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High anaemia prevalence and its causes in children aged 6-23 months in rural Qinghai, China: findings from a cross-sectional study

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ORIGINAL ARTICLE**Title page****High anaemia prevalence and its causes in children aged 6-23 months in rural****Qinghai, China: findings from a cross-sectional study**

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ABSTRACT

Objective: To investigate the current situation of anaemia among children aged 6-23 months in a rural county in China, and to explore the influencing factors and the main causes of anaemia.

Design: A cross-sectional study.

Setting: Huzhu County in Qinghai Province, China

Participants: We selected 38 sampled villages using Proportional to Population Size sampling method. We surveyed all the eligible children aged 6-23 months and their caregivers in each sampled village.

Primary and secondary outcomes measures: The prevalence of anaemia, the influencing factors of anaemia, the laboratory tests for causes of anaemia, including serum ferritin, sTfR, folic acid, Homocysteine and Vitamin B12.

Results: A total of 754 children aged 6-23 months and their caregivers were surveyed, and 183 anaemic children aged 12-23 months were collected venous blood sample. The anaemia prevalence of children aged 6-23 months in Huzhu County was 59.1%. Children of younger age (OR=0.968, 95%CI 0.940, 0.998), Tibetan nationality (OR=3.123, 95%CI 1.473,6.623)and not introducing meat (OR=0.698, 95%CI 0.499,0.976) were more likely to be anaemic. More than 80% of children with anaemia were due to iron deficiency, and 20.2% of them had both iron and folic acid deficiencies.

Conclusions: The anaemia prevalence of children aged 6-23 months in Huzhu County

was high and children of younger age, Tibetan nationality and not introducing meat were more likely to be anaemic. The main cause of anaemia was nutritional anaemia, with the vast majority being iron deficiency. Interventions of feeding counseling and nutrients supplements are appropriate and should be further strengthened.

Strengths and limitations of this study: The strengths of our study are as follows. Firstly, we proved that iron deficiency was the leading cause of anaemia for young children in Huzhu county. Secondly, we identified specific age and ethnic groups of children who were more vulnerable to be anaemic. Thirdly, we found a rebound of anaemia prevalence based on continuous data collection since 2012. All these findings will provide guidance for the future implementation of the on-going interventional program. Our study also has several limitations. Firstly, the study took place only within one rural Chinese county and caution is needed when generalizing the findings from this study to other settings. Secondly, the sample size was small. Thirdly, we only explored nutritional causes of child anaemia, so the anaemic causes of for nearly 20% of children were still unknown.

Trial registration number: ChiCTRPRC12002444 (Feb 15th, 2016; Version1).

Main text

INTRODUCTION

Childhood anaemia has long been a major public health problem worldwide. A systematic analysis of population-representative data suggested that anaemia prevalence in children younger than 5 years was 43% and there were 273 million children with anaemia globally in 2011¹. The national anaemia prevalence in children under 5 in China fluctuated between 12% and 23% between 1990 and 2005 and then decreased from 19.3% in 2005 to 12.6% in 2010². National Nutrition and Health

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4 Surveillance in 2013 showed that the prevalence of anaemia among children aged 0-
5 5 years was 11.6% across the country, 10.6% in urban areas and 12.4% in rural areas
6 respectively. However, huge regional differences exist, with rural Qinghai province
7 being the highest (27.5%)³. In some rural areas, anaemia prevalence in children aged
8 6-23 months was higher than 30%^{4,5}.

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15 In the public health perspective, anaemia is defined by the World Health Organization
16 as a hemoglobin concentration 2 SDs below the mean hemoglobin concentration for
17 a normal population of the same gender and the same age group⁶. Anaemia has
18 irreversible adverse effects on childhood growth and development, even their
19 working abilities in adulthood⁷. Many studies suggested an association between
20 anaemia and impaired psychomotor development; impaired cognitive functions such
21 as concentration, intellectual status, memory and scholastic skills; psychological and
22 behavioral disorders such as attention-deficit/hyperactivity disorder (ADHD)⁸ and
23 autism spectrum disorder (ASD)⁹; decreased physical activity¹⁰. Meanwhile, anaemia
24 has been confirmed to be associated with impaired renal function, increased
25 absorption of lead, and impaired immunity¹¹. The Global Burden of Disease (GBD)
26 2000 report estimated that anaemia resulted in 68.4 million years lived with disability
27 (YLD), accounting for 8.8% the total number of all cases of disability¹², and the GBD
28 2004 update had similar findings, which exerted a substantial economic burden¹³.

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45 Our previous study in Qinghai Province found that the prevalence of anaemia in rural
46 areas was much higher than the national average level. In 2012, the prevalence of
47 anaemia among children aged 6-23 months in Huzhu, Minhe and Guinan County was
48 71.1%, 56.1% and 86.3%, respectively¹⁴. We carried out a controlled interventional
49 study in Huzhu and Guinan county from 2012 to 2014. And all children aged 6-23
50 months in the intervention county, Huzhu County, received YingYangBao (a
51 domestically produced multiple micronutrient powders for infants and young children)
52 and their caregivers received infant feeding counseling from trained village doctors.
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4 The study found that the anaemia prevalence significantly decreased from 71.1% to
5 47.8% in Huzhu County ¹⁵. WHO defines the prevalence of anaemia in the population
6 less than 20% as a mild public health problem, 20% to 40% as a moderate public health
7 problem, and the prevalence of anaemia in the population \geq 40% as a serious public
8 health problem ¹⁶. Therefore, childhood anaemia in Huzhu County is still a serious
9 public health problem in spite of the dramatic reduction of anaemia prevalence after
10 the intervention, and more efforts are needed to further decrease anaemia
11 prevalence and improve the nutrition status of children.
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21 There are many reasons for anaemia: acute and chronic infections that result in
22 inflammation (including chronic blood loss caused by hookworm infection or
23 schistosomiasis); nutritional anaemia caused by iron deficiency and other
24 micronutrient deficiencies, especially of folic acid, vitamin B12 and vitamin A; and
25 genetically inherited traits such as thalassemia ¹⁷. Although it is generally accepted
26 that nutrients deficiency is the leading cause of child anaemia, proportions of anaemic
27 causes in specific areas are usually unknown. Since interventions based on
28 improvement of child feeding and nutrients supplements are only effective for
29 nutritional anaemia, understanding the status of anaemia-related nutrients (iron, folic
30 acid and vitamin B12) is crucial to estimate the potential effectiveness of nutritional
31 interventions. The purpose of this study is to investigate the current situation of
32 anaemia among children aged 6-23 months in Huzhu County, and to explore the
33 influencing factors and the main causes of anaemia, so as to develop more appropriate
34 strategies for combating this intractable public health issue.
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50 **METHODS**

