Under-utilized approaches to control anaemia in developing countries

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1. General introduction

Anaemia

1. Extent of the problem
Precise values of the global prevalence of anaemia and iron deficiency are difficult to estimate. During the International Nutritional Anemia Consultative Group (INACG) Symposium in Durban in March 1999 it was stated by Dr. Bruno de Benoist (WHO) that 2 billion people suffered from anaemia\(^1\).

Based on evidence from iron supplementation trials, it is estimated that, on average, 50% of anaemia globally is caused by iron deficiency\(^2\). Not all iron deficiency is so severe that it will cause anaemia and it is estimated that there are about 2.5 times more iron-deficient non-anaemic than iron-deficient-anaemic individuals\(^3\).

In total, 0.8 million (1.5%) deaths worldwide are attributable to iron deficiency\(^2\).

Morbidity associated with iron deficiency and/or anaemia is a major public health problem. Iron deficiency anaemia, particular when severe has been associated with: reduced levels of energy and productivity\(^4\), reproductive failure in women (peri-natal mortality, pre-term delivery, low birth weight)\(^5\), maternal death\(^6\), reduced immuno-competence\(^7\), pica\(^7\), decreased cognitive function in children\(^8\), increased incidence of accidents at work\(^8\), increased child mortality\(^9\) and impaired psychomotor development in children. However a recent review of studies on the effect of iron deficiency on cognitive development in children concluded that in children < 2 years the evidence was not convincing and in children aged > 2 years the evidence was reasonable convincing but not conclusive\(^10\).

The burden of a disease is often expressed as disability-adjusted life years (DALYs). One DALY can be considered as one lost year of healthy life and is a summary measure of the health of a population. The DALYs associated with iron deficiency anaemia are estimated to be a loss of about 35 million healthy life years (2.4% of global DALYs)\(^2\).

2. Definition
Anaemia is defined on the basis of the haemoglobin concentration in the blood. The cut-off values proposed by the World Health Organisation are shown in table 1.\(^"\)
Table 1  Haemoglobin and haematocrit values used to define anaemia in populations at sea level

<table>
<thead>
<tr>
<th>Age or gender group</th>
<th>Haemoglobin below (g/L)</th>
<th>Haematocrit below (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children 6 months – 5 years</td>
<td>110</td>
<td>33</td>
</tr>
<tr>
<td>Children 6 – 11 years</td>
<td>115</td>
<td>34</td>
</tr>
<tr>
<td>Children 12 – 13 years</td>
<td>120</td>
<td>36</td>
</tr>
<tr>
<td>Nonpregnant women</td>
<td>120</td>
<td>36</td>
</tr>
<tr>
<td>Pregnant women</td>
<td>110</td>
<td>33</td>
</tr>
<tr>
<td>Men</td>
<td>130</td>
<td>39</td>
</tr>
</tbody>
</table>

To adjust for altitude a correction is required as outlined in table 2.\(^{12}\)

Table 2  Correction estimates for altitude adjustment

<table>
<thead>
<tr>
<th>Meters above sea level</th>
<th>Haemoglobin correction (g/L)</th>
<th>Haematocrit correction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>914 – 1218</td>
<td>+ 2.0</td>
<td>+ 0.5</td>
</tr>
<tr>
<td>1219 – 1523</td>
<td>+ 3.0</td>
<td>+ 1.0</td>
</tr>
<tr>
<td>1524 – 1827</td>
<td>+ 5.0</td>
<td>+ 1.5</td>
</tr>
<tr>
<td>1828- 2133</td>
<td>+ 7.0</td>
<td>+ 2.0</td>
</tr>
</tbody>
</table>

3. Vulnerable groups
The groups with a high prevalence of anaemia are pregnant women and the elderly, both with estimated prevalences of 50%. In other groups the highest prevalence is reported amongst infants and children of 1-2 years of age, with prevalence estimates of about 50%. School-age children, non-pregnant women, adolescents and pre-school children have estimated prevalences of 40%, 35%, 30-55% and 25% respectively\(^{12}\). These are average figures and certain groups within populations, such as the socially deprived, may have much higher prevalences. These measurements indicate that many people are vulnerable during extended parts of their life.

4. Causes
The causes for anaemia can be divided into a number of main categories: nutrient deficiencies, infection and genetic disorders, or multi-factorial aetiologies.
4.1 Deficiencies in iron, vitamin A, folate, vitamin B

Iron deficiency is the most important nutritional cause of anaemia and often relates to an inadequate iron intake and/or poor bioavailability of iron. Iron is present in the food in two forms: haem iron and non-haem iron. Haem iron is present in meat, poultry, fish and blood products and has good absorption (25%). Non-haem iron is found in a variety of sources: vegetables, animal non-haem iron and contaminant iron originating from soil, dust, water, iron supplementation, or from iron leached into food prepared in iron pots or utensils. This non-haem iron equilibrates in different proportions with, the common pool of non-haem iron, from which a variable amount of iron is absorbed. The amount of iron that is absorbed, and is thus bioavailable, is influenced by several factors: the iron status of the individual, promoting ligands (ascorbic acid, fructose, cysteine), inhibiting ligands (polyphenol, phytates, phosphoprotein).

Vitamin A deficiency has been associated with anaemia in children and pregnant women. A trial in pregnant women in Indonesia reported that iron supplementation combined with vitamin A supplementation gave a better haematological response than either alone. A further study from Indonesia observed that vitamin A deficient infants and mothers were 2.5 and 3.8 times respectively at greater risk of anaemia, and 2.4 and 4.8 times greater risk of iron deficiency. In a study where adult male volunteers were made vitamin A deficient their haemoglobin concentrations fell from approximately 150 g/L to less than 110 g/L within 12 months. Supplementation with iron had no effect on correcting their anaemia, which responded when vitamin A supplements were also taken. Vitamin A is believed to improve absorption and mobilisation of iron and enhance host immunity decreasing the risk and/or severity of infections which contribute to the development of anaemia.

