Abstract

Background: Hepatitis and HIV are the most common co-infections in tuberculosis (TB) patients and may have an effect on the liver enzymes in these co-infected TB patients. This study aimed to determine the prevalence of Hepatitis B, C and HIV in patients infected with TB in Western Cameroon and assess the effects of co-infection on their liver function.

Materials and Methods: All TB infected patients referred to the Tuberculosis Research centres, from November 2014 to July 2015, and who gave their consent were screened for Hepatitis B surface antigen (HBsAg) & Hepatitis C virus (VHC) antibodies using enzyme linked immunoabsorbent assay (ELISA). HIV infection was confirmed using a combination of two rapid tests, namely, Combaids and Tridot. All HIV positive patients were on antiretroviral therapy during the study period. The data was entered and analysed using statistical Package for social sciences 21 (SPSS– 21), and the means and proportions were calculated.

Results: Of the 189 tuberculosis patients recruited in this study, HBsAg were detected in 24 (12.7%), anti-VHC antibodies in 8 (4.23%), HIV antibodies in 62 (32.8%), HBsAg and anti-VHC antibodies in 1 (0.5%), HBsAg and HIV antibodies in 9 (4.9%), and anti-VHC and HIV antibodies in 2 (1.1%). Estimation of liver enzymes in all co-infected and TB patients showed that a substantial proportion of our patients had normal ALP and GGT levels whereas a substantial proportion of our patients had abnormal levels of ALT and AST with patients having up to two to three-fold. All the study groups had higher baseline AST and ALT values with VHB co-infected groups having the biggest mean values.

Conclusions: The prevalence of hepatitis B and C coinfection was fairly high in this largely heterosexual population supporting the use of more careful screening methods for these viruses in tuberculosis persons in these regions. High levels of transaminases were found in our study population suggesting that all TB patient should be screened for VHB, VHC and HIV infections, then monitored carefully following the initiation of therapy.
Tuberculosis remains a leading health problem in both developing and developed countries [5]. The World Health Organization (WHO) estimated that there were 9.6 million new cases of TB globally in 2014, and 1.5 million deaths [8]. This is increased by HIV which is recognized alongside as a leading cause of death worldwide. In 2013, 1.5 [1.4–1.7] million people died from HIV-related causes globally. In the same year there were approximately 35.0 [33.2–37.2] million people living with HIV with 2.1 [1.9–2.4] million people becoming newly infected globally. Sub-Saharan Africa is the most affected region, with 24.7 [23.5–26.1] million people living with HIV that accounts for almost 70% of the global total of new HIV infections [9].

According to a recent report by the World Health Organization, Cameroon is a relatively high-TB-burden country in Central Africa with an estimated prevalence of all form including HIV was 319 [153–344]/100,000 and the incidence rate with HIV case was 238 [117–283]/100,000/100,000 resident and a case-detection rate of 60% [10].

Globally, the prevalence of VHB and VHC infections among patients with TB has not been extensively investigated, and very limited data on the rate of VHB and VHC co-infection among patients with TB exists [11]. Hepatotoxicity is the major adverse effect of three of the first line anti-TB agents: isoniazid, rifampin, and pyrazinamide [12]. Chronic liver disease raises a risk of hepatotoxicity during anti-tuberculosis treatment, which is three to five times more than that in TB patients who do not have viral infection [13]. Similarly, fourteen fold increase in the risk of anti-TB hepatotoxicity has been reported in HIV and VHC co-infected patients [14]. Patients with increased susceptibility to the hepatotoxic effects of first-line treatment regimens represent special populations and need to be identified prior to therapy initiation and monitored more carefully. Although three of the first-line drugs, rifampin, pyrazinamide, and isoniazid are known to be hepatotoxic [15–20], the patient characteristics that confer greater risk of treatment-associated liver injury are poorly understood. Older age, concurrent or chronic alcohol use, hepatitis C, hepatitis B, and HIV virus infection have been found to increase the risk of drug induce hepatotoxicity (DIH) [21-23]. Until definitive studies are conducted, caution suggests that patient populations should be screened for the above-mentioned characteristics and monitored carefully following the initiation of therapy [24]. Therefore, this study was conducted to address the prevalence of VHB, VHC and HIV infections in patients with TB and its impact on the incidence of anti-tuberculosis therapy induced hepatotoxicity.