51 **Study design**

52 This study was conducted as a cross-sectional survey of children aged 6-23 months
53 and their caregivers in Huzhu county, Qinghai province. Proportional to Population
54 Size (PPS) sampling method was used to select sampled villages in the county, and all
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4 the eligible children aged 6-23 months and their caregivers in each sampled village
5 were surveyed. The HemoCue Hb 301 analyzer (HemoCue, Lake Forest, CA, USA) was
6 used to collect children's fingertip peripheral blood by using microcuvettes (for blood
7 samples) to detect hemoglobin levels of children in the all sampled villages. 4-7 days
8 after the survey, anaemic children screened out by HemoCue Hb 301 analyzer were
9 called to Huzhu Maternal and Child Health and Family Planning Service Center to draw
10 venous blood sample for further blood routine test and laboratory tests.
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20 **Study setting**

21 Qinghai Province lies in northwest China, with an area of around 720,000 km². By the
22 end of 2017, the resident population of the province was 5,838,000, of which the
23 resident population of rural areas was 2,808,400, accounting for 46.9%, and the ethnic
24 minority population was 2,854,900, accounting for 47.7%. Qinghai Province has 34
25 counties and 439 townships. The Qinghai resident per capita disposable income in
26 2017 was ¥9,462 (US\$1363.13)¹⁸ for rural people, which was far lower than the
27 national level (¥13,432(US\$1935.06))¹⁹.
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37 Huzhu County is located in the northeastern part of Qinghai Province and 27.9% of its
38 population is part of an ethnic minority, which includes Tu, Tibetan, Hui and other 28
39 ethnic minorities²⁰. Huzhu County covers an area of 3,424 km². The county governs
40 19 townships with 294 villages, with a total population of 401,540, of which the rural
41 population accounted for 76.0%. By the end of 2017, the resident per capita
42 disposable income in the rural area was ¥9810 (US\$1414.91)²¹.
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51 **Participants**

52 Children aged 6-23 months and their caregivers were invited to participate in this
53 survey. We excluded children with a structural or genetic birth defect such as neural
54 tube defects, congenital heart disease or phenylketonuria or caregivers who refused
55 to participate.
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Survey instrument

We used the questionnaire, the maternal, newborn and child health household survey (MNCH HHS) questionnaire, to collect household information and infant feeding practices in each sampled village.

Sample size and Sampling

The sample size required were calculated based upon estimated anaemia prevalence in Huzhu County. We expected to draw 200 venous blood sample of anaemic children aged 12-23 months. According to the estimated anaemia prevalence of 35% in Huzhu County, we calculated the total number of children aged 12-23 months surveyed was 571. And according to the age composition of local children, the number of children aged 6-12 months was about half of the number of children aged 12-23 months. Hence a sample size of 856 children aged 6-23 months would be sufficient in this study.

We understood the average number of children aged 6-23 months in each villages of Huzhu County from the local Maternal and Child Health Family Planning Service Centre. Proportional to Population Size (PPS) sampling method was used to select 38 sampled villages to meet our sample size requirements in the county. We surveyed all the eligible children aged 6-23 months and their caregivers in each sampled village.

Training of Interviewers

Staff from the Capital Institute of Pediatrics in Beijing were supervisors for this survey, and 25 students were recruited from Qinghai Institute of Health Sciences as interviewers. We provided them training for two days before fieldwork, which included communication skills, explanation of questionnaires, demonstration, role plays, field practice, and group discussions. In addition, 4 of them were trained on measuring hemoglobin levels with a HemoCue Hb 301 analyzer. After the training, a half-day field practice was held in a village clinic. Any problems arising during the field

practice were discussed and solved directly.

Data collection

We carried out the survey from July 23th to 27th 2018. In every surveyed township, staff notified the village doctors of the sample villages in advance, then the village doctor called the caregivers to take their children to the village clinic for investigation. Firstly, interviewers introduced the aim of the survey to the mothers or other caregivers and obtained written informed consent from them. Then the interviewers questioned them following the instructions.

We used smartphones with the household survey questionnaire set up in specially developed software to record data ²². Four teams of interviewers carried out the survey, with 6 surveyors and 1 supervisor in each team. Data for each questionnaire were uploaded into an Excel database via the Internet server. Once the interview was completed, the special surveyor in each team measured hemoglobin with a HemoCue Hb 301 analyzer (HemoCue, Lake Forest, CA) by drawing around 10 ul finger blood.

Children aged 12-23 months screened as anaemia by HemoCue Hb 301 analyzer were informed to go to the Huzhu County Maternal and Child Health Family Planning Service Centre for further tests from July 31 to August 1 2018. About 4-5ml venous blood of each children were collected by experienced nurses and placed into two tubes. The first 1-2ml blood sample was collected into a labeled EDTA-K2 coated tube for blood routine test using whole blood. And the second 3ml blood sample was collected (without removing the needle) into a labeled vacuum separating tube for serum ferritin(SF), soluble transferrin receptor(sTfR), C-reactive protein(CRP), α -1 acid glycoprotein(AGP), vitamin B12, Homocysteine(HCY), and folic acid concentration using serum.

Blood routine test and blood centrifugation were completed in the local laboratory.

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4 The blood sample in labeled vacuum separating tubes were placed for 30 minutes,
5 then were centrifuged at 1500 turn/minute for 15 minutes. The serum was separated
6 into 1, 2 and 3 cryotubes using disposable pipettes.
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11 After the field work, all the blood samples were immediately stored at -70°C and
12 transported as soon as possible to Nutritional Institute for Institute for Nutrition and
13 Health, China Center for Disease Control and Prevention for further laboratory testing.
14 Repeated freezing and thawing were strictly avoided during transportation and
15 storage.
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23 **Laboratory analysis**

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25 The blood routine test was conducted using automatic blood cell analyzer (Horiba,
26 ABX Micros 60-OT, France) in local laboratory. SF, vitamin B12 and serum folic acid
27 were analyzed by Roche Cobas e601 analyzer (Germany) using
28 electrochemiluminescence immunoassay (ECLIA). sTfR, CRP, AGP were analyzed by
29 Hitachi 7600-110 chemistry autoanalyzer (Japan) using immunoturbidimetry and HCY
30 was analyzed by Hitachi 7600-110 chemistry autoanalyzer (Japan) using enzymatic
31 cycling assay.
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41 **Data management and statistical analysis**

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43 Data of questionnaires were automatically transformed and pooled into a Microsoft
44 Excel sheet. After the data cleaning, we converted the database into a database file
45 (dbf) for the final analysis.
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51 We carried out statistical analysis with SAS 9.2 for Windows. The median (Q1, Q3) was
52 used to describe the age in years of mothers and grandparents of children. Mean and
53 standard deviation (SD) was used to describe the values of serum ferritin and body
54 iron. Percentages were presented in binary or categorical variables. We used the
55 Pearson χ^2 -test and Fisher exact test to compare binary and categorical variables.
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Moreover, we carried out a logistic regression analysis to identify factors associated with children's anaemia in this survey. All relevant factors were first selected by univariate logistic analysis. Only those that were significant in the final multivariate model are presented. We present Odds Ratios (OR) and 95% confidence intervals (CI) and considered two-tailed P-values of <0.05 for a significant difference.