Folate deficiency causes megaloblastic anaemia, but the extent to which it influences the global prevalence of anaemia is unclear. Folate deficiency occurs during periods of rapid growth, e.g. during foetal growth and in adolescents and pregnant women. The association of folate deficiency with neural tube defects is well described in industrialised countries and partly relates to the occurrence of the genetic polymorphism methylene tetrahydrofolate reductase deficiency, which is common amongst Caucasian populations (6 – 12%). This is one of the important indications for pre-conceptional folate supplements. Other reasons for considering folate supplementation include reduction of pre-term delivery and reduction of raised plasma homocysteine levels, which are associated coronary heart disease and stroke. A recent review on folate deficiency found little evidence that folate deficiency was a public health problem in many developing countries. However folate requirements are increased secondary to chronic malaria haemolysis. This is particularly important in areas were pregnant women have marginal folate intakes.

Vitamin B deficiency causes megaloblastic anaemia. This vitamin is only found in animal products and a deficiency can develop due to dietary inadequacy, malabsorption or disturbed metabolism. There is little information on the
Vitamin B₂ status of many developing country populations, although deficiency of the vitamin has recently been reported to be frequent in pregnant women in Africa.²⁵

4.2 Infection
There are several mechanisms by which infection causes anaemia. Acute infection can lead to anaemia if the person is dependent on a rapid rate of erythropoiesis, which is depressed by acute infection. Haemolysis can also result from infection through one of a number of mechanisms such as disseminated intravascular coagulation (DIC), microangiopathic haemolysis following septicaemia or viraemia and immunological or idiosyncratic mechanisms.²⁴

Chronic infection can result in the anaemia of chronic disorders which may relate to one of several mechanisms such as the sequestration of iron into the reticuloendothelial system (RES), reduction of red cell life span and depression of the production of erythropoietin.²⁴ Related mechanisms may also explain the anaemia of chronic non-infective inflammatory disorders, eg rheumatoid arthritis.²⁶

Common infections important in the aetiology of anaemia are malaria, helminthiic and schistosome infection, as well as tuberculosis and human immunodeficiency virus (HIV).

4.2.1 Malaria
It is estimated that in 1998 almost 300 million people experienced a clinical episode of malaria and in 2001 it was estimated that 1.1 million people died due to malaria, and of these deaths more than 85% occurred in Africa.²⁻²⁷ The DALYs associated with malaria in 2001 were approximately 42 million healthy life years of which 85% occurred in Africa. In Africa the DALYs lost due to malaria constituted 10% of all DALYs lost due to disease in the continent.²

There are four plasmodium species which cause malaria in humans: *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale* and *Plasmodium malariae*. *Plasmodium falciparum* is the main cause of morbidity and mortality.

The usual way of infection in humans is by a bite of a infected female *Anopheline* mosquito, which injects saliva containing malaria sporozoites. These sporozoites gain access to the circulation and then to the hepatocytes where they develop, asexually into pre-erythrocytic schizonts. Within the erythrocyte the parasite (trophozoite) develops into a erythrocytic schizont. These schizonts contain merozoites which, are released into the blood stream by rupture of infected erythrocytes. In *Plasmodium vivax* and *Plasmodium ovale* dormant hepatic stages also occur, which is the mechanism explaining the prolonged incubation period and relapse with these species.

When parasitised red cells rupture the erythrocytic schizonts release new merozoites into the blood. Some of the merozoites do not develop into trophozoites but into gametocytes. These are the infective forms to *Anopheline* mosquitoes, which when ingested form a zygote which penetrates the mosquitoes midgut.
There it encysts as an oocyst and grows by asexual division. The mature sporozoites then migrate to the salivary glands of the mosquito.\textsuperscript{28}

Malaria causes anaemia by several different mechanisms. It exerts a positive selection pressure on the prevalence of thalassaemias, sickle cell disorders and glucose-6-phosphate dehydrogenase (G6PD) deficiency. These genetic disorders offer variable degrees of protection against \textit{Plasmodium falciparum} malaria, although they may also directly cause anaemia.\textsuperscript{29-34} \textit{Plasmodium falciparum} malaria also gives rise to haemolysis and dyerythropoiesis.\textsuperscript{35-36} The survival of uninfected erythrocytes is reduced possibly due to immune complexes which are absorbed onto the surface of red cells leading to either phagocytosis, or complement-mediated lysis.\textsuperscript{37}

In areas with stable malaria transmission, eg hyperendemic and holoendemic areas children and pregnant women (especially adolescent primigravidae) are most at risk for severe malaria.\textsuperscript{38} In areas with unstable malaria transmission, all age groups are at risk for severe malaria and epidemic malaria can occur.

\subsection*{4.2.2 Helminthic and Schistosome infections}

It has been estimated that approximately 1.2 billion people are infected with hookworms (\textit{Ancylostoma duodenale} and \textit{Necator americanus}) and one billion people with whipworm (\textit{Trichurus trichiura}). Of these 98 million people with hookworms and 130 million with \textit{Trichurus trichiura} have clinically demonstrable morbidity.\textsuperscript{39} The DALYs associated with these two nematode infections in 2001 were approximately 1.6 million and 1.8 million respectively. In the World Health Report (2002) hookworm and \textit{Trichurus} infections were reported to have caused 4000 and 2000 deaths world wide during 2001.\textsuperscript{4} Undoubtedly the mortality, caused indirectly by anaemia or other co-morbidity, is much higher.

Hookworms are nematodes. The adult worm is about 1 cm long and lives in the small intestine. Eggs are passed by the female worm into the intestinal lumen and are excreted into environment. Under the right conditions the infective third stage larvae develop within one week. These infective larvae can survive in the soil for months. Infection of humans most often occurs when infective larvae penetrate the intact skin, or when larvae from \textit{Ancylostoma duodenale} are swallowed and directly reach the small intestine. Hookworm infections cause anaemia by inducing chronic blood loss from the small bowel which occurs when the hookworm attaches itself to the gut mucosa to feed on blood. Hookworms, when feeding, secrete an anticoagulant that causes the lesions to continue to bleed even after the worms have finished feeding. A single \textit{Ancylostoma duodenale} or \textit{Necator americanus} causes an average blood loss of 0.08 ml and 0.031 ml respectively per day.\textsuperscript{39} The intensity of the hookworm infection, the extent of the lesions, as well as the daily absorption of iron, the size of the body iron stores, the iron requirement of the body and co-infections may all negatively influence iron balance. These factors highlight that children are likely to be at greatest risk and women in their child bearing years because of their high iron requirements and their risk of hookworm infection.
Trichuris trichiura are 3 – 4.5 cm long and the anterior part of their body is thread-like and allows attachment to the mucosa of the large intestine. They live mostly in the caecum, but with severe infection may spread throughout the large intestine to the ileum. They do not feed on blood but on tissue juices. Eggs are passed by female worms into the intestinal lumen and are then excreted. Under the right conditions eggs become embryonated and infective in about 2 weeks. Infection of humans occurs through swallowing infective eggs which hatch when the larvae reach the intestines.

