルスの主媒介蚊でもある．熱帯や亜熱帯で活動するネッタイシマカには越冬の概念がなく，ボウフラは水温が 10℃以上あればそのままさなぎになり羽化する．一方，日本のようなネッタイシマカが生息しない温帯地域ではヒトスジシマカが媒介する．

デング熱国内流行のリスクを下げるためには，デ

ングウイルスの媒介蚊であるヒトスジシマカの絶対数を減らす必要がある．その最も有効な対策は幼虫対策である．日本ではヒトスジシマカは成虫のまま越冬できず，卵の形で越冬する．したがって秋に越冬卵を産ませないように産卵場所を除去する．春には幼虫が成虫にならないように，幼虫（ボウフラ）のいるたまり水をひっくり返して無くし，雨水マスのようなところには幼虫成長抑制剤（IGR）を入れるといった対策を実施する．ヒトスジシマカは池やプールのような大きなたまり水に産卵することはないので，水を抜くなどの必要はない．それでもデング熱輸入症例が増加する夏季には，デング熱国内発生リスクは高まる．特に代々木公園のように多くの人が集まり，さまざまな催し物の多い公園のようなところはそのリスクは高い．ヒトスジシマカに刺されないためには，夏季の野外活動では，蚊に刺されないように長袖，長ズボンを着用する．あるいはDEETを含む虫よけ剤を使用する必要がある．

ただし，ネッタイシマカは海外から航空機に紛れて，国際空港までは来ている[10]．ネッタイシマカには越冬の概念がなく，水温が10℃以下にならないたまり水があればボウフラは生きることが可能である．したがって冬がある日本では定着できる可能性は低い．しかし，一部の地域や地下の駅構内などには，水温が10℃以下にならないたまり水が存在するのも事実である．ネッタイシマカの国内定着を防止することも重要である．

文献

1）木庭密樹：デング熱に於ける耳鼻咽喉科的領域の変化．耳鼻咽喉科　1932；5 (1)：1―10.
2）Taniguchi T, Fujino T, Inoki S, Okuno Y：Studies on the experimental inoculation of dengue fever. Medical Journal of Osaka Univ. 1951；2 (2)：1―36.
3）堀田　進：デングウイルス研究60年の回顧．ウイルス．2001；51 (1)：105―7.
4）Hotta S. Dengue epidemics in Japan, 1942-1945. J Trop Med Hyg. 1953；56 (4)：83.
5）Rosen L：Further Observations on the Mechanism of Vertical Transmission of Flaviviruses by Aedes Mosquitoes. Am J Trop Med Hyg. 1988；39 (1)：123―6.
6）Ito M, Mukai RZ, Takasaki T, Kotaki A, and Kurane I：Antibody-dependent enhancement of dengue virus infection in vitro by undiluted sera from monkeys infected with heterotypic dengue virus. Arch Virol. 2010；155 (10)：1617―24.
7）Omatsu T, Moi ML, Hirayama T, Takasaki T, Nakamura S, Tajima S, *et al.*：Common marmoset (Callithrix jacchus) as a primate model of dengue virus infection：development of high levels of viraemia and demonstration of protective immunity. J Gen Virol. 2011；92 (Pt 10)：2272―80.
8）Halstead SB, Nimmannitya S, Cohen SN：Observations related to pathogenesis of dengue hemorrhagic fever. IV. Relation of disease severity to antibody response and virus recovered. Yale J Biol Med. 1970；42 (5)：311―28.
9）Jain A, Chaturvedi UC：Dengue in infants：an overview. FEMS Immunol Med Microbiol. 2010；59 (2)：119―30.
10）Sukehiro N, Kida N, Umezawa M, Murakami T, Arai N, JinnaiT, *et al.*：First Report on Invasion of Yellow Fever Mosquito, Aedes aegypti, at Narita International Airport, Japan in August 2012. Jpn J Infect Dis. 2013；66 (3)：189―94.