All individual hemoglobin values were adjusted using WHO recommendations based on the altitude of the surveyed villages where children lived. An adjusted hemoglobin lower than 110g/l¹⁶ was defined as anaemia and was used to calculate the prevalence of anaemia. A hemoglobin concentration of 90–110 g/L was defined as mild anaemia, <90 g/L as moderate anaemia or severe anaemia. Cut-offs for elevated CRP and AGP were >5mg/l and >1mg/l, respectively²³. If one of these two indicators were elevated, the children surveyed were classified as infected children. Serum ferritin concentration <12ug/l and <30ug/l were used to define iron deficiency in non-infected children and infected children, respectively. The children with concurrent anaemia and iron deficiency were diagnosed with iron deficiency anaemia²⁴. A plasma folic acid concentration of <4ng/ml was used to define folic acid deficiency according to WHO guidelines²⁵. Vitamin B12 deficiency was defined as <197pg/ml. Cut-offs for elevated sTfR and HCY were 8.3mg/l and 14umol/l, respectively²⁶.

Body iron stores (BI) were estimated by applying Cook's formula as follows: $\text{body iron(mg/kg)} = -[\log(\text{R/F ratio}) - 2.82290] / 0.1207$. The R/F ratio was soluble transferrin receptor (sTfR) / serum ferritin (SF)²⁷. Among them, sTfR needs to be transformed by the following formula: $\text{Flowers sTfR} = 1.5 * \text{Roche sTfR} + 0.35\text{mg/l}$ ²⁸. The positive value means iron surplus in stores and the negative value means iron deficit in tissues.

Ethical considerations

The study was approved by the Ethics Committee of the Capital Institute of Pediatrics

(reference no.2018017). All interviewees read the Information Sheet and provided written consent on behalf of the children involved in our study.

Participant and public involvement

The participants and the public were not involved in the design, recruitment and conduct of the study. There are no plans to disseminate the study findings to the study participants.

RESULTS

The flowing chart of this study is shown in **Figure 1**. A total of 754 children aged 6-23 months and their caregivers were surveyed, with 444 being anaemic. 183 children aged 12-23 months agreed to draw venous blood for further laboratory tests, and 52 of them were found acute and/or chronic infections (CRP > 5 mg/l in blood routine test or AGP > 1 g/l in laboratory test).

Characteristics of 754 surveyed children and their caregivers are shown in **Table 1**. Nearly all main caregivers of the children surveyed were mothers and grandparents, and about 70% of mothers were Han nationality, followed by Tu and Tibetan nationality. More than 60% of mother attended junior high school, and the proportion of mothers who were illiterate was only 4.0%. More than half of grandparents were still illiterate. The main source of household income was working outside the county, followed by agriculture-related work such as growing crops, vegetables and animal husbandry.

Table1 Characteristics of surveyed children and their caregivers (N=754)

Characteristic	Percentage or median
Children	
Age, %(n)	
6-11months	32.8(247)

12-17months	32.6(246)
18-23months	34.6(261)
Sex, %(n)	
Boy	52.1(393)
Girl	47.9(361)
Main caregivers, %(n)	
Mother	48.7(367)
Grandparent	46.4(350)
Father	4.8(36)
Other	0.1(1)
Mothers	
Age in years (median (Q1, Q3))	29(26,31)
Nationality, %(n)	
Han	69.9(523)
Tu	22.8(171)
Tibetan	6.3(47)
Hui	0.3(2)
Others	0.7(5)
Education, %(n)	
Illiterate	4.0(30)
Primary school	13.8(103)
Junior high school	61.6(461)
Senior high school or above	17.4(130)
Do not know	3.2(24)
Grandparents	
Age in year (median (Q1, Q3))	54(50,59)
Education, %(n)	
Illiterate	60.3(211)
Primary school	22.6(79)
Junior high school	15.1(53)
Senior high school	1.1(4)
Do not know	0.9(3)
Household income, %(n)	
Working outside the county	89.2(673)

Agriculture-related work	6.4(48)
Self-employed	2.9(22)
Others	1.2(9)
Do not know	0.3(2)

Table 2 shows the prevalence of anaemia and hemoglobin levels by age and severity in this survey. Most of anaemic children were mildly anaemic, accounting for 76.9% of the total. The prevalence of anaemia in the 18 to 23-month group was significantly lower than that in the 6 to 11-month group ($p=0.0026$) and the 12 to 17-month group ($p=0.0261$). The median hemoglobin levels in the 6 to 11-month group and 12 to 17-month group were lower than that in the 18 to 23-month group (116g/l vs. 121 g/l, $p<0.0001$ and $p=0.0004$ respectively).

Table 2 Prevalence of anaemia and hemoglobin level by age and severity

	Anaemia prevalence (N=751) *			Hemoglobin level (median, (Q1, Q3))
	Mild anaemia	Moderate or severe	Total	
	(%, n)	anaemia (%, n)	(%, n)	
6-11m	49.8(123)	15.0(37)	64.8(160)	116(106,125)
12-17 m	51.2(126)	10.2(25)	61.4(151)	116(107,125)
18-23 m	43.4(112)	8.1(21)	51.6(133)	121(111,130)
Total	48.1(361)	11.1(83)	59.1(444)	117(108,127)

*Three children refused to measured hemoglobin levels.

Table 3 shows the results of a univariate logistic analysis of anaemia prevalence in this survey. Older children were less likely to suffer from anaemia than younger children ($p=0.0028$). The prevalence of anaemia in children with Tibetan nationality was significantly higher than those with Han nationality ($p<0.0001$). The prevalence of anaemia in children who consumed iron-rich or iron-fortified foods was significantly lower than that in children who did not consume these foods ($p=0.0150$). The prevalence of anaemia in children who were given meat was also significantly lower

than that in children without meat ($p=0.0077$). Furthermore, the anaemia prevalence of children achieving minimum dietary diversity was significantly lower than that of children who did not meet the standard ($p=0.0163$).

Table 3 Univariate logistic analysis of the prevalence of anaemia

Factors	Sample	Case	Anaemia	P-value
Child's age				0.0028
Nationality				
Han	521	291	55.9	
Hui	2	0	0	0.9799
Tu	170	108	63.5	0.0836
Tibetan	47	38	80.9	0.0016
Other	5	3	60.0	0.8570
Children aged 6-23 given iron-rich or iron-fortified food				
Yes	475	265	55.8	0.0150
No	276	179	64.9	
Introduction of meat				
Yes	210	108	51.4	0.0077
No	541	336	62.1	
Minimum dietary diversity				
Yes	364	199	54.7	0.0163
No	387	245	63.3	

The results of multivariate logistic analysis of anaemia prevalence in surveyed children is shown in **Table 4**. Older children (OR=0.968, 95%CI 0.940, 0.998) and those consuming meat (OR=0.698, 95%CI 0.499,0.976) were associated with lower anaemia levels, whereas children of Tibetan nationality (OR=3.123, 95%CI 1.473,6.623) were more likely to be anaemic.