Anaemia is caused by mucosal irritation by the attached worms and bleeding occurs from the inflamed colonic mucosa. There may be an association with secondary parasitic or bacterial infection. Heavy infections with Trichuris trichiura can cause a blood loss of up to 4 ml per day.\textsuperscript{24} The highest prevalence is in children of primary school age who play in contaminated soil.\textsuperscript{39}

It is estimated that 200 million people worldwide are infected with schistosomes of whom 120 million have clinical symptoms and 20 million have severe debilitating symptoms.\textsuperscript{40} The DALYs associated with schistosomiasis in 2001 were approximately 1.7 million healthy life years. Accurate mortality rates are difficult to obtain. In the World Health Report (2002) schistosomiasis was said to have caused 15,000 deaths worldwide in 2001.\textsuperscript{2} Again, as with infection with hookworm and Trichuris trichiura, the true mortality rate, caused by anaemia or other co-morbidity, is undoubtedly higher.

Schistosomes are trematodes, of 1 – 2 cm length. There are five species which infect humans, of which the three commonest are: Schistosoma mansoni, Schistosoma japonicum and Schistosoma haematobium. Schistosoma mansoni and Schistosoma japonicum inhabit the venules of the mesenteric veins and Schistosoma haematobium the vesical plexus. The first two species cause intestinal schistosomiasis and the latter urinary schistosomiasis. The female worms shed their eggs which migrate through the wall of the intestinal or urinary tract and are excreted from the body. When they are placed in fresh water they hatch and a ciliated miracidium emerges. The miracidium needs to enter a suitable intermediate host (snail species) to develop further over several weeks into a cercaria, an infective form to humans. Cercariae penetrate the skin and migrate to the liver where they develop into their mature form. Subsequently male and female worms couple and migrate to their final habitat.

All three Schistosome species cause anaemia but this is most firmly established for Schistosoma haematobium.\textsuperscript{11} Schistosoma haematobium causes anaemia because of haematuria which can be macroscopic and reaching even 60 – 80 ml of blood daily.\textsuperscript{24} Schistosoma mansoni and Schistosoma japonicum cause anaemia through several mechanisms: blood loss from ulcers and polyps, anaemia of chronic inflammation and from the complications of severe disease.

The highest prevalence of schistosomiasis is found in children of primary school age and in specific occupational groups (eg fishermen, irrigation workers).\textsuperscript{40}
4.2.3 Tuberculosis

Mycobacterium tuberculosis infections in 1997 had a global prevalence estimated at 1.86 billion people. In 2001 the incidence rate of tuberculosis was estimated at 8.7 million people annually, of which 3.8 million were smear-positive cases. The DALYs associated with tuberculosis were estimated as 36 million healthy life years.\textsuperscript{1,41-42} For 2001 it was estimated that tuberculosis caused 1.6 million deaths, many of which were related to HIV infection.\textsuperscript{3}

Infection in humans occurs through inhalation of Mycobacteria in droplets of sputum from a patient, or ingestion of Mycobacterium bovis in food or milk.\textsuperscript{24} Tuberculosis causes anaemia through chronic inflammation and nutrient deficiencies of vitamin B\textsubscript{12}, folate and iron due to malabsorption and/or anorexia (secondary to tuberculosis). Metastatic fibrocystic granulomas in the bone marrow may also impair erythropoiesis.\textsuperscript{24} A rare cause is severe haemolytic anaemia in disseminated tuberculosis.\textsuperscript{43}

Especially at risk for tuberculosis are children and individuals with reduced immuno-competence, especially related to HIV infection. The HIV/AIDS epidemic is driving the tuberculosis epidemic which is further complicated by increasing numbers of multidrug resistant tuberculosis cases.\textsuperscript{44} The World Health Organisation in 1993 declared tuberculosis a global health emergency.

4.2.4 HIV infection

It is estimated that currently 42 million people worldwide are infected with the human immunodeficiency virus (HIV) and in 2002 five million people became newly infected with HIV and 3.1 million died due to AIDS.\textsuperscript{45}

The DALYs contributed by HIV/AIDS worldwide were 88 million healthy life years, which is 6% of the DALYs from all diseases. In Africa where 70% of people with HIV/AIDS live, the DALYs lost due to HIV/AIDS were 18.8% of the total DALYs lost due to disease in that continent.\textsuperscript{2}

Human immunodeficiency virus is a RNA virus belonging to the lentivirus group. Two distinct types occur in humans, type 1 and type 2. Type 1 is the most prevalent in the world and is further divided in groups and subtypes.\textsuperscript{45}

Infection in humans occurs when virus particles enter the body due to trauma during unprotected sexual contact (vaginal and anal), administration of infected blood or blood products, through contaminated instruments (needles etc) and transmission from mother to child (before, during or after birth). Heterosexual and mother to child transmission are the most important routes of infection in Africa.\textsuperscript{45}

In a United States study anaemia in HIV infected people was significantly associated with increased risk for death and that who recovered from anaemia had significantly higher median survival.\textsuperscript{46} Anaemia is common in people infected with HIV and is often caused by cytopenia, which can have several causes, eg intercurrent infections and drugs given in the treatment of AIDS. The most consistent haematological defect that occurs in HIV infected individuals is a regenerative bone marrow failure.\textsuperscript{47} Why this bone marrow failure occurs is not clearly understood. It could be an effect of direct infection of the haematopoietic
precursor cells as has been shown with megakaryocytes, however for stem cells this has not been shown.\textsuperscript{48-50} A study by Shen at al showed that multiple precursors in the haematopoietic development could be infected, but not stem cells due to a blockade at the level of viral-cell membrane fusion and entry.\textsuperscript{51} Though stem cells can not be infected directly, indirect effects by HIV infection have been suggested and seem to be due to HIV infection of the stromal layers in the bone marrow.\textsuperscript{51,52}

4.3 Genetic disorders
Thalassaemia and sickle cell disease are important genetic causes of anaemia. Haemolysis associated with glucose-6-phosphate dehydrogenase deficiency is also an important contributor.