J Infect Chemother 21 (2015) 272–276

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

journal homepage: http://www.elsevier.com/locate/jic

Original article

Comparison of clinical characteristics and laboratory findings of malaria, dengue, and enteric fever in returning travelers: 8-year experience at a referral center in Tokyo, Japan

Satoshi Kutsuna*, Kayoko Hayakawa, Yasuyuki Kato, Yoshihiro Fujiya, Momoko Mawatari, Nozomi Takeshita, Shuzo Kanagawa, Norio Ohmagari

National Centre for Global Health and Medicine, Disease Control and Prevention Center, Japan

ARTICLE INFO

Article history:
Received 2 June 2014
Received in revised form
28 November 2014
Accepted 9 December 2014
Available online 18 December 2014

Keywords:
Dengue fever
Malaria
Typhoid fever
Enteric fever
C-reactive protein

ABSTRACT

Background: Without specific symptoms, diagnosis of febrile illness in returning travelers is challenging. Dengue, malaria, and enteric fever are common causes of fever in returning travelers and timely and appropriate treatment is important. However, differentiation is difficult without specific diagnostic tests.
Methods: A retrospective study was conducted at the National Centre for Global Health and Medicine (NCGM) from April 2005 to March 2013. Febrile travelers returning from overseas who were diagnosed with dengue, malaria, or enteric fever were included in this study. Clinical characteristics and laboratory findings were compared for each diagnosis.
Results: During the study period, 86 malaria, 85 dengue, and 31 enteric fever cases were identified. The mean age of the study cohort was 33.1 ± 12 years and 134 (66.3%) study participants were male. Asia was the most common area visited by returning travelers with fevers (89% of dengue, 18.6% of malaria, and 100% of enteric fever cases), followed by Africa (1.2% of dengue and 70.9% of malaria cases). Clinical characteristics and laboratory findings were significantly different among each group with each diagnosis. Decision tree models revealed that returning from Africa and CRP levels <10 mg/L were factors specific for diagnosis of malaria and dengue fever, respectively.
Conclusion: Clinical manifestations, simple laboratory test results, and regions of travel are helpful to distinguish between dengue, malaria, and enteric fever in febrile returning travelers with non-specific symptoms.

1. Introduction

A GeoSentinel review of over 42,000 ill-returned travelers highlighted that malaria, dengue fever (DF), and enteric fever (EF) were the most common causes of febrile illness in returning travelers during 2007–2011, accounting for 28.7%, 14.6%, and 4.6% fever cases, respectively [1]. The clinical manifestations of these diseases, including fever, headache, arthralgia, myalgia, and gastrointestinal symptoms, are non-specific and overlapping. Therefore, it is challenging to diagnose these diseases without specific tests. In Japan, a limited number of clinics perform specific tests to differentiate these diseases, such as malaria smear tests or rapid diagnostic tests for DF. The number of people who travel abroad is increasing due to the globalization of economy and tourism [2]; thus, early disease diagnosis is important.

We have previously reported the clinical characteristics of DF and malaria cases in our institute from 2005 to 2010 [3], and differences in laboratory findings between DF and malaria cases from 2005 to 2013 [4]. The current study included the same sample set of patients with DF and malaria, and extended the observations of our previous studies. The sample set was used to assess differences in clinical characteristics, including the location where the disease (DF, malaria, or EF) was contracted, duration of stay at the location, and clinical manifestations of the diseases in travelers. These characteristics were used to design a flow chart to distinguish between DF, malaria, and EF.

* Corresponding author. National Centre for Global Health and Medicine, Disease Control and Prevention Center, Address: 1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan. Tel.: +81 3 3202 7181; fax: +81 3 3207 1038.
E-mail address: sonare.since1192@gmail.com (S. Kutsuna).

http://dx.doi.org/10.1016/j.jiac.2014.12.004
1341-321X/

Table 1
Countries where patients with dengue fever were infected. 13 patients who visited more than one endemic country were excluded.

Country	Number of patients	Country	Number of patients	Country	Number of patients
Southeast Asia		**South Asia**		**Africa**	
Philippines	19	India	9	Benin	1
Indonesia	16	Bangladesh	3	**Oceania**	
Thailand	4	Pakistan	2	Papua New Guinea	1
Cambodia	3	Sri Lanka	2	Tahiti	1
Malaysia	2			Solomon Islands	1
Myanmar	2			Tonga	1
East Timor	2			**Latin America**	
Viet Nam	1			Mexico	1
				Brazil	1

To our knowledge, no other study has compared the usefulness of clinical characteristics and general laboratory findings to differentiate these diseases. The aim of this study was to describe differences in clinical characteristics and laboratory findings and to design decision tree models to diagnose DF, malaria, and EF at the first hospital presentation.