Materials and Methods

Study area

This study was carried out at the TB unit of the Regional Hospital Bamenda [RHB] situated in the Nord-West Region of Cameroon and at the TB unit of the “Centre Médical d’Arrondissement (CMA) de Baleng Bafoussam” located in the West Region of Cameroon. The two hospitals have dedicated adult pulmonology wards primarily for admission of TB patients during the intensive phase of treatment.

Enrolled in this study were patients who were admitted on the pulmonology wards during the study period and who had a confirmed microscopic diagnosis of pulmonary TB. All the patients who refused informed consent and minors who provided assent but whose guardians/legal representatives refused to consent were excluded from the study.

This was a transversal cross sectional study in which patients with a microscopically confirmed diagnosis of pulmonary TB admitted on the TB wards of the Regional Hospital Bamenda (RHB) and CMA Baleng Bafoussam were consecutively recruited during the study period from November 2014 to July 2015. Collectively, these two study centres include more than 80% of the state’s incident TB cases and have dedicated adult pulmonology wards primarily for admission of TB patients during the intensive phase of treatment.

Ethical considerations

Ethical clearance for this study was obtained from the Ethics Review and Consultancy Committee of the Cameroon Bioethics Initiative [CAMBIN] under the reference number CBI/294/ERCC/CAMBIN on the 16th of September 2014. An authorisation to collect and analyse blood samples was also obtained from the Regional Delegation of Public Health Bamenda and the Baleng Medicalised Centre in Bafoussam. All participants were fully informed of the study goals, procedures, potential harm and benefits, cost as well as the finality of the study. They willingly provided informed consent either by signing or placing their thumbprint on the consent form after being satisfied with responses to all questions asked the investigator. Information was provided in English, French or interpreted in the local dialect by a hospital volunteer independent of the study team.

Sample size

In the present study, a convenient sample of 189 sputum positive pulmonary TB patients who visited the health facilities and who provided consent prior to the onset of any study procedure were included. These sample size was designed according to the prevalence of the co-infection between these two diseases which is 1% to 18%.

Laboratory investigation and specimen collection

All case records were investigated for clinical history (including history of intravenous drug use, other risk behaviours), physical examination findings and laboratory investigation results.

Venous blood samples of about 2–5 ml were collected aseptically from each participant by the assigned laboratory technician. These blood specimens were appropriately transported to the Laboratory for further processing to obtain sera which was then used for testing VHC antibodies, VHB antigens, HIV antibodies and the measurement of...
Prevalence of VHB, VHC and HIV among the TB patients

Using ELISA to test for hepatitis B surface antigen (HBsAg) (Hepalisa, J. Mitra & Co. Ltd/Biotech INC, India), anti-VHC antibodies (Micro Lisa, J. Mitra & Co. Ltd/Biotech INC, India) and Human immunodeficiency virus chart documentation of serologic testing and the cut off values considered as positive, HBsAg were detected in 24 (12.7%), anti-VHC antibodies in 8 (4.23%), HIV antibodies in 62 (32.8.0%), HBsAg and anti-VHC antibodies in 1 (0.53%), HBsAg and HIV antibodies in 9 (4.9%) and anti-VHC and HIV antibodies in 2 (1.1%) patients as shown in table 2.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total</th>
<th>Age (Years) Means ± SD</th>
<th>Sex Male Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB VHB</td>
<td>14</td>
<td>35.14 ± 14.816</td>
<td>14 0</td>
</tr>
<tr>
<td>TB VHC</td>
<td>5</td>
<td>66.00 ± 9.823</td>
<td>4 1</td>
</tr>
<tr>
<td>TB VHB VHC</td>
<td>1</td>
<td>35.00 ± 0.00</td>
<td>1 0</td>
</tr>
<tr>
<td>TB VHB HIV</td>
<td>9</td>
<td>38.67 ± 7.07</td>
<td>5 4</td>
</tr>
<tr>
<td>TB VHC HIV</td>
<td>2</td>
<td>61.00 ± 4.24</td>
<td>1 1</td>
</tr>
<tr>
<td>TB VHB VHC HIV</td>
<td>0</td>
<td>00 ± 00</td>
<td>0 0</td>
</tr>
<tr>
<td>TB HIV</td>
<td>62</td>
<td>38.29 ± 11.34</td>
<td>29 33</td>
</tr>
<tr>
<td>TB</td>
<td>91</td>
<td>39.41 ± 15.42</td>
<td>64 27</td>
</tr>
</tbody>
</table>