4.3.1 Thalassaemia
Thalassaemias are inherited disorders of haemoglobin synthesis which occur as a result of disorders in the rate of the synthesis of the globin chains that form the haemoglobin molecule. Depending on which globin chain is affected, α or β, there are two main variants of thalassaemia: α or β thalassaemia.

α-thalassaemia occurs in the Mediterranean region, Africa, Middle East and South East Asia. α\textsuperscript{0} and α\textsuperscript{+} thalassaemia gene frequencies vary between 5\% and 70\% in these areas.\textsuperscript{53} α-thalassaemia is divided into five genotypes: heterozygous α\textsuperscript{+} thalassaemia (-α/αα), homozygous α\textsuperscript{+} thalassaemia (-α/-α), heterozygous α\textsuperscript{0} thalassaemia (-/-α), heterozygous α\textsuperscript{0} thalassaemia/α\textsuperscript{+} thalassaemia (-/-α) and homozygous α\textsuperscript{0} thalassaemia (-/-). These genotypes are expressed in four clinical types: silent carrier (-α/αα), α thalassaemia trait ((-α/-α) or (-/-αα)), haemoglobin H disease (−/-α) and haemoglobin Bart's hydrops fetalis.

Silent carriers are clinically normal and have only a small reduction in their mean haemoglobin (3.4 g/l) compared with the reference population, as shown for example in a study conducted in Nigeria.\textsuperscript{54,55} Sometimes their haemoglobin level is normal as in a Brazilian study.\textsuperscript{56}

People with α-thalassaemia trait usually have a lower haemoglobin than the reference population and this manifest as a mild anaemia.\textsuperscript{57} Haemoglobin H disease gives rise to haemoglobin values between 70 to 100 g/l, but these values can be further reduced when a haemolytic crises occurs due to infection or exposure to oxidant drugs or pregnancy.\textsuperscript{58} Haemoglobin Bart's hydrops fetalis is a condition which is not compatible with life, and these children die in the third trimester of gestation or shortly after birth.

Anaemia in thalassaemia results from haemolysis and reduced haemoglobin production. α-thalassaemia offers some degree of protection from severe malaria. This was first postulated by JBS Haldane in 1949 with reference to β-thalassaemia. Its protective effect against malaria was considered an explanation for the high prevalence in malarious areas.\textsuperscript{59} Later research has shown that people with α-thalassaemia trait have, depending on their genotype, [(-/-αα) or (-α/-α)], between 34\% and 60\% protection against severe malaria.\textsuperscript{60}
Although the mechanisms for this protection are still incompletely understood, immunological mechanism are probably important.\textsuperscript{59}

\(\beta\)-thalassaemia occurs in populations in the Mediterranean region, Middle East, India and South East Asia.\textsuperscript{53} They are classified on clinical grounds into three groups: thalassaemia major, thalassaemia intermedia and thalassaemia minor.

In thalassaemia major there is severe anaemia (haemoglobin 20-80 g/l) and there are skeletal abnormalities due to the massive expansion of the bone marrow cavity, as a result of increased iron absorption. Due to decreased iron utilisation, iron is stored in the tissues leading to iron overload. Marked hepato-splenomegaly occurs. Individuals with this disease require regular blood transfusion during childhood. Survival until adulthood is compromised especially without regular transfusions.\textsuperscript{24} Thalassaemia intermedia has a clinical picture varying from a moderate haemolytic anaemia to that resembling thalassaemia major. Thalassaemia minor may have no clinical symptoms except a mild anaemia in the range of 90 – 110 g/l.\textsuperscript{24} Anaemia is caused by haemolysis and decreased red blood cell production.

\(\beta\)-thalassaemias have a protective effect against malaria. In a study in Liberia it was found that children with thalassaemia minor showed a relative resistance to malaria.\textsuperscript{30} Although the mechanism for this protection are not completely understood they probably relate to immunological factors and direct effect of fetal haemoglobin.\textsuperscript{59}

\textbf{4.3.2 Sickle cell disease}

Sickle cell disease results from a point mutation on the \(\beta\) globin gene, which leads to the production of an aberrant \(\beta\) globin (\(\beta^S\)). If combined with normal \(\alpha\) globin chains this forms sickle haemoglobin (HbS). The homozygous state for the sickle cell gene leads to the clinical picture of sickle cell disease in which only sickle haemoglobin (HbS) is produced together with variable amounts of fetal haemoglobin. The heterozygous state for the sickle cell gene leads to the clinical picture of sickle cell trait in which about 60% normal haemoglobin is produced and 40% haemoglobin S.\textsuperscript{60}

In 1992 world wide prevalence was approximately 78 million carriers of sickle cell trait, with 65 million (90.3%) in Africa. In Africa gene frequencies of HbS of over 0.15 have been reported, indicating that over 30% of the individuals have sickle cell trait.\textsuperscript{61}

Sickle cell disease causes anaemia which in the steady state results in average haemoglobin values of 60 –100 g/l, or lower during anaemic crises. These crises are often caused by malaria, acute splenic sequestration and folate deficiency. Aplastic crises can result in life-threatening anaemias.\textsuperscript{24} Conversely sickle cell trait causes no anaemia and there are no clinical abnormalities.

Anaemia is caused because the sickle haemoglobin forms rod-like fibres in a de-ox y configuration, which causes the erythrocytes to sickle. This sickling leads to increased rigidity, aggregation in the microcirculation and augmented interaction with vascular endothelium, leading to haemolysis of red cells and phagocytosis within the reticuloendothelial system (RES).
There is extensive evidence which indicates that sickle cell trait is protective against *Plasmodium falciparum* malaria. Recent research indicates that the protective effect reduces mortality from *Plasmodium falciparum* malaria and the severity of malaria infection.\textsuperscript{31,32,60}

### 4.3.3 G6PD deficiency

Glucose-6-phosphate dehydrogenase (G6PD) is the most important enzyme which can be deficient in the red cell. G6PD reduces nicotinamide adenine dinucleotide phosphate (NADP) into reduced nicotinamide adenine dinucleotide phosphate (NADPH). NADPH is used to regenerate reduced glutathione from oxidized glutathione. Reduced glutathione protects both the red cell membrane and haemoglobin against oxidation.