2. Patients and methods

This retrospective study was conducted at the National Centre for Global Health and Medicine (NCGM), a tertiary care governmental general hospital in Tokyo, Japan with about 900 inpatient beds which houses a travel clinic that is also a GeoSentinel Network site. NCGM functions as a referral hospital for returned travelers. Febrile returned travelers who visited NCGM during the period (April 2005 through March 2013) and were diagnosed with malaria, dengue, or EF were included in the study. Patients without fever at the first presentation were excluded. Demographic information including age, sex, nationality, and possible source of infection as well as reasons for travel, including business, leisure, visiting friends or relatives (VFR), volunteering, resease, expatriation or other reasons, were analyzed. Each country was classified according to geographical region, including Asia, Africa, Oceania, and South America. If 2 or more countries were visited, then all visited countries were included in the data. Clinical manifestations (rash, diarrhea, nausea/vomiting, headache, arthralgia, and myalgia) and laboratory data (white blood cell, WBC; hematocrit, Ht; platelet, Plt; total bilirubin, T-bil; aspartate aminotransferase, GOT; glutamate oxaloacetate transaminase, GPT; glutamate pyruvate transaminase, LDH; and C-reactive protein; CRP) at the first presentation were collected.

Table 2
Countries where patients with malaria were infected. Of confirmed cases, 56 were due to *Plasmodium falciparum* (*Pf*), 20 were *P. vivax* (*Pv*), 8 were *P. ovale* (*Po*), 1 was *P. malariae* (*Pm*), and 1 was *P. knowlesi* (*Pk*) infection. 8 patients (5*Pf*, 2*Pv*, 1*Po*) who visited more than one endemic country were excluded.

Country	Number of patients	Country	Number of patients	Country	Number of patients
Oceania		**South Asia**		**Africa**	
Papua New Guinea	4 (1 *Pf*, 3 *Pv*)	India	6 (6 *Pv*)	Ghana	13 (11 *Pf*, 2 *Po*)
Solomon islands	1 (1 *Pf*)	Pakistan	2 (2 *Pv*)	Nigeria	9 (9 *Pf*)
Latin America				Uganda	7 (3 *Pf*, 4 *Po*)
Brazil	2 (2 *Pv*)			Benin	4 (4 *Pf*)
French Guiana	1 (1 *Pv*)	**Southeast Asia**		Sierra Leone	3 (3 *Pf*)
Ecuador	1 (1 *Pv*)	Indonesia	3 (2 *Pf*, 1 *Pv*)	Guinea	3 (3 *Pf*)
		Malaysia	2 (1 *Pv*, 1 *Pk*)	Cameroon	3 (2 *Pf*, 1 *Po*)
		Myanmar	1 (1 *Pf*)	Zambia	2 (2 *Pf*)
				Burkina Faso	2 (2 *Pf*)
				Malawi	2 (2 *Pf*)
				Kenya	1 (1 *Pf*)
				Rwanda	1 (1 *Pv*)
				Togo	1 (1 *Pf*)
				Senegal	1 (1 *Pf*)
				Cote d'Ivoire	1 (1 *Pf*)
				Mali	1 (1 *Pf*)
				Mozambique	1 (1 *Pm*)

Dengue was confirmed by real-time polymerase chain reaction (PCR) (TaqMan RT-PCR), IgM-capture ELISA, IgG ELISA performed at the National Institute of Infectious Diseases in Tokyo, Japan, and a rapid diagnostic test that detected the viral non-structural 1 antigen (Standard Diagnostics Inc., Korea) performed at NCGM.

Malaria was confirmed by combined conventional microscope examination of Giemsa-stained thin blood films and rapid diagnostic tests (BinaxNOW Malaria Test, Binax, Inc. Maine, USA); *Plasmodium* species were confirmed by PCR if parasite morphology was not diagnostic. Laboratory diagnoses were performed at the Research Institute of the National Centre for Global Health and Medicine.

EF was confirmed by blood or stool culture of *Salmonella enterica* serotype Typhi or paratyphi A in the setting of a compatible clinical illness.

All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 20 (2011, IBM Corp., Armonk, NY, USA). The sensitivity and specificity of the decision trees were calculated using a diagnostic test calculator (MedCalc Software; http://www.medcalc.org/calc/diagnostic_test.php). The Mann–Whitney U test was used to compare continuous variables. A two-sided P value <0.05 was considered statistically significant. The study protocol was approved by the Ethics Committee at National Center for Global Health and Medicine (approved number: NCGM-G-001648-00).