Table 4 Multivariate logistic analysis of anaemia prevalence

Independent variable	Influential factors of anaemia			
	β	Wald	P	OR (95%CI)

Child' age	-0.0321	4.4930	0.0340	0.968(0.940,0.998)
Nationality				
Hui –Han	-13.6385	0.0006	0.9797	<0.001
Tu-Han	0.3038	2.7371	0.0980	1.335(0.945,1.942)
Tibetan-Han	1.1388	8.8166	0.0030	3.123(1.473,6.623)
Other-Han	0.0294	0.0010	0.9745	1.030(0.169,6.260)
Introduction of meat	-0.3595	4.4118	0.0357	0.698(0.499,0.976)

Results of the laboratory tests for 183 children are shown in **Table 5**. There were 113 children with iron deficiency, accounting for 61.7%. The mean serum ferritin concentration in anaemic children was significantly lower than that in non-anaemic children ($10.2 \pm 9.6\text{ng/ml}$ vs. $22.6 \pm 15.9\text{ng/ml}$, $p < 0.0001$). The body iron store in anaemic children was significantly lower than that in non-anaemic children ($-3.1 \pm 4.4\text{mg/kg}$ vs. $2.1 \pm 3.3\text{mg/kg}$, $p < 0.0001$). 32.6% of children with anaemia had elevated sTfR, significantly higher than that in children without anaemia (4.3%, $p < 0.0001$). At the same time, the proportion of folic acid deficiency in anaemic children was also significantly higher than that in non-anaemic children (20.2% vs. 5.3%, $p = 0.0024$). There was no vitamin B12 deficiency either in anaemic or non-anaemic children.

Table 5 Results of laboratory tests

	Anaemic children (N=89)	Non-anaemic children (N=94)	Total (N=183)	<i>p</i>
Serum ferritin(ug/l)				
Mean \pm SD	10.2 ± 9.6	22.6 ± 15.9	16.6 ± 14.6	<0.0001
<12ug/l for non-infected children or <30ug/l for infected children (% , n)	80.9% (72)	43.6% (41)	61.7% (113)	<0.0001
sTfR (>8.3mg/l) (% , n)	32.6% (29)	4.3% (4)	18.0% (33)	<0.0001

Body iron store (mean \pm SD)	-3.1 \pm 4.4	2.1 \pm 3.3	-0.45 \pm 4.56	<0.0001
Folic acid (<4ng/ml) (% , n)	20.2% (18)	5.3% (5)	12.6% (23)	0.0024
Vitamin B12 (<197pg/ml) (% , n)	0.0% (0)	0.0% (0)	0.0% (0)	-
Homocysteine (>14umol/l) (% , n)	4.5% (4)	1.1% (1)	2.7% (5)	0.1425

Figure 2 shows causes of anaemia. 80.9% of children with anaemia were due to iron deficiency, and 20.2% of them had both iron and folic acid deficiencies. The causes of 19.1% anaemic children were unknown.

DISCUSSION

Main findings

The anaemia prevalence of children aged 6-23 months in Huzhu County was 59.1% and most of them were mildly anaemic. The prevalence of anaemia in the 18 to 23-month group was significantly lower than that in the 6 to 11-month group ($p=0.0026$) and the 12 to 17-month group ($p=0.0261$). Meanwhile, children of younger age, Tibetan nationality and not introducing meat were more likely to be anaemic. 80.9% of children with anaemia were due to iron deficiency, and 20.2% suffered from both iron deficiency and folic acid deficiency. The prevalence of iron deficiency among all children was 61.7% and 43.6% of non-anaemic children also had iron deficiency. Body iron stores in all children tested averaged -0.45 ± 4.56 mg/kg. The proportion of microcytic hypochromic anaemia (MCH, MCV and MCHC were lower than normal value) was 13.1%. And the specificity of the combination of MCV + MCH + MCHC in the diagnosis of iron deficiency anaemia was 100%, but its sensitivity was only 17.8%.

Influencing factors of anaemia

Children aged 6-11 months are in the transition period from exclusive breastfeeding to complementary feeding, during which the storage iron from birth is depleted and complementary foods become the main source of iron, therefore, they were more

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4 likely to suffer from anaemia. In our study, anaemia prevalence of children at this age
5 is the highest (64.8%).
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9 Our analysis showed that eating meat was a protective factor of child anaemia,
10 however, few caregivers gave meat to their children of this age due to the wrong
11 beliefs that they could not digest meat. In addition, some caregivers did not know how
12 to prepare meat for young infants, especially at the very beginning of complementary
13 feeding ²⁹, thus infant feeding counseling should include these specific issues to
14 provide caregivers accurate knowledge and help them solve problems, such as
15 demonstration of the preparation of meat instead of just giving information.
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24 Compared with other nationalities, children of Tibetan nationality, who accounted for
25 about 10% in Huzhu County, were more likely to be anaemic¹⁴, probably because their
26 special customs and dietary habits with the main complementary food for children
27 being zanba (a local ethnic food consisting mainly of carbohydrates) and porridge,
28 which contain very few irons^{30, 31}. At the same time, poor family economic conditions
29 would also make it unaffordable to feed animal food to their children ³².
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39 **The causes of anaemia**

40 The causes of anaemia can generally be summarized into three categories: nutritional
41 anaemia, infectious diseases and genetic hemoglobin disorders. Nutritional anaemia
42 results from insufficient bioavailability of hemopoietic nutrients (iron, vitamin B12,
43 vitamin A and folic acid) needed to meet the demands of hemoglobin and erythrocyte
44 synthesis and decreased absorption enhancers such as vitamin C. Infectious diseases
45 include soil-transmitted helminths, malaria and schistosomiasis. Genetic hemoglobin
46 disorders include thalassemia and hemoglobin variants etc¹⁷. Many previous studies
47 have found that iron deficiency may be the most common cause of anaemia ^{1, 6, 17, 33}.
48 Our study confirmed that 80.9% of anaemic children aged 12-23 months in Huzhu
49 County were due to iron deficiency. The prevalence of iron deficiency among all
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4 children was 61.7% and body iron stores in all children tested averaged only -
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6 0.45±4.56 mg/kg.
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10 Iron deficiency (ID) is a state in which iron is insufficient to maintain normal
11
12 physiological functions of tissues such as blood, brain, and muscle. If iron deficiency
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14 lasts too long or is serious enough, it can result in iron deficiency anaemia (IDA)⁶. In
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16 addition to the important role of oxygen carrier in the heme group of hemoglobin,
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18 iron also exists in many key proteins in cells, such as cytochromes, myoglobin, neural
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20 transmitters, various enzymes and coenzymes³⁴. Therefore, iron deficiency not only
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22 causes anaemia, but also has many other adverse effects, especially on children in
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24 growth and development.
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27 Iron deficiency is often found in association with a deficiency of folic acid. Combined
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29 folic and iron deficiency may occur in preterm infants who are fed unfortified formula
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31 based on evaporated milk. Other study indicated that infants fed on goat's milk were
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33 also at risk³⁵. Our study found that 20.2% of children suffered from both iron
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35 deficiency and folic acid deficiency. Hence, attentions also need to be paid to the
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37 deficiency of folic acid. There was no vitamin B12 deficiency either in anaemic or non-
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39 anaemic children tested. However, causes of the remaining 19.1% of anaemic children
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41 were still unknowns and may need further explorations.
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45 **Recommendations on reducing nutritional anaemia**

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47 Deficiencies of iron and folic acid were the main causes of children's anaemia in Huzhu
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49 County, therefore, previous interventions of feeding counseling and nutrients
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51 supplements are appropriate and should be further strengthened.
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54 (1) Improving traditional delivery channels for infant and young child feeding (IYCF).