G6PD deficiency affects about 400 million people worldwide, with the highest prevalence in tropical Africa, the Middle East, sub-tropical Asia and the Mediterranean region. It is caused by a mutation on the gene that codes for glucose-6-phosphate dehydrogenase which is located on the X chromosome and over 400 different mutations of this gene have been found.\textsuperscript{60}

G6PD deficiency is classified into class I to V according to the G6PD activity present ranging from nearly absent to increased (> 150%).\textsuperscript{24} Only G6PD deficiencies from class I to III cause anaemia. G6PD deficiency class I gives the clinical situation of a non-spherocytic haemolytic anaemia. Class II and III give rise to intermittent haemolysis and less severe intermittent haemolysis respectively. These intermittent episodes can occur in the neonatal period and be induced by infections, consumption of certain foods eg favism and drugs.\textsuperscript{24}

Anaemia is caused by the inability of the red cell to protect itself from oxidation of the red blood cell membrane and haemoglobin, which leads to lysis of the red cell.

G6PD deficiency is protective against *Plasmodium falciparum* malaria. This has been shown in cross-sectional and case-control studies.\textsuperscript{62,63} The mechanisms of this protective effect are not fully understood, but it has been suggested that the impaired anti-oxidant defence in ring-stage parasitised erythrocytes, which are G6PD deficient, leads to membrane damage and consequently increased phagocytosis of these parasitised cells.\textsuperscript{34}

### 4.4 Multi-factorial anaemia

A list of causes of anaemia could suggest an oversimplified picture of the problem of anaemia in the world. This could give the impression that anaemia is caused by distinct and separate causes, however several factors may contribute to anaemia in any particular individual.

Van den Broek and Letsky showed in a study amongst 150 anaemic pregnant women in South Malawi that only 23 % were iron deficient, whereas a further 58% were deficient in iron and a second micronutrient, or deficient in other micronutrients only. An important cause is chronic inflammation which may occur with nutritional deficits.\textsuperscript{35}

A review by Corbett et al on the effect that malaria, sexually transmitted diseases and tuberculosis have on the HIV epidemic concluded that interactions are
common. For example HIV increases the risk for clinical malaria parasitaemia in pregnant women. Whitworth et al reported in a study from Uganda that HIV positive non-pregnant adults also have an increased frequency of clinical malaria and parasitaemia. These findings require confirmation. Malaria may have a direct effect on HIV, as a study by Hoffman et al reported that HIV infected adults with acute malaria had seven times higher concentration of HIV-1 RNA in their blood compared to HIV positive adults without acute malaria. This was reversible with effective malaria treatment. There are no clear-cut findings in children on the interaction of HIV and malaria.

HIV also reactivates latent tuberculosis and increases the risk of progressive disease and in this way fuels the tuberculosis epidemic. Antelman et al reported that in HIV infected pregnant women, infectious diseases, and possibly vitamin A deficiency, contributed to their anaemia.

5 Anaemia control strategies

One of the most important aspects which needs to be taken into account with every intervention, is the acceptability of that intervention to the target population. An intervention can have a high efficacy and be cost effective, but if it is not also acceptable to the population then little can be expected from it.

Reduction of poverty is an important general strategy to reduce anaemia, as increased access to (health) education and health care is more likely to improve when poverty is reduced. Also the affordability of specific interventions (eg insecticide treated bed nets) should increase. Poverty is an important reason for the reduced consumption of animal products which are good sources for highly bio-available iron and vitamin A.

5.1 Supplementation

Iron supplements have been shown to be an effective intervention amongst pregnant women by improving haemoglobin and iron status. They should be combined with malaria chemoprophylaxis in malaria endemic regions. Also in children studies have shown that iron supplements are effective in improving haemoglobin values and reducing anaemia. A recent Cochrane review (including 7 studies) on the effectiveness of iron therapy for improving psychomotor development and cognitive function in children (under three years) with iron deficiency anaemia concluded that there was no convincing evidence for this to be the case for changes discernable after a short time and that long term follow-up research is necessary to determine their long term effects. There is a increasing number of studies which report on the effects of iron supplementation given to older children and indicate that there is an influence on cognitive function.

There are still many constraints in iron supplementation programmes which prevent them making their full public health impact. These constraints relate to supply and logistics, service delivery systems, training of staff, the form of iron supplement and compliance. Some programmes in the Pacific and Asian regions have reported that their most important constraints were related to the supply of iron supplements and logistics. There is some concern that iron supplementation
increases malaria morbidity. A review by Shanker et al reported small increases in malaria prevalence due to iron supplementation, although this was accompanied by a significant improvement of haemoglobin levels and a reduction of the risk of severe malaria by 50%.77

Vitamin A supplementation has been given routinely as oral supplementation post-partum and at child health centres. An effective route is to deliver vitamin A supplements with the EPI programmes, or by routine supplementation with vitamin A every 4-6 months to young children.12

5.2 Fortification

Food fortification with iron has large potential to improve the iron status of whole communities and is a long term, cost-effective intervention.78 An attractive aspect of this intervention is that to be effective it does not require changes in the customary diet, or compliance in an individual. In developing countries in areas where many households live from subsistence farming, food fortification programmes may have limited effect due to the small amount of commercially processed foods consumed by those households.78

It is essential with food fortification that the appropriate food vehicle is selected and that this fulfils certain criteria. Some of the criteria are: the known frequency and average consumption of the vehicle by the targeted population; risk of over-consumption; change in the organoleptic qualities of the vehicle due to the added iron; possibility to process the fortification of the vehicle centrally, and the price of the fortified product is affordable to the target population.78,79 The cooperation of the government is important to support the food fortification project and if necessary to change, or make legislation which permits, regulates and requires the iron fortification of certain foods.78

Bioavailability of the selected iron compound with which the food is fortified is important but other factors should be taken in account. These relate to shelf life, ease of storage and costs. There are four formulas that contain iron and are suitable to be added to food. Ferrous sulphate, the availability of which is reduced by phytates and tannins in the diet. The shelf life is limited to approximately 6 months in bulk cereals and it causes taste and colour changes in some foods. Ferrous fumarate is relatively expensive and has a limited shelf life. Ferrous lactate can be used only in liquid foods and is expensive. Elemental iron is inexpensive but needs to have a small particle size to ensure good absorption.12,79 These constraints are an important reason why food fortification programmes have not made as great an impact as anticipated. A new and promising form of iron for use in iron fortification programmes is sodium iron ethylenediaminetraacetic acid (iron-EDTA). This substance, compared to the other available iron forms, is better absorbed and less sensitive to food iron inhibitors. An important step in promoting the use of iron-EDTA would be the acquiring of GRAS status (generally recognised as safe) from the Food and Drug Administration for wheat flour fortified with iron-EDTA in the United States.79