the other groups although the difference was not significant (P=0.3128). The age groups [22–35], 30 (41.1%) and [36–55], 34 (46.6%) were the most infected with HIV although the difference was not significant (P=0.197). There were more patients with primary level of education, 4 (50.00%) positive to VHB compared to other groups although the difference was not significant (P=0.098). Similarly, patients with primary level of education, 38 (52.1%) were the most positive to HIV infection compared to patients of the other groups although the difference was not significant (P=0.708). In addition, patients with secondary level of education, 15 (62.1%) were the most positive to VHB compared to the other groups although the difference was not significant (P=0.255). The married and unmarried participants in this study were highly infected with VHB compared to the widowed although the difference was not significant (P=0.48). However, married participants were the most positive to HIV infection compared to the other groups although the difference was not significant (P=0.504). Similarly, married participants were the most positive to VHC compared to the other groups although the difference was not significant as shown in table 3.

The baseline values of liver enzymes of all the infected and co-infected patients are shown in table 4.

The evaluation of hepatic enzymes (GGT, ALT, AST, and ALP) in the 189 patients showed that, only 2 patients with TB–VHB, 2 patients with TB–HIV and 4 patients had more than two-fold elevations in GGT, whereas 2 patients with TB–HIV and 5 patients with TB had more than three-fold elevations in GGT. One (1) patient with TB–VHB–HIV, 1 patient with TB–VHC–HIV and 1 patients with TB–VHC–HIV had more than three-fold elevations in ALT.

### Table 3: Comparison in socio-demographic characteristics between the different groups of co-infected TB patients in Western Cameroon.

<table>
<thead>
<tr>
<th>SEX</th>
<th>INFECTIONS</th>
<th>VIH</th>
<th>VHB</th>
<th>VHC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>négative</td>
<td>positive</td>
<td>Négative</td>
</tr>
<tr>
<td>Female</td>
<td>38 (52.1%)</td>
<td>29 (26.1%)</td>
<td>4 (16.7%)</td>
<td>65 (39.6%)</td>
</tr>
<tr>
<td>Male</td>
<td>35 (47.9%)</td>
<td>82 (73.9%)</td>
<td>20 (83.3%)</td>
<td>99 (60.4%)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.001</td>
<td>0.029</td>
<td>0.13</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>AGE</th>
<th>INFECTIONS</th>
<th>VIH</th>
<th>VHB</th>
<th>VHC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤ 21 year</td>
<td>2 (2.7%)</td>
<td>12 (10.7%)</td>
<td>3 (12.5%)</td>
</tr>
<tr>
<td></td>
<td>22-35 year</td>
<td>30 (41.1%)</td>
<td>43 (38.4%)</td>
<td>10 (41.7%)</td>
</tr>
<tr>
<td></td>
<td>36-55 years</td>
<td>34 (46.6%)</td>
<td>37 (33.0%)</td>
<td>10 (41.7%)</td>
</tr>
<tr>
<td></td>
<td>&gt; 55 years</td>
<td>7 (9.6%)</td>
<td>20 (17.9%)</td>
<td>1 (4.2%)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.197</td>
<td>0.01</td>
<td>0.318</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Level of education</th>
<th>INFECTIONS</th>
<th>VIH</th>
<th>VHB</th>
<th>VHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0 (0.0%)</td>
<td>4 (3.6%)</td>
<td>0 (0.0%)</td>
<td>4 (2.4%)</td>
</tr>
<tr>
<td>Primary</td>
<td>38 (52.1%)</td>
<td>51 (45.5%)</td>
<td>9 (37.5%)</td>
<td>81 (49.4%)</td>
</tr>
<tr>
<td>Secondary</td>
<td>31 (42.5%)</td>
<td>53 (47.3%)</td>
<td>15 (62.5%)</td>
<td>71 (43.1%)</td>
</tr>
<tr>
<td>University</td>
<td>4 (3.6%)</td>
<td>4 (5.5%)</td>
<td>0 (0.0%)</td>
<td>8 (4.9%)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.708</td>
<td>0.255</td>
<td>0.098</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Matrimonial status</th>
<th>INFECTIONS</th>
<th>VIH</th>
<th>VHB</th>
<th>VHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unmarried</td>
<td>29 (39.7%)</td>
<td>45 (40.2%)</td>
<td>12 (15.8%)</td>
<td>64 (84.2%)</td>
</tr>
<tr>
<td>Married</td>
<td>43 (58.9%)</td>
<td>64 (57.1%)</td>
<td>12 (15.8%)</td>
<td>96 (88.9%)</td>
</tr>
<tr>
<td>widowed</td>
<td>1 (1.4%)</td>
<td>3 (2.7%)</td>
<td>0 (0.0%)</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.504</td>
<td>0.48</td>
<td>0.095</td>
<td></td>
</tr>
</tbody>
</table>

