Background. Anaemia has been reported to affect 20 - 75% of children in South Africa. The range suggests the effects that geography, health, and socio-economic status can have on the observed prevalence of anaemia within a specific community. Our objective was to investigate the prevalence of anaemia in children aged under 5 presenting for well-child examinations at a community health centre in Thohoyandou, Limpopo Province.

Design. A cross-sectional observational study was carried out in June and July 2007. Caregivers participated in a brief interview where demographic, health and nutritional information was collected. A blood sample was collected from each child, and haemoglobin levels were assessed with a point-of-care haemoglobin testing system. Anaemia was defined as having a haemoglobin value <2 standard deviations below age-altitude adjusted normal values.

In developing countries and resource-poor settings around the world, anaemia affects 46 - 66% of children; 80% of all children in these countries will suffer some degree of anaemia before their 18th birthday. The effects of childhood anaemia have been investigated across many disciplines, and range in severity from mild and reversible developmental impairment to greater risks of infection to mortality rates as high as 12.5%. Anaemia among clinically well under-fives attending a community health centre in Venda, Limpopo Province. We aimed to study the prevalence of anaemia among children visiting a community health centre in Thohoyandou, Limpopo Province. We also present preliminary results of a follow-up study conducted between June and August 2008 investigating the prevalence of anaemia among mother-child pairs in Thohoyandou.

Methods

Patient selection

Participants were recruited from children <60 months old receiving routine care at Thohoyandou Health Centre (THC) in Thohoyandou between June and July 2007. Communication with subjects and their families was assisted by Tshivenda-speaking assistants. The study was introduced at the conclusion of routine care and, if the child’s parent or caregiver was interested, they were given time to ask questions and voice concerns. Informed consent was obtained. Children were excluded if they were clinically ill or >60 months old.

Results. Three-quarters (39/52 – 75%) of children were anaemic. Girls were significantly more likely to be anaemic than boys (20/20 v. 19/32 respectively; p=0.001). Anaemic children were significantly less likely to be underweight compared with their peers (32/38 v. 5/12 respectively; p=0.007). There was no significant association between anaemia and infection with Helicobacter pylori (p=0.729), intestinal helminths (p=1.000) or food insecurity (p=0.515).

Conclusion. We found a striking prevalence of anaemia among clinically well children <5 years old in Thohoyandou, Limpopo Province. The rates of anaemia were higher than those found in previous studies conducted in similar settings in South Africa. Future work should focus on aetiologies and interventions.
Study design

The first encounter took place immediately after consent was given by the caregiver. Caregivers provided demographic information and a focused medical history (Table I), were given a 50 ml screw-top sample jar, and asked to collect and return a stool sample as soon as possible. At the second encounter, the stool sample was collected, a haemoglobin level was obtained from capillary blood using a HemoCue 201+ point-of-care haemoglobin testing system, and household food security was assessed using the Household Food Insecurity Access Scale (HFIAS) survey. Subjects and their families were reimbursed for travel expenses. Data were recorded on paper encounter forms and transferred to a password-protected electronic database.

When it became apparent that few subjects returned for their second encounter, several were contacted; most cited childcare or domestic responsibilities as obstacles to revisiting. Therefore the first encounter was amended to include haemoglobin testing, administration of the HFIAS survey and, if possible, stool collection; patients were also given travel reimbursement in advance. If stool collection were not possible during the first encounter, subjects and their caregivers were asked to return the sample at a future date. If samples were not returned within a week, caregivers were phoned and encouraged to return the sample. If the caregiver were unreachable or still unable to return to the clinic, study personnel made home visits to collect the samples.

In June 2008, investigators conducted a follow-up study investigating the prevalence of anaemia among mother-child pairs. Pairs were recruited from two clinic sites: THC and Pfanani Clinic (PC), both in suburban Thohoyandou. Informed consent was obtained. Children were excluded if <6 months or >60 months old.