A wide range of foods can be fortified, eg wheat or maize flour, sugar, salt, condiments and soy sauce. These interventions are aiming to improve the iron
status of the general population. Since infants and young children, who are especially at risk for iron deficiency, do not consume these foods, or in insufficient quantity, it is also important to fortify foods and products, which are consumed by them in adequate amounts. This is the reason why cereals, milk and modified infant formula's need to be fortified.78

There are not many studies which have measured the efficacy and/or effectiveness of iron fortification on iron status. One trial in which iron and iodine fortified salt was used in school children showed a significant increase in haemoglobin.80 In Ghana infants between the age 6 to 12 months were given a cereal-legume blend fortified with iron and other micronutrients while a control group received an unfortified cereal-legume blend. In the group which received fortified food the prevalence of low ferritin values decreased from 18% to 11%, while in the control group the prevalence increased from 19% to 55%.81

In Venezuela in 1993 maize and wheat flour were enriched with iron and other micronutrients and in 1994 the prevalence of anaemia in the age group 7 to 15 years had decreased from 19% to 9%.82 These studies showed that food fortification can be used to fortify foods with several micronutrients.

There are sometimes concerns raised about the risk that iron fortified staple foods may lead to iron overload especially in susceptible people. The general consensus is that the amount of iron ingested from these products, while enough to improve the iron status in a population, is too small to pose a significant risk for this to occur, even in people homozygous for haemochromatosis. Also from a public health point of view it is likely that the potential benefit of an iron intervention, to a predominantly iron-deficient population, vastly outweighs any risk this may pose for a few individuals.79,83

5.3 Dietary improvements
Strategies targeting dietary improvements could be especially important and effective as a strategy as they can encompass several deficiencies at the same time. For example a higher intake of animal products improves intake of riboflavin, vitamin B12, iron, zinc and vitamin A.83 Altering diets so that they contain more iron promoting ligands such as meat, fish and vitamin C could be a useful intervention, although more research is required before these interventions can be implemented in programmes.12

With regard to vitamin A, a promising food is red palm oil. It is extremely rich in carotene (approximately 500 µg/g) and vitamin E (approximately 33 mg/g), and because it is a fat, it increases the energy density of the food.12,84 Some trials of its use in humans have shown that it is effective in raising the serum retinol in children, lactating mothers as well as in their infants.85,86 One of the biggest challenges is that for this intervention to be successful it is necessary to change peoples dietary habits and/or cooking habits.

5.4 Under-utilized interventions to control iron deficiency
The fact that iron deficiency and iron deficiency anaemia, despite current strategies, continue to be major public health problems shows clearly that there is
a need for innovative interventions. Two such interventions are: cooking in iron pots/utensils and delayed umbilical cord clamping.

5.4.1 Cooking in iron pots/utensils
A non-food source, which is a potentially low cost sustainable intervention, is the use of cast iron utensils for cooking. A number of studies have shown their utility for increasing the iron content of food.\textsuperscript{87-93} Martinez and Vannucchi reported, using a rodent model, that iron added in this manner was bioavailable\textsuperscript{94}, and two randomised controlled trials in humans have demonstrated beneficial effects of consumption of food prepared in iron pots.\textsuperscript{92,95} It is still unclear what form of iron is added to the food during preparation and how it binds to components of the diet, such as phytate and polyphenols. Ferric oxide and hydroxide, are the most common forms of iron that contaminate food, but both are very poorly absorbed.\textsuperscript{13} Iron that is solubilised from iron cooking pots and utensils will enter the common non-haem iron pool, and its availability for absorption (potential bioavailability) will be determined by the composition of the meal and physiological factors primarily relating to the lumenal conditions of the gastrointestinal tract.\textsuperscript{96} More field research is needed to assess the value of this type of intervention under different settings. Laboratory studies are also needed to determine if and how much iron content increases in foods used in areas with a high prevalence of iron deficiency. Until now only one laboratory study has been conducted on African foods.\textsuperscript{92} Further work on the variables, which influence the amount of iron that is added to food, is also required. Factors already known to be important include: cooking time, food moisture content and pH. Others may be important but have not been systematically studied, for example, the effect of continuous use of iron utensils and the effect of the addition of oil in food preparation.

5.4.2 Delayed umbilical cord clamping
Delayed cord clamping or placental transfusion, increases the volume of blood transferred from placenta to the neonate and thus increases the total body iron content. This will help to prevent (iron deficiency) anaemia in later infancy. The distribution of blood at birth as reflected in the infant : placenta ratio is 2:1, which increases to a infant : placenta ratio of 4:1 after a three minutes delay in cord clamping.\textsuperscript{97,98} Recently van Rheenan and Brabin reviewed the efficacy of delayed cord clamping for preventing infant anaemia in developing countries.\textsuperscript{99} They concluded that there are indications that children, especially those at risk for iron deficiency, would profit from delayed cord clamping. However few studies have been conducted which have studied the long-term effects of delayed cord clamping and more studies are required to determine these.\textsuperscript{99} It is also unknown the extent to which the use of delayed cord clamping is already practiced, especially amongst populations at risk for iron deficiency and iron deficiency anaemia. Complications related to neonatal hyperbilirubinaemia require further study.
5.5 Reduction of infection

5.5.1 Malaria

There are several strategies that can be used to control malaria: vector control by means of residual spraying and/or larval control, insecticide treated mosquito nets, malaria chemoprophylaxis and improved malaria treatment.

1. Vector control

Non-selective residual spraying is no longer a recommended strategy in vector control and should only be used in well defined high- or special risk situations. Spraying should then be restricted to places where the risk of transmission is highest. Resistance to insecticides develops mostly because of their agricultural use and not due to their use in control operations for vector control. Especially important in this is the recently found kdr resistance mechanism in malaria vectors. This kdr mechanism results from a point mutation in sodium channels, which are the target sites for DDT and pyrethroids. Resistance to them is of great concern since pyrethroids are currently the only insecticides available for treating materials with insecticides. Recently the impact of this kdr mechanism on the effectiveness of pyrethroid-treated bed nets has been studied and no impact of this mutation on the repellent and killing effects has been found.