### Table 4: Baseline values of liver enzymes among TB, hepatitis co-infected and HIV co-infected patients.

<table>
<thead>
<tr>
<th>Co-infection</th>
<th>Total</th>
<th>GGT (U/l)</th>
<th>ALAT (U/l)</th>
<th>ASAT (U/l)</th>
<th>PAL (U/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean SD</td>
<td>Mean SD</td>
<td>Mean SD</td>
<td>Mean SD</td>
</tr>
<tr>
<td>TB VHB</td>
<td>14</td>
<td>39.46 ± 38.55</td>
<td>316.00 ± 335.61</td>
<td>424.50 ± 284.39</td>
<td>50.52 ± 90.58</td>
</tr>
<tr>
<td>TB VHC</td>
<td>5</td>
<td>187.85 ± 352.84</td>
<td>281.57 ± 228.09</td>
<td>316.57 ± 194.23</td>
<td>46.59 ± 47.37</td>
</tr>
<tr>
<td>TB VHB VHC</td>
<td>1</td>
<td>16.57 ± 00</td>
<td>10.50 ± 00</td>
<td>567.88 ± 00</td>
<td>31.71 ± 00</td>
</tr>
<tr>
<td>TB VHB HIV</td>
<td>9</td>
<td>45.55 ± 60.58</td>
<td>350.78 ± 277.46</td>
<td>464.82 ± 355.20</td>
<td>105.99 ± 152.49</td>
</tr>
<tr>
<td>TB VHC HIV</td>
<td>2</td>
<td>13.81 ± 14.85</td>
<td>181.56 ± 82.29</td>
<td>441.00 ± 270.99</td>
<td>40.66 ± 45.81</td>
</tr>
<tr>
<td>TB VHB VHC HIV</td>
<td>0</td>
<td>00 ± 00</td>
<td>00 ± 00</td>
<td>00 ± 00</td>
<td>00 ± 00</td>
</tr>
<tr>
<td>TB HIV</td>
<td>62</td>
<td>32.74 ± 46.79</td>
<td>397.84 ± 339.87</td>
<td>389.57 ± 365.04</td>
<td>107.46 ± 123.82</td>
</tr>
<tr>
<td>TB</td>
<td>91</td>
<td>47.88 ± 133.99</td>
<td>396.01 ± 320.58</td>
<td>469.24 ± 330.41</td>
<td>69.44 ± 88.81</td>
</tr>
</tbody>
</table>