3. Results

Characteristics of DF, malaria, and EF are shown in Tables 1–4. Clinical manifestations of these diseases were compared and odds ratios calculated (Table 5). Laboratory findings were compared using the Mann–Whitney U-test for each diagnosis group (Table 6).

The flow chart for determining DF, malaria, and EF at the first hospital presentation are shown in Fig. 1. "Returning from Africa" had a sensitivity of 72.09% (95% confidence interval [95% CI] = 61.38–81.23%) and specificity of 99.14% (95% CI = 95.27–99.86%) to predict malaria as opposed to the other 2 diseases (Box A). "Returning from elsewhere than Africa" combined with "CRP < 10 mg/L" had a sensitivity of 76.47% (95% CI = 66.02–84.99%) and specificity of 98.29% (95% CI = 93.95–99.74%) to predict DF as opposed to the other 2 diseases (BOX B). "Returning from elsewhere than Africa" combined with "CRP > 10 mg/L" had a sensitivity of 96.77% (95% CI = 83.24–99.46%) and specificity of 75.44% (95% CI = 68.28–81.69%) to predict EF as opposed to the other 2 diseases (BOX C). "Returning from Africa" or "Returning from elsewhere than Africa" with "CRP >10 mg/L" had a sensitivity of 98.84% (95% CI = 93.67–99.81%) and specificity of 57.76% (95% CI = 48.24–66.87%) to predict malaria as opposed to the other 2 diseases (BOX A + C). The combination of "Returning from elsewhere than Africa," "CRP > 10 mg/L," "Returned from South Asia," and "Platelet count < 15 cells/mm^3" had a sensitivity of 51.61% (95% CI = 33.07–69.83%) and specificity of 99.42% (95%

Table 3
Countries where patients with enteric fever were infected. 5 patients who visited more than one endemic country were excluded.

Country	Number of patients	Country	Number of patients	Country	Number of patients
Southeast Asia		**South Asia**		**Middle East**	
Cambodia	2	India	16	Turkey	1
Indonesia	1	Bangladesh	5		
Myanmar	1				

Table 4
Characteristics of dengue, malaria, and enteric fever in returning travelers.

	DF (n = 85)	Malaria (n = 86)	EF (n = 31)
Age	32.8 ± 12.9	34.0 ± 11.4	31.4 ± 11.6
Male (%)	50 (58.8)	64 (73.6)	22 (71.0)
Japanese (%)	79 (92.9)	59 (67.8)	29 (93.5)
Place disease contracted[a]			
Africa	1 (1.4%)	55 (70.1%)	0 (0%)
Southeast Asia	49 (68.1%)	6 (7.7%)	4 (15.4%)
South Asia	16 (22.2%)	8 (10.3%)	21 (80.8%)
Oceania	4 (5.6%)	5 (6.4%)	0 (0%)
Latin America	2 (2.8%)	4 (5.1%)	0 (0%)
other areas	0 (0%)	0 (0%)	1 (3.8%)
Reason for travel			
Business	34 (41.5%)	28 (33%)	13 (41.9%)
Leisure	38 (46.3%)	28 (33%)	13 (41.9%)
VFR	3 (3.7%)	21 (24.4%)	0 (0%)
Volunteer	4 (4.9%)	2 (2.3%)	2 (6.5%)
Research	0 (0%)	1 (1.2%)	1 (3.2%)
other	3 (3.7%)	6 (7.0%)	2 (6.5%)
Duration of stay			
<1 month	68 (81.0%)	36 (43.4%)	13 (48.1%)
≥1 month	16 (19.0%)	47 (56.6%)	14 (51.9%)

DF, dengue fever; EF, enteric fever; VFR, visiting friends or relatives.
[a] Patients (DF; n = 13, malaria; n = 8, EF; n = 5) who visited more than one endemic country were excluded.

CI = 96.77–99.90%) to predict EF as opposed to the other 2 diseases (BOX D). Malaria cases in Box C were predominantly non-falciparum malaria (3 *Pf*, 18 *Pv*, and 1 *Pk*).

4. Discussion

Japan has reported no domestic cases of malaria for 50 years. Although approximately 200 imported cases of DF are reported in recent years [5], an autochthonous case was not confirmed for 70 years in Japan [6]. On August 26, 2014, an autochthonous case of DF in a patient without any history of overseas travel was reported in Tokyo. As of October 31, 2014, 160 autochthonous cases have been confirmed [7]. Small numbers of EF cases in Japan have been occasionally reported [8]. During 2000 and 2010, the mean annual number of imported cases of malaria, DF, and EF were 73.5, 78.5, and 44.5, respectively [8,9].