Due to the problems with resistance to insecticides, there is a renewed interest in larval control which can be obtained by insecticides, use of larvivorous fish and environment management.\(^{100}\)

2. Insecticide-treated mosquito nets

Insecticide-treated mosquito nets (ITBN) are an effective intervention and in Africa their use is moving from a research/project base towards operational implementation.\(^{100}\)

Their use has been shown to improve haemoglobin levels in children and reduce parasite prevalence and parasite density.\(^{101,102}\) They also reduce acute morbidity and mortality, especially in the youngest children.\(^{102,103}\) A Cochrane review of the effect of insecticide-treated bed nets for preventing malaria, which included 18 studies, found that ITBN have a protective efficacy of 17% against mortality when compared with no, or plain nets. Further ITBN reduced the incidence of mild malarial episodes by 48% and 34% when compared with controls using no nets, or untreated nets respectively.\(^{104}\)

Goodman et al reviewed the cost effectiveness of ITBN in malaria control and found them to be cost effective for very-low-income countries with high transmission.\(^{105}\) An important factor in the costs are whether bed nets need to be provided and distributed, or if only insecticide treatment is required. In the first situation the cost effectiveness range was US$ 410 per DALY averted, in the latter US$ 1985 per DALY averted.\(^{105}\) However efficacy and cost effectiveness alone do not make this intervention a successful malaria control strategy. Also important is the acceptability of this intervention to the population and
hindrance of their acceptability should be removed, eg higher price and poor availability. Schellenberg et al showed that with social marketing of ITBN in Tanzania, in a population of 480,000 people, the coverage percentage of infants using a ITBN increased from 10% to 50% over three years.\textsuperscript{106}

Since ITBN protect individuals against sporozoite inoculations a concern is that by inhibiting the development of immunity severe morbidity and mortality due to malaria could be delayed until a older age. That is why, as pointed out by Coleman et al, long-term surveillance of a population is necessary to detect an increased incidence of severe malaria in older children.\textsuperscript{107}

3. MALARIA CHEMOPROPHYLAXIS
Malaria chemoprophylaxis in children can increase haemoglobin levels, reduce mortality, parasitaemia, incidence of clinical malaria, number of hospital admissions and number of out patient visits.\textsuperscript{108-111} Intermittent sulphadoxine-pyrimethamine (SP) treatment has been given to children with success, reducing the incidence of clinical malaria and severe anaemia.\textsuperscript{112} Verhoef et al found that intermittent iron supplementation in children, in addition to intermittent treatment with SP, did not lead to greater gains in haemoglobin concentration.\textsuperscript{113} An explanation for these different findings could be the difference in malaria transmission rates in the countries where these studies took place.

Intermittent SP treatment is not malaria chemoprophylaxis in the strictest sense but does provide a significant protective effect. Malaria chemoprophylaxis in children has been shown to be a cost-effective intervention, however fears concerning spread of drug resistance and impairment of the development of acquired immunity in children has prevented this intervention being widely implemented.\textsuperscript{105,115} Also in pregnant women intermittent sulphadoxine-pyrimethamine treatment (SP) was effective. It reduced parasitaemia and severe anaemia in the women as well as increasing mean birth weight and decreasing the incidence of low birth weight.\textsuperscript{116,117}

4. IMPROVED MALARIA CHEMOTHERAPY
Improved malaria chemotherapy can be obtained by timely change to alternative first line anti-malarial treatment when drug resistance to the current first line anti-malarial is unacceptably high. This was achieved in Malawi where Bloland et al reported in 1993 that there was severe resistance to Chloroquine.\textsuperscript{118} Malawi introduced sulphadoxine-pyrimethamine (SP) as the new first-line treatment for uncomplicated \textit{Plasmodium falciparum} malaria in 1993. Verhoef et al reported two years later a effectiveness of SP of 90.5% in uncomplicated \textit{Plasmodium falciparum} malaria.\textsuperscript{119} There are no clear and undisputed thresholds for failures of first-line treatment above which the first-line anti-malarial treatment should be changed. A threshold of 25% has been proposed.\textsuperscript{120}

An innovative and promising way to improve malaria chemotherapy is pre-packaging of anti-malarial drugs. Yeboah-Antwi et al reported that compared with non-pre-packed anti-malarial drugs, the group which received the pre-
packed drugs had a 20% higher compliance with medication, a 50% reduction in total costs and a 61% reduction in waiting time.20 The data further seemed to suggest that pre-packaging lead to time saving for the health professionals as more people were weighed than in the group which received no pre-packed drugs (98.3% versus 26.1%).

Other interventions which could improve malaria chemotherapy are training of special groups, eg mothers to ensure prompt and adequate treatment of malaria and making second-line anti-malarials available to health centres.12112

5.5.2 Helminthic and Schistosome infections
If there is political commitment, social development, epidemiological information and sustained integrated interventions it is possible to reduce or even eliminate the public health consequences of helminth or schistosome infections. This has already been achieved in several countries.123 Both types of infection are closely related to poverty and are often endemic in the same population.40

Chemotherapy for helminth and schistosome infections is the cornerstone of the strategy to control morbidity due to these infections.40 The World Health Organisation recommends mass treatment of children in schools when Schistosome or helminth infection prevalence exceeds 50%.19-40 Where hookworm infection and/or schistosomiasis are endemic, it is recommended to consider giving presumptive treatment, to everybody who presents with severe anaemia.11 It is also recommended to treat all adults and children older than 5 years at least once a year against hookworm, and in areas where schistosomiasis is endemic, to treat all school children annually who report blood in their urine.12

Hookworm or schistosomiasis chemotherapy needs to be supported by other interventions such as the provision of water and sanitation, snail control and health education. All these interventions are aimed at breaking the life cycle of these parasites at different places and consequently to prevent transmission.