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56 IYCF is one of the key strategies to lower the risk of iron-deficiency anaemia in early
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58 infancy^{17, 36}. Previous studies found that inappropriate IYCF practices were common in
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4 many rural areas in China^{37, 38}, for instance, complementary foods generally contained
5 mainly carbohydrates and lacked protein and fat³⁹, or were introduced to children too
6 early or too late, or were given in too small amounts or not frequently enough^{40, 41}.
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11 The information-motivation-behavior skills (IMB) model indicates that information
12 can be transformed into action that can motivate individuals and eventually influence
13 their attitudes and behaviors^{42, 43}. In China, information and knowledge about infant
14 feeding was mainly disseminated through the traditional rural three-tier healthcare
15 system (county-township-village). Village doctors were responsible to provide face-to-
16 face infant and young child feeding counseling to caregivers^{14, 44}. Our previous study
17 in Qinghai found that training village doctors to deliver health education information
18 could be effective in improving caregivers' feeding practice¹⁵. Therefore, we should
19 continue making use of the traditional health information dissemination system and
20 measures need to be taken to further improve the quality of services, for example,
21 conducting regular refresh training and supervision, providing monetary incentives to
22 village doctors, and more importantly, tailoring IYCF information to the local feeding
23 problems and special dietary habits instead of barely giving general knowledge.
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39 (2) Exploring new channels for delivering IYCF information.

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41 Nowadays, mobile phones and the Internet have spread to millions of households in
42 China. Data showed that, by the end of 2017, there were 1417.49 million mobile
43 phone users and 772 million Internet users, of which 753 million were smartphone
44 Internet users. The Internet penetration rate reached 55.8%, of which 35.4% was in
45 rural areas¹⁹. Social media and smartphones have become new channels for
46 information acquisition, and these have been widely used in many health education
47 researches⁴⁵⁻⁴⁷. A systematic review proved the feasibility of delivering eHealth
48 interventions to improve health literacy skills among people with different health
49 conditions, risk factors and socio-economic backgrounds⁴⁸. However, using eHealth or
50 mHealth methods to deliver complementary feeding information in China is rarely
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4 reported. Therefore, further studies are needed to assess the feasibility and
5 effectiveness of such delivery channels in improving IYCF knowledge and practice in
6 rural China.
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10 11 (3) Strengthening nutrients supplements (YYB program). 12

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14 Nutrients supplements have been commonly accepted as effective interventions in
15 reducing child anaemia worldwide^{15, 49-54}. In China, a domestically produced multi-
16 nutrient powders (MNPs) for infants and young children called Ying Yang Bao (YYB)
17 was developed, and a study conducted in Gansu Province from 2001 to 2004 to test
18 the effectiveness of this complementary food supplement, showed that the use of YYB
19 could significantly reduce the anaemia prevalence⁵¹. Our controlled interventional
20 study in Huzhu and Guinan county from 2012 to 2014 found that the anaemia
21 prevalence decreased more in the intervention county (receiving YYB) than in the
22 control county (not receiving YYB, 71.1% to 47.8% vs 86.3% to 75.3%, respectively)¹⁵.
23 However, the YYB compliance needs to be improved⁵⁵ to further increase the
24 effectiveness of the interventional program. In addition, the sustainability of the YYB
25 programs also needs to be evaluated to find constraining factors and solutions since
26 the anaemia prevalence in Huzhu County went up from 47.8% in 2014 to in 59.1%
27 2018.
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43 44 **CONCLUSIONS** 45

46 The anaemia prevalence of children aged 6-23 months in Huzhu County was 59.1%
47 and children of younger age, Tibetan nationality and not introducing meat were more
48 likely to be anaemic. 80.9% of children with anaemia were due to iron deficiency, and
49 20.2% of them suffered from both iron and folic acid deficiencies. Therefore, previous
50 interventions of feeding counseling and nutrients supplements are appropriate and
51 should be further strengthened.
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Strengths and limitations of this study

The first strength of our study is that we proved that iron deficiency was the leading cause of anemia for young children in Huzhu county, and therefore confirm the appropriateness of the on-going interventional program. Secondly, we found specific age and ethnic groups of children who were more vulnerable to be anemic, and it implies that the future program implementation should pay more attentions to these groups. In addition, although this study was reported as a cross-sectional survey, we have collected data continuously since 2012, and the rebound of anemia prevalence suggested that sustainability issues of the current programs should be identified and addressed.

Our study has several limitations. Firstly, the study took place only within one rural Chinese county and caution is needed when generalizing the findings from this study to other settings. Secondly, although we surveyed all the eligible children aged 6-23 months and their caregivers in each sampled village, the sample size was still small. Thirdly, we only explored nutritional causes of child anemia, so the anemic causes of for nearly 20% of children were still unknown.

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Authorship statement

The study was initiated, conceptualized, and supervised by JSH, SYC and YFZ. LJW, YWH, QW and WW collected and analyzed data. LJW conducted laboratory tests. YFZ, YWH, and QW participated in the explanation and discussion of the results. The

manuscript was drafted by YWH, reviewed and revised by YFZ, QW, SYC and JSH. All authors read and approved the final manuscript.

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The survey was funded by the United Nations Children's Fund (UNICEF). The founder was involved in study design, data interpretation, preparation of the manuscript, and decision to publish.

Competing interests

None declared.

Data showing statement

Additional data can be accessed via the Dryad data repository at <https://datadryad.org/> with the doi: 10.5061/dryad.57v2100