The region of travel varied by disease. Malaria patients were typically infected in Africa (70.1%), while patients with DF and EF mainly contracted the diseases in Asia (90.3% and 96.2%, respectively). Among malaria species, *Plasmodium falciparum* malaria patients were mostly contracted in Africa (90.1%), while patients with vivax malaria mainly contracted infections in regions other than Africa, including South Asia (44.4%), Latin America (22.2%), Oceania (16.7%), and Southeast Asia (11.1%). All patients with P. ovale malaria contracted the disease in Africa. Patients with DF and EF were typically infected in Southeast Asia (68.1%) and South Asia (80.8%), respectively. This trend matches the epidemiological information reported by GeoSentinel surveillance [1]. Regions of travel are major clues for disease diagnosis. VFR was the third most common reason for travel among patients with malaria, but a minor reason for travel for the patients with DF and EF. A U.S. malaria surveillance report from 1997 to 2011 found VFR to be the third most common reason for travel in malaria patients (17% of 10,032 reported cases) [10]. VFR travelers have increased exposure to travel-related diseases, including malaria [11]. Although individuals born and raised in highly malaria-endemic areas develop a relative immunity, they lose this immunity after long periods in non-endemic countries [12]. These high-risk populations should obtain pre-travel consultation for vaccinations and malaria prophylaxis.

While the clinical manifestations of these diseases are similar, their relative frequencies differ. Headache and arthralgia are more frequent in DF, and diarrhea is more frequent in typhoid fever than the other diseases. While it is a specific manifestation of DF, rash is mainly observed in later stages and not frequently reported in early stages [13].

Disease-specific differences in laboratory findings were observed for WBC, Plt, T-bil, and CRP. Patients with DF had significantly lower WBC counts than those with the other 2 diseases. Leukopenia is a common finding in DF and a useful diagnostic feature [14]. Leukopenia in DF usually peaks on days 3–7 of illness, but is not always significant in the febrile phase [13]. Thrombocytopenia was observed in DF and malaria patients [14,15]. In our study, platelet counts were lower in patients with malaria compared to patients with dengue at the first presentation. Because platelet counts during DF infections are typically lowest 3–6 days from onset, when fever is about to decrease [13], the count of thrombocyte could be normal at the first presentation. Taylor et al. reported hyperbilirubinemia to be the most diagnostic finding for malaria in returning travelers [16], with a sensitivity of 38% and specificity of 95%. Our study results agree with this report: we found increased T-bil levels to be a distinguishing laboratory finding in malaria patients compared to DF and EF patients.

CRP measures acute phase reactants; markedly elevated CRP levels are strongly associated with infection. CRP levels may also be elevated in patients with viral infections, although typically not to the degree seen in patients with bacterial infections [17,18]. Premaratna et al. [19] reported 12 DF patients with CRP levels <12 mg/

Table 5
Clinical manifestations of dengue, malaria, and enteric fever in returning travelers.

	DF	Malaria	EF	(DF vs. Malaria)	(DF vs. EF)	(Malaria vs. EF)
Clinical characteristics, number (%)				Odds ratio (95% Confidence interval)		
Rash	25 (29.4)	1 (1.2)	1 (3.2)	35.4 (4.7–268.6)	12.5 (1.6–96.7)	0.4 (0.2–5.8)
Diarrhea	20 (23.5)	18 (20.9)	14 (45.2)	1.2 (0.6–2.4)	0.4 (0.2–0.9)	0.3 (0.1–0.8)
Nausea/vomiting	16 (18.8)	17 (19.8)	6 (19.4)	1.0 (0.4–20)	1.0 (0.3–2.7)	1.0 (0.4–2.9)
Headache	65 (77.3)	54 (65.1)	14 (45.2)	1.8 (0.9–3.6)	4.2 (1.7–9.9)	2.3 (1.0–5.2)
Arthralgia	51 (60.7)	23 (28.0)	8 (25.8)	4.0 (2.1–7.6)	4.4 (1.8–11.1)	1.1 (0.4–2.9)
Myalgia	17 (20.0)	11 (12.8)	3 (9.8)	1.6 (0.7–3.8)	2.4 (0.7–8.9)	1.5 (0.4–5.7)

DF, dengue fever; EF, enteric fever.