The study comprised one visit. Assisted by Tshivenda-speaking research assistants, the mothers were asked to answer a survey assessing age, gender, recent health, nutrition, and household factors pertinent to anaemia. Haemoglobin levels were tested as in the 2007 study.

Laboratory methods

Stool samples were stored in the clinic freezer immediately after collection, transported to the University of Venda (UNIVEN)’s Department of Microbiology laboratory, and stored at -20°C. Once a week, new samples were thawed and screened for presence of hookworm oocysts by light microscopy, using the oocyst concentration method. Results were validated after completion of the study by a random cross-check of 10 samples by an independent microscopist. DNA was extracted and purified from each sample, using the QIAGEN Stool DNA Purification Kit. Polymerase chain reaction (PCR) analysis was carried out on each DNA sample to determine the presence or absence of *H. pylori* infection.

Patient follow-up and treatment

Caregivers were notified of their child’s results by post in the form of typed letters in English and Tshivenda that detailed the child’s haemoglobin level and hookworm status. Patients were instructed to return to THC for treatment if the child were anaemic and/or positive for hookworms. The costs of treatment were covered by the health centre as per local protocol.

Clinical definitions

**Anaemia.** Normal haemoglobin levels vary as a child ages from birth to 60 months, and in children who live at sea level compared with those at an altitude of 1500 m, as in our patient population. Therefore, we defined anaemia in both studies as haemoglobin falling >2 SD below the mean of age-specific, altitude-adjusted haemoglobin values.

**Food security.** Food security is defined as ‘all people at all times have both physical and economic access to sufficient food to meet their dietary needs for a productive and healthy life’.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Anaemic (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>19/32 (59.4)</td>
<td>0.001</td>
</tr>
<tr>
<td>Female</td>
<td>20/20 (100)</td>
<td></td>
</tr>
<tr>
<td>Food security</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secure</td>
<td>25/32 (78.1)</td>
<td>0.515</td>
</tr>
<tr>
<td>Severely insecure</td>
<td>13/19 (68.4)</td>
<td></td>
</tr>
<tr>
<td>Dewormed in previous 6 months?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2/3 (66.7)</td>
<td>1.000</td>
</tr>
<tr>
<td>No</td>
<td>37/49 (75.5)</td>
<td></td>
</tr>
<tr>
<td>Vitamin supplements in previous 6 months?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>8/12 (66.7)</td>
<td>0.466</td>
</tr>
<tr>
<td>No</td>
<td>31/40 (77.5)</td>
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</tr>
<tr>
<td>Breastfeeding?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>30/39 (76.9)</td>
<td>0.714</td>
</tr>
<tr>
<td>No</td>
<td>9/13 (69.2)</td>
<td></td>
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<tr>
<td>Current hookworm infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>1/1 (100)</td>
<td>1.000</td>
</tr>
<tr>
<td>Negative</td>
<td>38/51 (74.5)</td>
<td></td>
</tr>
<tr>
<td>Current <em>H. pylori</em> infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>10/14 (71.4)</td>
<td>0.729</td>
</tr>
<tr>
<td>Negative</td>
<td>29/38 (73.1)</td>
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</tr>
<tr>
<td>Stunted</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>22/31 (71.0)</td>
<td>0.497</td>
</tr>
<tr>
<td>No</td>
<td>14/17 (82.4)</td>
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<tr>
<td>Underweight</td>
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<td></td>
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<tr>
<td>Yes</td>
<td>5/12 (41.7)</td>
<td>.007</td>
</tr>
<tr>
<td>No</td>
<td>32/38 (84.2)</td>
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<tr>
<td>Wasted</td>
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<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3/3 (100)</td>
<td>0.560</td>
</tr>
<tr>
<td>No</td>
<td>32/44 (72.7)</td>
<td></td>
</tr>
</tbody>
</table>

*Table I. Specific variables evaluated and their relation to prevalence of anaemia*
Growth and development. Anthropometric data were obtained from patient growth cards, when available, and plotted on World Health Organization (WHO) growth curves. Z scores were calculated by comparing weight-for-age, length-for-age, and weight-for-height. Patients >2 SD below the mean were classified as underweight, stunted, and wasted, respectively.22

Ethical approval
Ethical clearance was granted by the ethical review boards of each participating institution.