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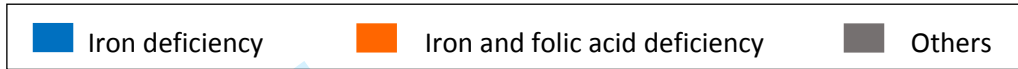
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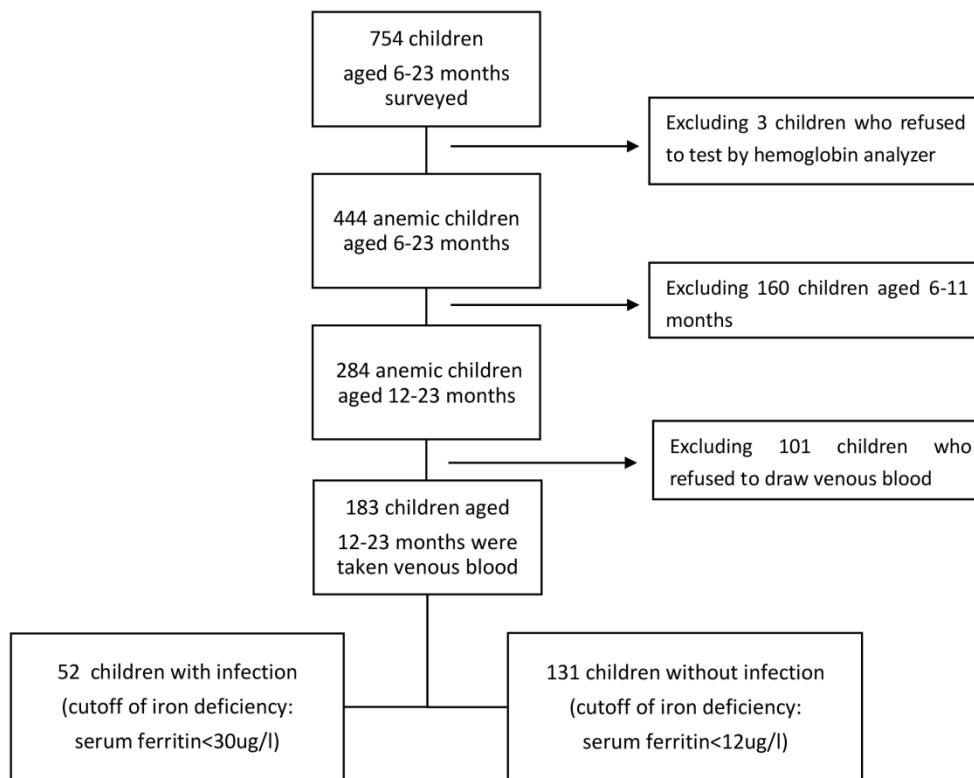
Figure legends

Figure 1 Flowchart of study procedures

Figure 2 Causes of anaemia



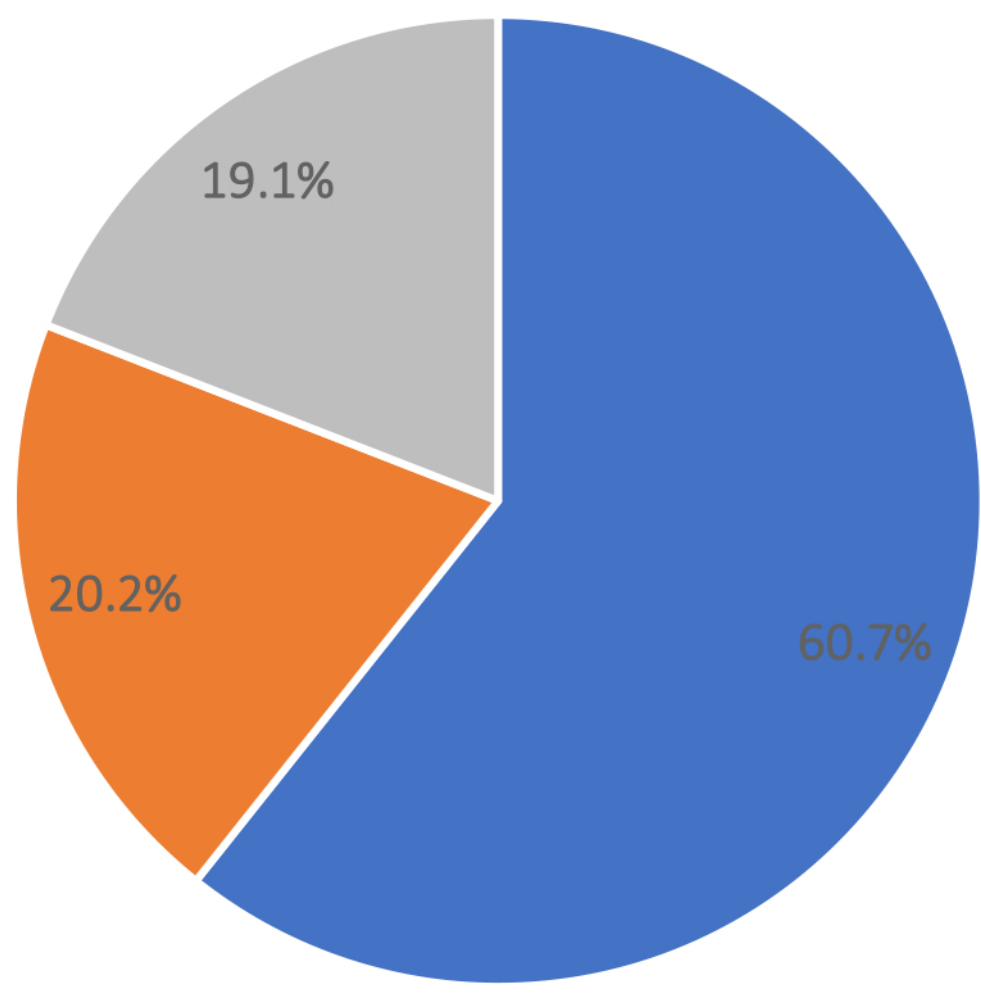
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Flowchart of study procedures

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causes of anaemia

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	3-4
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	6-7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants	7
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	11
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	9-10
Bias	9	Describe any efforts to address potential sources of bias	8-10
Study size	10	Explain how the study size was arrived at	8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	N/A
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	10-11
		(b) Describe any methods used to examine subgroups and interactions	N/A
		(c) Explain how missing data were addressed	10-11
		(d) If applicable, describe analytical methods taking account of sampling strategy	10-11
		(e) Describe any sensitivity analyses	N/A
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	12;27
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	27
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	12-13
		(b) Indicate number of participants with missing data for each variable of interest	14
Outcome data	15*	Report numbers of outcome events or summary measures	15-16
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	N/A
		(b) Report category boundaries when continuous variables were categorized	12-14
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarise key results with reference to study objectives	17
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	21-22
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	17-21
Generalisability	21	Discuss the generalisability (external validity) of the study results	21-22
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	22

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

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ORIGINAL ARTICLE**Title page****Prevalence and causes of anaemia in children aged 6-23 months in rural Qinghai,****China: findings from a cross-sectional study**

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ABSTRACT

Objective: To investigate the current situation of anaemia among children aged 6-23 months in a rural county in China, and to explore the influencing factors and the main causes of anaemia.

Design: A cross-sectional study.

Setting: Huzhu County in Qinghai Province, China

Participants: We selected 38 sampled villages using Proportional to Population Size sampling method. We obtained the name list of children aged 6-23 months in each sampled village and planned to survey all the eligible children aged 6-23 months and their caregivers.

Primary and secondary outcomes measures: The prevalence of anaemia, the influencing factors of anaemia, the laboratory tests for biological causes of anaemia, including serum ferritin, sTfR, folic acid, Homocysteine and Vitamin B12.

Results: A total of 754 children aged 6-23 months and their caregivers were surveyed, and 183 anaemic children aged 12-23 months were collected venous blood sample. The anaemia prevalence of children aged 6-23 months in Huzhu County was 59.1%. Children of younger age (OR=0.968, 95%CI 0.940, 0.998), Tibetan nationality (OR=3.123, 95%CI 1.473,6.623) and not introducing meat (OR=0.698, 95%CI 0.499,0.976) were more likely to be anaemic. More than 80% of children with anaemia were due to iron deficiency, and 20.2% of them had both iron and folic acid deficiencies.