Table 6
Laboratory findings of dengue, malaria, and enteric fever in returning travelers.

	DF	Malaria	EF	(DF vs. Malaria)	(DF vs. EF)	(Malaria vs. EF)
Laboratory findings at first presentation, median (IQR)				P value		
WBC (/mm^3)	2780 (2020–3610)	4920 (3800–6280)	5220 (3870–6810)	**<0.001**	**<0.001**	0.576
Hct (%)	41.8 (39.6–45.2)	39.1 (36.5–43.6)	40.8 (36.3–43.4)	**<0.001**	**0.01**	p = 0.934
Plt (× 10^3/μL)	119 (83.5–161)	78 (48–124)	175 (130–240)	**<0.001**	**<0.001**	**<0.001**
T-bil (mg/dl)	0.6 (0.5–0.7)	1.8 (0.8–2.5)	0.6 (0.5–0.8)	**<0.001**	0.446	**<0.001**
GOT (IU/L)	37.0 (28.0–61.8)	33.0 (25.0–45.0)	61.0 (40.0–91.0)	0.067	0.05	**<0.001**
GPT (IU/L)	27.0 (19.0–47.5)	33.0 (22.8–46.3)	60.5 (41.8–107)	0.296	**<0.001**	**<0.001**
LDH (IU/L)	256 (194–326)	323 (227–447)	387 (324–459)	**0.002**	**<0.001**	0.196
CRP (mg/dL)	0.5 (0.3–0.9)	8.1 (4.0–13.1)	5.9 (3.6–10.8)	**<0.001**	**<0.001**	0.148

DF, dengue fever; EF, enteric fever; IQR, interquartile range; WBC, white blood cell; Hct, hematocrit; Plt, platelet; T-bil, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; CRP, C-reactive protein.
Bold denotes a two-sided P value < 0.05 which was considered statistically significant.

L. CRP levels are also elevated in malaria patients, making this biomarker effective for assessing malaria severity and for follow-up. Choo et al. [20] reported that CRP increases in EF patients. The mean CRP value among 108 pediatric EF patients was 4.3 (12–150) mg/L. CRP levels are also elevated in malaria patients and useful in assessing malaria severity and follow-up [21,22]. We have reported that the CRP values of semi-immune patients were significantly higher than those of non-immune patients [4]. In the present study, a CRP cutoff value of 10 mg/L was predictive of malaria compared to DF, with a sensitivity of 97.7% (95% CI = 91.8–99.7%) and specificity of 76.5% (95% CI = 66.0–85.0%).

On the basis of patient data on places of endemic travel, clinical manifestations, and laboratory findings, we made the flow chart to distinguish between DF, malaria, and EF at the initial hospital