GGT= gamma glutamyl transferase; ALAT= alanine amino transferase; ASAT= aspartate amino transferase; ALP = Alkaline phosphatase
VHC – HIIV, 6 patients with TB – HIV and 8 patients had more than two-fold elevations in ALT whereas 8 patients with TB – VHB, 4 patients with TB – VHC, 6 patients with TB – VHB – HIIV, 1 patient with TB – VHC – HIIV, 42 patients with TB – HIIV and 67 patients with TB had more than three-fold elevations in ALT. Four (04) patients with TB – HIIV and 3 patients with TB had more than two-fold elevations in ALP whereas 1 patient with TB – VHB, 1 patient with TB – VHB – HIIV, 5 patients with TB – HIIV and 3 patients with TB had more than three-fold elevations in ALP. Two (02) patients with TB – VHB – HIIV, 8 patients with TB – HIIV and 10 patients with TB had more than two-fold elevations in AST, whereas 11 patients with TB – VHB, 4 patients with TB – VHC, 1 patients with TB – VHB – VHC, 6 patients with TB – VHB – HIIV, 2 patients with TB – VHC – HIIV, 41 patients with TB – HIIV and 69 patients with TB. The schematic repartition of patients having enzymatic alterations and according to the sex are represented in Figures 1 – 4 depending on the type of enzyme. It is seen from those figures that there were patients having more than two to three-fold elevation of AST and ALT.

Discussion

To our knowledge this is the first report conducted in Western Cameroon to investigate prevalence of VHB and VHC infections among TB/HIV positive and TB/HIV negative patients and the liver profile. The overall prevalence of VHB, VHC and HIV infections in the current study was 12.7%, 4.23% and 32.8.0% respectively. This prevalence of VHB (12.7%) is in agreement with the fact that Cameroon like other sub-Saharan African countries is a hyper-endemic area for hepatitis B infection (prevalence 8–20%) [25,26]. The seropositivity of VHB infection, (12.7%) found in this study was relatively higher than the 9.5% obtained in the study conducted by Nail et al., in Khartoum among TB patients, 9.4% obtained by Khalid and Bahaeldin in sudanese patients with HIV/TB co-infection, 8.92% obtained by Mengesha et al., in Ethiopia, and also higher than that reported from other studies carried out, in Thailand (9%), Georgia (4.3%), Pakistan (5.5%), Kurdistan (1.8%), and India (2.96%) [1,27–30,36-45]. However the 12.7% obtained from this study was less than the 15.3% reported by Abdallah et al., in Eastern Sudan and 18.4% reported in London by Nooredinvand et al. [31,36]. The present prevalence obtain in the present study was almost the same as the national prevalence which is 12.14% [32].The proportion of VHB/TB co-infection was found to be higher in males (10.58%) than females (2.12%). This finding is similar to other reports [27,33,34]. High rate of VHB infection among male gender in this setting might be related to the gender exposure difference between males and females. Interestingly, a male preponderance was documented recently in another study conducted among general population in Eastern Sudan [35]. The high frequency of VHB infection observed in this study may be explained by the higher national
prevalence but also lack of adherence to universal infection control measures including vaccination.

The prevalence of VHC infection in the present study was 4.23% which is higher than that reported by Nail et al. (3.5%), Abdallah et al., in Eastern Sudan (1%), and Khalid and Bahaeldin (1.9%). This was also higher than that from other reports carried out, in London (2.6%) by Nooradinov et al., and 0.9% in Kurdistan by Muayad et al. [1, 27, 31, 36, 43]. The prevalence of VHC infection among general population was reported as 4.3%–29.7% [37]. However, it was lower than that obtained from previous studies done by Reis NR et al. (7.5%) in Brazil, Richards et al. (22%) in Georgia, Akhtar et al. (9.1%) in Pakistan, Agha et al. (17.02%) in Egypt, Khalili et al. (27.45%) in Soudan, and Mengesha et al. (7.14%) in Ethiopia, [30,38-41,43,44]. The proportion of VHC/TB co-infection was found to be higher in males (3.17%) than females (1.06%). High rate of VHC infection among male gender in this setting might be related to the gender exposure difference between males and females.

The difference between the prevalence of VHB and VHC infections in the current study and other results may be attributed to use of different diagnostic techniques such as PCR, ELISA, and ICT as reported by Akhtar et al. [30]. Also the sample size may be responsible as recently reported by Abdallah et al., in the prevalence of VHB and VHC among healthy blood donors as 4, 3% and 3.1% respectively [42].