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4 **Conclusions:** The anaemia prevalence of children aged 6-23 months in Huzhu County
5 was high and children of younger age, Tibetan nationality and not introducing meat
6 were more likely to be anaemic. The main cause of anaemia was nutritional anaemia,
7 with the vast majority being iron deficiency. Interventions of feeding counseling and
8 nutrients supplements are appropriate and should be further strengthened.
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15 **Strengths and limitations of this study:** The first strength was that we proved that
16 iron deficiency was the leading biological cause of anaemia for young children in
17 Huzhu County. Secondly, we identified specific age and ethnic groups of children who
18 were more vulnerable to be anaemic.. Our study also has several limitations. Firstly,
19 the study took place only within one rural Chinese county and caution is needed when
20 generalizing the findings from this study to other settings. Secondly, the sample size
21 was small.
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31 **Trial registration number:** ChiCTRPRC12002444 (Feb 15th, 2016; Version1).
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35 Main text

40 INTRODUCTION

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42 Childhood anaemia has long been a major public health problem worldwide. A
43 systematic analysis of population-representative data suggested that anaemia
44 prevalence in children younger than 5 years was 43% and there were 273 million
45 children with anaemia globally in 2011¹. The national anaemia prevalence in children
46 under 5 in China fluctuated between 12% and 23% between 1990 and 2005 and then
47 decreased from 19.3% in 2005 to 12.6% in 2010². National Nutrition and Health
48 Surveillance in 2013 showed that the prevalence of anaemia among children aged 0-
49 5 years was 11.6% across the country, 10.6% in urban areas and 12.4% in rural areas
50 respectively. However, huge regional differences exist, with rural Qinghai province
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4 being the highest (27.5%)³. In some rural areas, anaemia prevalence in children aged
5 6-23 months was higher than 30%^{4,5}.
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10 In the public health perspective, anaemia is defined by the World Health Organization
11 as a hemoglobin concentration 2 SDs below the mean hemoglobin concentration for
12 a normal population of the same gender and the same age group⁶. Anaemia has
13 irreversible adverse effects on childhood growth and development, even their
14 working abilities in adulthood⁷. Many studies suggested an association between
15 anaemia and impaired psychomotor development; impaired cognitive functions such
16 as concentration, intellectual status, memory and scholastic skills; psychological and
17 behavioral disorders such as attention-deficit/hyperactivity disorder (ADHD)⁸ and
18 autism spectrum disorder (ASD)⁹; decreased physical activity¹⁰. Meanwhile, anaemia
19 has been confirmed to be associated with impaired renal function, increased
20 absorption of lead, and impaired immunity¹¹. The Global Burden of Disease (GBD)
21 2000 report estimated that anaemia resulted in 68.4 million years lived with disability
22 (YLD), accounting for 8.8% the total number of all cases of disability¹², and the GBD
23 2004 update had similar findings, which exerted a substantial economic burden¹³.
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39 Our previous study in Qinghai Province found that the prevalence of anaemia in rural
40 areas was much higher than the national average level. In 2012, the prevalence of
41 anaemia among children aged 6-23 months in Huzhu, Minhe and Guinan County was
42 71.1%, 56.1% and 86.3%, respectively¹⁴. We carried out a controlled interventional
43 study in Huzhu and Guinan county from 2012 to 2014. And all children aged 6-23
44 months in the intervention county, Huzhu County, received YingYangBao (a
45 domestically produced multiple micronutrient powders for infants and young children)
46 and their caregivers received infant feeding counseling from trained village doctors.
47 The study found that the anaemia prevalence significantly decreased from 71.1% to
48 47.8% in Huzhu County¹⁵. WHO defines the prevalence of anaemia in the population
49 less than 20% as a mild public health problem, 20% to 40% as a moderate public health
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4 problem, and the prevalence of anaemia in the population $\geq 40\%$ as a serious public
5 health problem¹⁶. Therefore, childhood anaemia in Huzhu County is still a serious
6 public health problem in spite of the dramatic reduction of anaemia prevalence after
7 the study, and more efforts are needed to further decrease anaemia prevalence and
8 improve the nutrition status of children.
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15 There are many reasons for anaemia: acute and chronic infections that result in
16 inflammation (including chronic blood loss caused by hookworm infection or
17 schistosomiasis); nutritional anaemia caused by iron deficiency and other
18 micronutrient deficiencies, especially of folic acid, vitamin B12 and vitamin A; and
19 genetically inherited traits such as thalassemia¹⁷. Although it is generally accepted
20 that nutrients deficiency is the leading cause of child anaemia, proportions of anaemic
21 causes in specific areas are usually unknown. Since interventions based on
22 improvement of child feeding and nutrients supplements are only effective for
23 nutritional anaemia, understanding the status of anaemia-related nutrients (iron, folic
24 acid and vitamin B12) is crucial to estimate the potential effectiveness of nutritional
25 interventions. The purpose of this study is to investigate the current situation of
26 anaemia among children aged 6-23 months in Huzhu County, and to explore the
27 influencing factors and the main causes of anaemia, so as to develop more appropriate
28 strategies for combating this intractable public health issue.
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45 **METHODS**

46 **Study design**

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48 This study was conducted as a cross-sectional survey of children aged 6-23 months
49 and their caregivers in Huzhu County, Qinghai province. Proportional to Population
50 Size (PPS) sampling method was used to select sampled villages in the county. We first
51 obtained the name list of children aged 6-23 months in each sampled village and
52 aimed to survey all the eligible children aged 6-23 months and their caregivers. The
53 HemoCue Hb 301 analyzer (HemoCue, Lake Forest, CA, USA) was used to collect
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4 children's fingertip peripheral blood by using microcuvettes (for blood samples) to
5 detect hemoglobin levels of children in the all sampled villages. After 4-7 days,
6 anaemic children screened out by HemoCue Hb 301 analyzer were called to Huzhu
7 Maternal and Child Health and Family Planning Service Center to draw venous blood
8 sample for further blood routine test and laboratory tests.
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15 **Study setting**

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17 Qinghai Province lies in northwest China, with an area of around 720,000 km². By the
18 end of 2017, the resident population of the province was 5,838,000, of which the
19 resident population of rural areas was 2,808,400, accounting for 46.9%, and the ethnic
20 minority population was 2,854,900, accounting for 47.7%. Qinghai Province has 34
21 counties and 439 townships. The Qinghai resident per capita disposable income in
22 2017 was ¥9,462 (US\$1363.13)¹⁸ for rural people, which was far lower than the
23 national level (¥13,432(US\$1935.06))¹⁹.
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33 Huzhu County is located in the northeastern part of Qinghai Province and 27.9% of its
34 population is part of an ethnic minority, which includes Tu, Tibetan, Hui and other 28
35 ethnic minorities²⁰. Huzhu County covers an area of 3,424 km². The county governs
36 19 townships with 294 villages, with a total population of 401,540, of which the rural
37 population accounted for 76.0%. By the end of 2017, the resident per capita
38 disposable income in the rural area was ¥9810 (US\$1414.91)²¹.
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48 **Participants**