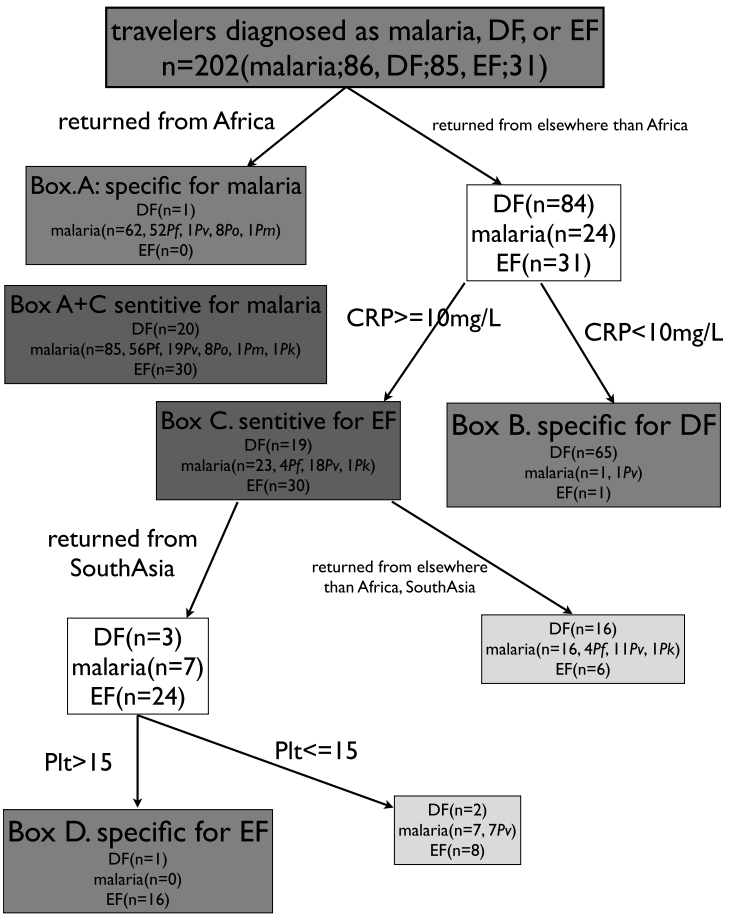


Fig. 1. The flow chart for determining dengue fever, malaria, and enteric fever for returned travelers at the first hospital presentation. DF: dengue fever, EF: enteric fever, CRP: C-reactive protein, Plt: platelet, *Pf: Plasmodium falciparum, Pv: Plasmodium vivax, Po: Plasmodium ovale, Pm: Plasmodium malariae, Pk: Plasmodium knowlesi.*

presentation. This decision tree is designed for high malaria and DF specificity and high malaria sensitivity because malaria is the most fatal of these diseases. "Returned from Africa" had a high specificity for malaria. Although DF and EF are also endemic to Africa, malaria is more prevalent. In the GeoSentinel Surveillance Network database from March 1997 to May 2011, DF and EF acquired in Africa was diagnosed in as few as 113 and 58 travelers with febrile illness, respectively, while 2789 travelers were diagnosed with malaria [23]. Travelers diagnosed with malaria returning from regions other than Africa were mainly infected with non-falciparum malaria, which is typically less severe than falciparum malaria. Febrile patients returning from regions other Africa with CRP levels <10 mg/L had a high specificity for DF, and patients returning from Africa or returning from elsewhere than Africa with CRP levels >10 mg/L had a high sensitivity of 98.84%. In this study, we found a CRP cutoff level of 10 mg/L to be useful for distinguishing DF from the other diseases in returned travelers with a high malaria and EF sensitivity.

Our study has some limitations. First, we compared only 3 diseases: malaria, DF, and EF. Although these diseases are the major causes of febrile illness in returning travelers, other diseases, such as schistosomiasis, hepatitis A, rickettsiosis, and leptospirosis, should be considered as differential diagnoses. Second, clinical manifestations were obtained from patient medical records. Doctors did not always describe clinical manifestation in detail, so some findings may be erroneously omitted. Third, geographical epidemiology could be changed by some factors, such as a large-scale outbreak of typhoid fever [24].

In conclusion, the specific region of travel, reason for travel, clinical manifestations, and simple laboratory test results could be helpful for improving the accuracy in diagnoses of DF, malaria, and EF in febrile returning travelers with non-specific symptoms. We found, leukopenia, and lower CRP levels in DF patients and increased T-bil in malaria patients to be the most diagnostic symptoms for those diseases.

Funding

This work was partly supported by funding from the Research on Emerging and Re-emerging Infectious Diseases by the Ministry of Health, Labour, and Welfare, Japan (H23-shinkou-ippan-006 and H24-shinkou-ippan-013).

Financial disclosure

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgments

We are grateful to the Department of Virology 1st, at the National Institute of Infectious Diseases for diagnosis of dengue fever, as well as to Dr. Shigeyuki Kano at the Department of Tropical Medicine and Malaria, Research Institute, in the National Center for Global Health and Medicine assisting with malaria diagnosis.