Results

2007 results
Ninety-two children were enrolled; 4 were withdrawn before data could be collected, 1 moved to Johannesburg, and 3 were withdrawn at the request of their fathers. Of the 88 patients remaining, 21 were lost to follow-up, leaving 67 who were screened for anaemia and assessed for food insecurity. Fifteen patients were excluded retrospectively for being clinically ill at the time of sample collection. In sum, 52 well children <5 years old were included in the study.

Thirty-two (61.5%) of the children were boys, and 39 (75%) were breastfed at the time of enrolment. Median household size was 5±3.6 inhabitants. The number of siblings ranged from 0 to 3.3 with a median of 1. Patients travelled 6±7.4 km to reach the clinic.

Thirty-nine (75%) out of the 52 children were found to be anaemic. The median haemoglobin concentration was 9.65±2.6 g/dl. A number of variables were assessed for significance among anaemic children (Table I). Girls were significantly more likely to be anaemic than boys (20/20 v. 19/32 respectively; p=0.001). Height data were available for 41 patients, and weight data for 51 patients. Anaemic children were significantly less likely to be underweight compared with their peers (32/38 v. 5/12 respectively; p=0.007). No significant association was observed regarding the remaining variables.

2008 results
Seventy-five mother-child pairs were enrolled; 4 pairs were subsequently excluded, 1 because the child’s gender was omitted and 3 because they were >60 months old. We analysed only prevalence rates of anaemia among children; nutritional information and the role of anaemia in mothers await final analysis.

Fifty-four (76%) out of the 71 children were found to be anaemic. There was no significant difference in anaemia prevalence between THC and PC (13/18 v. 41/53; p=0.75). The gender differences observed in 2007 were not observed in 2008.

Discussion

Childhood anaemia is treatable and preventable. Unfortunately, the resource-limited regions of the world bear the greatest burden of anaemia. Their paucity of resources to address anaemia necessitates robust surveillance programmes to identify at-risk populations and to monitor interventions to ensure effective utilisation of resources.23

We evaluated the prevalence of anaemia among clinically well children <5 years old visiting THC in Thohoyandou, Limpopo Province; to our knowledge, this is the first survey of its kind conducted in the region. Three-quarters of the participating children were anaemic. Our preliminary results from a follow-up study of the prevalence of anaemia among mother-child pairs in the same region a year later show a similar prevalence of anaemia (75% v. 76%) among children in the two studies. Intestinal helminths, H. pylori, food insecurity, concomitant illness and cited causes of anaemia did not significantly contribute to the rate observed in 2007. Anaemia was significantly more prevalent among girls in 2007.

SAVACG completed the largest study of anaemia in South Africa in 1994, observing anaemia in 20% of the 11 430 children <6 years old.9 While our prevalence finding and theirs differ strikingly, the communities they sampled varied greatly in geographical location and socio-economic status, and are therefore not a good comparison.

Agreement between our two data sets validates our argument that anaemia is highly prevalent among our study population. Further comparisons can be drawn from other studies. Faber et al observed a 65.2% prevalence of anaemia among children <2 years old in rural KwaZulu-Natal.10 Simultaneous dietary analysis pointed to diets high in carbohydrates but lacking animal protein, fruit and vegetables. Peri-urban settlements studied near Bloemfontein found 44 - 47% of children to be anaemic, also citing low iron intake as a probable aetiology.11 Nutrition is therefore a commonly cited cause of anaemia in children.