5.5.3 Tuberculosis
The main interventions for controlling tuberculosis are BCG vaccination, preventative treatment (chemoprophylaxis) and directly observed treatment, short-course (DOTS).124 BCG vaccination is given to approximately 100 million children a year. The current recommendation of the WHO is that children receive this vaccination at birth, or at first contact with the health services. There are criteria which if met, allow selective vaccination of high risk groups instead of routine vaccination of all children. BCG vaccinations are effective in preventing severe disease (tuberculous meningitis and military disease) in children. The effectiveness against adult pulmonary tuberculosis is a matter of controversy, and estimates vary between 0% to approximately 80%.124 DOTS is an effective strategy to assist in controlling the tuberculosis epidemic. It is a strategy that has five key components: political commitment to sustain tuberculosis control, case detection by sputum smear microscopy, directly observed taking of medication by patient for at least two months of the standardized treatment regimen of 6 – 8 months, a regular uninterrupted supply of all essential anti-tuberculosis drugs
and a standardized recording and reporting system. Burman et al conducted a cost effectiveness analysis of directly observed treatment vs self-administered therapy for treatment of tuberculosis. They found that cure and failure rates following self-administered therapy were 0.79 and 0.21, respectively, while cure and failure rates following DOT were 0.945 and 0.055, respectively. Their conclusion was that directly observed treatment was more cost-effective than self-administered therapy.

While single drug resistance in tuberculosis has already been reported from all surveyed countries, a growing concern is the increasing prevalence of multi-drug resistant tuberculosis which may become a significant problem. Single and multi-drug resistance tuberculosis has been inversely associated with DOTS and positively with the proportion of previously treated cases. This highlights the need for increasing the use of DOTS in the treatment of tuberculosis.

To further target multi-drug resistant tuberculosis the DOTS-plus strategy has been launched aiming at improving access to second-line tuberculosis drugs.

5.5.4 HIV

Prevention is at the moment the most important strategy for controlling the HIV epidemic in the world. There are many different interventions to prevent human immunodeficiency virus infection (HIV). From a public health point of view they can be divided into interventions which target different risk behaviours, different aspects of a given risk behaviour and different at-risk populations. Often used interventions are education to promote behaviour change, condom distribution, improved blood safety, improved treatment of sexual transmitted diseases, prevention of mother-to-child transmission and voluntary counselling and testing (VCT).

Some interventions aimed at treatment and care in HIV positive people are short-course treatment for new sputum smear positive tuberculosis patients, antibiotic prophylaxis for HIV-positive tuberculosis patients, home based care for people with AIDS, preventive therapy for tuberculosis and highly active antiretroviral therapy (HAART).

There is a great deal of research that has been undertaken and many studies published concerning HIV prevention. It is important to remember that a study can show that an intervention has significantly changed a specific outcome, eg number of treated sexual transmitted diseases. The epidemiological context in a specific situation determines if that intervention also has an impact on HIV incidence. This means that a programme that has been successful in one country may not be in another. Creese et al conducted a cost-effectiveness analysis of HIV/AIDS interventions in Africa. They showed that there are only a few studies undertaken and so this analysis has limitations. If cost-effectiveness is defined as a DALY gained for 50 USD or less, then most interventions in Africa meet this criteria. However the authors rightly point out that cost-effectiveness is not the same as affordability and that less cost-effective interventions may be demanded by governments.
5.6 Targeting genetic disorders

For thalassaemia, sickle cell disease and glucose-6-phosphate dehydrogenase deficiency there is no curative therapy. In β thalassaemia bone-marrow transplantation may carry a good prognosis if carried out early in life.60 The morbidity and mortality caused by these disorders can be greatly reduced by good health care. Prevention of these disorders is only possible through screening programmes, which face a lot of restraints especially in developing countries, and by counselling of people affected by these disorders.

6. The study site in Malawi

The field studies described in this thesis were carried out in the Shire Valley and Blantyre in southern Malawi. These sites were selected because of their known high prevalence of anaemia and/or the good working relationship with Malawian health authorities/hospitals.

Malawi is a republic, located in South East Africa and is bordered by Tanzania, Mozambique and Zambia (figure 1). The country covers an area of 118,484 square kilometres of which 24,208 sq km (+/- 20%) are lakes.
The official language is English and the national language is Chichewa. In 2000 the population was estimated to be approximately 11 million people. Most people live in the rural areas (85.3%). The life expectation, which has dropped considerably over the recent years, for women was 37.7 years and for men 37.3 years. The under five mortality rate was 186 per 1000 births and the infants mortality 115.4 per 1000 births. The total fertility rate was 6.10 children per women. The literacy rate amongst men is 61.3 % and amongst women 47.9%. Of the whole population 43.8 % lives under the poverty line. Malawi has a tropical climate with a rainy season from December until April. Malaria transmission occurs year round with the highest intensity during the rainy season. In 1993 Bloland reported a RIII resistance to chloroquine of 33% and it was decided to change the first-line treatment from chloroquine to sulphadoxine-pyrimethamine (SP). Verhoeff et al reported two years later a effectiveness of SP of 90.5% in uncomplicated Plasmodium falciparum malaria. The main sources of food are maize complimented with ground nuts and seasonally with Chinese cabbage or pumpkin leaves.

7. Study objectives
Anaemia continues to be a major public health problem with serious and detrimental effects, despite various efforts to reduce its magnitude.

To be able to improve the effectiveness of interventions against anaemia several factors are of importance. It is necessary that there is a clear understanding of the factors which cause anaemia and maintain it in a population. For the long-term effectiveness of an intervention, it is essential that the acceptability of that intervention in the target population is studied. This makes it possible to adapt, change or redirect an intervention for maximal effect in various communities. More attention should be given to interventions which are not reaching their full potential, in order to determine what is limiting their effectiveness. Innovative approaches are required, particularly with reference to low-cost strategies which are technically feasible and non-dependent on donor support.

The objective of this research was to increase the understanding of anaemia and its consequences in one of the poorest countries in the world and to evaluate and/or increase the body of evidence for the efficacy of some under-utilized control interventions. A further aim was to systematically review available data from relevant controlled trials in order to establish the relative merits, advantages and disadvantages of specific interventions.

Objectives:
1) To describe anaemia in relation to mortality in mothers and children.
2) To determine the haematological profile of communities living in southern Malawi.
3) To evaluate the efficacy of eating food prepared in iron pots for reducing iron deficiency anaemia.
4) To evaluate the acceptability, compliance and attitudes on the use of iron cooking pots in a community in which they were not traditionally used.
5) To complete a meta-analysis of the effectiveness of iron pot interventions.
6) To determine the iron content of staple Malawian foods when prepared in iron cooking pots.
7) To analyse the effects of malaria chemoprophylaxis in children on haematologic responses, morbidity, mortality, health service utilisation and rebound malaria immunity.


130. Malawi population and housing census. National Statistics Office, Zomba, Malawi