References

[1] Leder K, Torresi J, Libman MD, Cramer JP, Castelli F, Schlagenhauf P, et al. GeoSentinel surveillance of illness in returned travelers, 2007–2011. Ann Intern Med 2013;158:456–68.
[2] Kozarsky PE, Keystone JS. Introduction to travel medicine. In: Keystone JS, Kozarsky PE, Freedman DO, Nothdurft HD, Connor BA, editors. Travel medicine. 3rd ed. Edinburgh: Mosby. p. 1–3.
[3] Mizuno Y, Kato Y, Kano S, Takasaki T. Imported malaria and dengue fever in returned travelers in Japan from 2005 to 2010. Travel Med Infect Dis 2012;10:86–91.
[4] Kutsuna S, Hayakawa K, Kato Y, Fujiya Y, Mawatari M, Takeshita N, et al. The usefulness of serum C-reactive protein and total bilirubin levels for distinguishing between dengue fever and malaria in returned travelers. Am J Trop Med Hyg 2014;90:444–8.
[5] Takasaki T. Imported dengue fever/dengue hemorrhagic fever cases in Japan. Trop Med Health 2011;39:13–5.
[6] Oki M, Yamamoto T. Simulation of the probable vector density that caused the Nagasaki dengue outbreak vectored by Aedes albopictus in 1942. Epidemiol Infect 2013;141:2612–22.
[7] Kutsuna S, Kato Y, Moi ML, Kotaki A, Ota M, Shinohara K, et al. Dengue fever, Tokyo, 2014. Emerg Infect Dis 2015 [cited 2014 Nov 28. Epub ahead of print].
[8] Infectious Disease Surveillance Center; Typhoid fever 2010, http://idsc.nih.go.jp/disease/typhoid/typhoid2010.html, [accessed 17.04.14].
[9] Infectious Disease Surveillance Center, Annual Surveillance Data (page 1: notifiable diseases), http://idsc.nih.go.jp/idwr/ydata/report-Ea.html, [accessed 17.04.14.
[10] Harvey K, Esposito DH, Han P, Kozarsky P, Freedman DO, Plier DA, et al., Centers for Disease Control and Prevention (CDC). Surveillance for travel-related disease–GeoSentinel Surveillance System, United States, 1997–2011. MMWR Surveill Summ 2013;62:1–23.
[11] Keystone J. Immigrants returning home to visit friends and relatives. In: Brunette GW, Kozarsky P, Magill AJ, Shlim DR, Whatley A, editors. CDC health information for international travel. New York, NY: Oxford University Press. p. 547–551.
[12] Hellgren U. Approach to the patient with malaria. In: Keystone J, Freedman DO, Kozarsky P, editors. Travel medicine. 3rd ed. St Louis: Saunders; 2013. p. 173–7.
[13] Dengue: guidelines for diagnosis, treatment, prevention and control – 2009 new edition.
[14] Potts JA, Rothman AL. Clinical and laboratory features that distinguish dengue from other febrile illnesses in endemic populations. Trop Med Int Health 2008;13:1328–40.
[15] Kain KC, Harrington MA, Tennyson S, Keystone JS. Imported malaria: prospective analysis of problems in diagnosis and management. Clin Infect Dis 1998;27:142–9.
[16] Taylor SM, Molyneux ME, Simel DL, Meshnick SR, Juliano JJ. Does this patient have malaria? JAMA 2010;304:2048–56.
[17] Krüger S, Ewig S, Papassotiriou J, Kunde J, Marre R, von Baum H, et al. CAPNETZ Study Group. Inflammatory parameters predict etiologic patterns but do not allow for individual prediction of etiology in patients with CAP: results from the German competence network CAPNETZ. Respir Res 2009;10:65.
[18] Simon L, Gauvin F, Amre DK, Saint-Louis P, Lacroix J. Serum procalcitonin and C-reactive protein levels as markers of bacterial infection: a systematic review and meta-analysis. Clin Infect Dis 2004;39:206–17.
[19] Premaratna R, Bailey MS, Ratnasena BG, de Silva HJ. Dengue fever mimicking acute appendicitis. Trans R Soc Trop Med Hyg 2007;101:683–5.
[20] Choo KE, Davis TM, Henry RL, Chan LP. Serum C-reactive protein concentrations in Malaysian children with enteric fever. J Trop Pediatr 2001;47:211–4.
[21] Hurt N, Smith T, Tanner M, Mwankusye S, Bordmann G, Weiss NA, et al. Evaluation of C-reactive protein and haptoglobin as malaria episode markers in an area of high transmission in Africa. Trans R Soc Trop Med Hyg 1994;88:182–6.
[22] Paul R, Sinha PK, Bhattacharya R, Banerjee AK, Raychaudhuri P, Mondal J. Study of C reactive protein as a prognostic marker in malaria from Eastern India. Adv Biomed Res 2012;1:41.
[23] Mendelson M, Han PV, Vincent P, von Sonnenburg F, Cramer JP, Loutan L, et al., GeoSentinel Surveillance Network. Regional variation in travel-related illness acquired in Africa, March 1997–May 2011. Emerg Infect Dis 2014;20:532–41.
[24] Imanishi M, Kweza PF, Slayton RB, Urayai T, Ziro O, Mushayi W, et al. Household water treatment uptake during a public health response to a large typhoid fever outbreak in Harare, Zimbabwe. Am J Trop Med Hyg 2014;90:945–54.