The HFIAS was used to assess access to food among households of our patients, 37% of whom came from severely food-insecure households. However, this factor alone did not seem to contribute to the development of anaemia. We did not evaluate dietary intake in 2007, and results are still pending of the dietary intake evaluated in 2008.

The effect of illness on anaemia is well documented, and we controlled for this by excluding children who were clinically ill at the time of enrolment. We investigated the effects of specific infections on the development of anaemia. Although intestinal helminths often cause anaemia in tropical areas, they did not appear to play a role in 2007. Only one child was found to have hookworms at the time of stool analysis. This low prevalence was observed previously in the area (Stanley: personal communication), and could be a result of both studies having taken place in the dry season. Children who had been dewormed in the previous 6 months were at no less risk for becoming anaemic than those without previous treatment. Intestinal helminths do not appear to play a role in the prevalence of anaemia during the dry season.

Malaria causes anaemia in Africa and is endemic in Limpopo Province, where transmission rates are lowest during June, July and August, and lowest in patients aged 0 - 4 years.24 Given the timing and target demographics of our study, we do not feel that malaria played a significant role in our results.

A 56% prevalence of H. pylori was demonstrated among children <5 years old in Thohoyandou.25 As a link has been shown between anaemia and H. pylori infection among young children, we tested for H. pylori by PCR analysis of stool sample.
samples. Our prevalence disagreed with previous findings, probably owing to differences in our patient populations; other studies were of children with acute diarrhoeal illness, while ours were well children. H. pylori is more likely to be found in the stools of the acutely ill and malnourished than in healthy individuals, which could account for these differences.26

Our most striking finding was the significant difference in prevalence of anaemia between girls and boys in 2007. This result was not replicated in 2008. This inconsistency may be explained by the fact that most patients in the 2008 study were from Pfanani. While the girls from THC were more likely to be anaemic than boys from THC in both years, the girls at Pfanani were not more likely than boys to be anaemic.

There is little literature on the relationship between gender and anaemia in South Africa. Nutritional profiles among Kenyan children also demonstrated higher rates of anaemia among girls, attributing the differences observed to social feeding practices.27 While social norms could result in differential intake of iron-rich foods between genders, we did not assess this aspect. Subsequent studies should include detailed dietary analysis and engage experts in sociology and anthropology to better understand this complex issue.

This study has several limitations. The lack of red blood cell (RBC) indices prevents further classification of anaemia, which could have helped in finding an aetiology. The original study did not include this because of uncertainty regarding laboratory facilities, cost, and a lack of understanding of the region’s anaemia burden. Another concern was the quantity of blood that was required for a complete blood count (CBC), and its implications for already anaemic children. Our sample size was also small; however, the reproducibility of our findings minimises this as a limitation. We also did not control for HIV infection. Although 20% of women in Limpopo are HIV positive, prevention of mother-to-child transmission (PMTCT) is covered in approximately 60% of cases. Therefore, it was felt that the likelihood of seropositive children enrolling was low. Future studies should plan to overcome these logistical and clinical obstacles.

While the ultimate cause is not apparent, the most probable infectious causes (hookworm and malaria) are unlikely. H. pylori, another possible cause, was also not associated with anaemia. Therefore, the paucity of iron-rich foods in the diets of children in the area may play a role in the high prevalence of anaemia. Future studies should evaluate the relationship of anaemia and gender, and aim to profile the dietary intake of children in the region.

This study would not have been possible without the support of Matron Vho Angie Mutondwa of THC. We thank Nurses Mathabela and Rambani for lessons on the people, history and health of Thohoyandou, and for their vital role in patient enrolment and sample collection. We thank Vhonani Nethsandama, of the University of Venda, for her logistical support and advice. This study was a product of collaboration between University of Venda and University of Virginia Center for Global Health, a relationship facilitated by Professor Robert Swap and the South African Virginia Networks and Associations (SAVANA). Both institutions provided us with wisdom, support and guidance, and we are deeply grateful.

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