Estimation of Alanine aminotransferase (ALT), Aspartate transaminase (AST), Alkaline Phosphatase (ALP) and gamma-glutamyltransferase (GGT) are an inexpensive and invasive means of assessing liver disease as it reflects the activity of hepatotropic viruses and status of liver before and during therapy with various hepatotoxic drugs. A substantial proportion of our patients had normal ALP and GGT levels whereas a substantial proportion of our patients had abnormal levels of ALT and AST with patients having up to two to three-fold. All the study groups had higher baseline ALT and AST values with VHB co-infected groups having the biggest mean values. 68.34% male and 66.67% of female participants had more than three-fold ALT while 73.34% of male and 66.67% of female participants had more than three-fold AST. This slight difference may be due to hormonal differences between males and females but could also be due to the proportion of males and females in the present study where males were more represented.

In the present study an increase in hepatic transaminase values to more than 2 times the upper limit of normal (>50 IU) occurred in 57.14% of TB–VHB, 20% of TB–VHC, 77.78% of TB–VHB–HI, 100% of TB–VHC–HI, 77.42% of TB–VHB and 82.42% of TB for ALT and 78% of TB–VHB, 80% of TB–VHC, 88.89% of TB–VHB–HI, 100% of TB–VHC–HI, 100% of TB–VHB–VHC, 79.03 of TB–HI and 86.81% of TB for AST.

Comparing the results obtained in our study to those from previous studies, we observed that the high levels of transaminases are comparable to those obtained by Subir et al. 2013 [46], but differ to those obtained by Padmapriyadarsini et al. (2006) where substantial proportions of patients had normal ALT and AST levels before treatment [47].

The high levels of transaminases obtained in our study could be explained by the fact that a good number of our study participants were HIV patients (38.6%) who have been on ARV treatment for a long time. It has been shown that HIV attacks the liver cells directly [48], causing cell death and the release of the cellular contents into the surrounding medium, of which the enzymes constitute 20% [49]. This may be responsible for the increase in the serum liver enzymes in the infected patients. As a support to our findings, various studies have shown a significant increase in the serum AST, ALT and ALK–P activities in the ART naïve [50]. Moreover the values are more in the cases with tuberculosis. This may be due to the degeneration of the connective tissue of the liver [51] and it may also be the result of the hepatobiliary obstruction that had occurred in the subjects. This high levels of transaminases may also be due to the fact that, in our society, access to treatment is still difficult due to poverty and the fact that the majority of the population hardly visit the hospital for appropriate treatment unless their health is critical. They always concentrate on taking over-the-counter drugs which in general are drugs from unknown origin, not well preserved and most of the time are expired drugs that can lead to severe hepatotoxicity and cirrhosis as in the case of our patients.

It has been reported that VHB and VHC infections are independently and significantly associated with incidents of hepatotoxicity [52,53] but also HIV–Antiretroviral Therapy [54]. Thus co–infection of tuberculosis, HIV, VHB and VHC increase the risk of hepatotoxicity particularly during treatment of tuberculosis. Therefore, it is important to identify them so as to reduce morbidity and delay mortality.

One of the limitations of this study was that the risk factors and social behaviours for VHB and VHC among TB patients were not assessed but also the sero–prevalence of VHB and VHC were not confirmed by polymerase chain reaction (PCR). So, further study is needed to determine the risk factors of VHB and VHC among tuberculosis patients.

Conclusion

This study documents high prevalence of VHB and VHC infections among TB infected patients; suggesting more careful screening for these viruses in TB positive persons. Further, we have shown a high level of transaminases in our study population. Therefore it should be mandatory to screen every TB patient for VHB and VHC and a careful follow up should be done during TB treatment.

Acknowledgements

We wish to thank all the participants who sacrificed their time and donated their blood for this study. We also thank particularly the Director, and Nurses of Regional Hospital Bamenda and “Centre Médical d’Arrondissement de Bafoussam” for their contributions and assistance during sample collection and analysis.

Authors’ contributions

CBT, and LFS conceived the study. LFS and OIND carried out sample analysis and data collection. IMA and RB participated in analysis of the samples, data management and statistics. MN supervised the field study. LFS, OIND and ECW drafted the manuscript. All authors reviewed the manuscript and approved the final version prior to submission.

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