49 Children aged 6-23 months and their caregivers were invited to participate in this
50 survey. We excluded children with a structural or genetic birth defect such as neural
51 tube defects, congenital heart disease or phenylketonuria or caregivers who refused
52 to participate.
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58 **Survey instrument**

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4 We used the adapted WHO Maternal, Newborn and Child Health Household Survey
5 (MNCH HHS, unpublished, 2009) to collect household information and infant feeding
6 practices in each sampled village.
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10 11 **Sample size and Sampling**

12 The sample size required were calculated based upon estimated anaemia prevalence
13 in Huzhu County. We used the sample size calculation for proportion in single cross-
14 sectional survey to estimate the sample for our study. Based on 35% of expected
15 anaemia prevalence for children aged 6-23 months in Huzhu County, 5% of desired
16 absolute precision, and 2 of design effect, we calculated the sample size of 699.
17 Meanwhile, we also expected to draw 200 venous blood sample of anaemic children
18 aged 12-23 months. According to the estimated anaemia prevalence of 35% in Huzhu
19 County, we calculated that the total number of children aged 12-23 months needed
20 was 571. We assumed that the number of children equally distributed in three age
21 groups (6-11 months, 12-17 months, and 18-23 months), and calculated that 285
22 children aged 6-11 months were needed. Hence a sample size of 856 children aged 6-
23 23 months was used in this study.
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39 We knew the average number of children aged 6-23 months in each villages of Huzhu
40 County from the local Maternal and Child Health Family Planning Service Centre.
41 Proportional to Population Size (PPS) sampling method was used to select 38 sampled
42 villages to meet our sample size requirements in the county. We obtained the name
43 list of children aged 6-23 months in each sampled village and planned to survey all the
44 eligible children and their caregivers.
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52 **Training of Interviewers**

53 Staff from the Capital Institute of Pediatrics in Beijing were supervisors for this survey,
54 and 25 students were recruited from Qinghai Institute of Health Sciences as
55 interviewers. We provided them training for two days before fieldwork, which
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3 included communication skills, explanation of questionnaires, demonstration, role
4 plays, field practice, and group discussions. In addition, 4 of them were trained on
5 measuring hemoglobin levels with a HemoCue Hb 301 analyzer. After the training, a
6 half-day field practice was held in a village clinic. Any problems arising during the field
7 practice were discussed and solved directly.
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13 **Data collection**

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15 We carried out the survey from July 23th to 27th 2018. In every surveyed township,
16 staff notified the village doctors of the sample villages in advance, then the village
17 doctor called the caregivers to take their children to the village clinic for investigation.
18 Firstly, interviewers introduced the aim of the survey to the mothers or other
19 caregivers and obtained written informed consent from them. Then the interviewers
20 questioned them following the instructions.
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31 We used smartphones with the household survey questionnaire set up in specially
32 developed software to record data ²². Four teams of interviewers carried out the
33 survey, with 6 surveyors and 1 supervisor in each team. Data for each questionnaire
34 were uploaded into an Excel database via the Internet server. Once the interview was
35 completed, the special surveyor in each team measured hemoglobin with a HemoCue
36 Hb 301 analyzer (HemoCue, Lake Forest, CA) by drawing around 10 ul finger blood.
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45 Children aged 12-23 months screened as anaemia by HemoCue Hb 301 analyzer were
46 informed to go to the Huzhu County Maternal and Child Health Family Planning Service
47 Centre for further tests from July 31 to August 1 2018. About 4-5ml venous blood of
48 each children were collected by experienced nurses and placed into two tubes. The
49 first 1-2ml blood sample was collected into a labeled EDTA-K2 coated tube for blood
50 routine test using whole blood. And the second 3ml blood sample was collected
51 (without removing the needle) into a labeled vacuum separating tube for serum
52 ferritin(SF), soluble transferrin receptor(sTfR), C-reactive protein(CRP), α -1 acid
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glycoprotein(AGP), vitamin B12, Homocysteine(HCY), and folic acid concentration using serum.

Blood routine test and blood centrifugation were completed in the local laboratory. The blood sample in labeled vacuum separating tubes were placed for 30 minutes, then were centrifuged at 1500 turn/minute for 15 minutes. The serum was separated into 1, 2 and 3 cryotubes using disposable pipettes.

After the field work, all the blood samples were immediately stored at -70°C and transported as soon as possible to Nutritional Institute for Institute for Nutrition and Health, China Center for Disease Control and Prevention for further laboratory testing. Repeated freezing and thawing were strictly avoided during transportation and storage.

Laboratory analysis

The blood routine test was conducted using automatic blood cell analyzer (Horiba, ABX Micros 60-OT, France) in local laboratory. SF, vitamin B12 and serum folic acid were analyzed by Roche Cobas e601 analyzer (Germany) using electrochemiluminescence immunoassay (ECLIA). sTfR, CRP, AGP were analyzed by Hitachi 7600-110 chemistry autoanalyzer (Japan) using immunoturbidimetry and HCY was analyzed by Hitachi 7600-110 chemistry autoanalyzer (Japan) using enzymatic cycling assay.

Data management and statistical analysis

Data of questionnaires were automatically transformed and pooled into a Microsoft Excel sheet. After the data cleaning, we converted the database into a database file (dbf) for the final analysis.

We carried out statistical analysis with SAS 9.2 for Windows. The median (Q1, Q3) was

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4 used to describe the age in years of mothers and grandparents of children. Mean and
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6 standard deviation (SD) was used to describe the values of serum ferritin and body
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8 iron. Percentages were presented in binary or categorical variables. We used the
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10 Pearson χ^2 -test and Fisher exact test to compare binary and categorical variables.

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13 Moreover, we carried out a logistic regression analysis to identify factors associated
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15 with children's anaemia in this survey. All relevant factors were first selected by
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17 univariate logistic analysis, including child's age, child's sex, parents' age, parents'
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19 nationality, parents' education, parents' job, whether parent worked outside the
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21 county, whether children aged 6-23 months had been given iron-rich or iron-fortified
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23 foods during last 24 hours, whether children aged 6-23 months had been given meat
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25 during last 24 hours, minimum dietary diversity, whether child had coughed, fever or
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27 diarrhea in the past two weeks, and whether children aged 6-23 months had been
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29 given YYB 5 bags or more. Only those that were significant in the final multivariate
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31 model are presented. We present Odds Ratios (OR) and 95% confidence intervals (CI)
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33 and considered two-tailed P-values of <0.05 for a significant difference.

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36 We calculated the feeding practice indicators based on the WHO guideline "Indicators
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38 for Assessing Infant and Young Child Feeding Practices"²³, which based on the 24 hour
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40 recall method. And all individual hemoglobin values were adjusted using WHO
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42 recommendations based on the altitude of the surveyed villages where children lived.
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44 An adjusted hemoglobin lower than 110g/L¹⁶ was defined as anaemia and was used
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46 to calculate the prevalence of anaemia. A hemoglobin concentration of 90–110 g/L
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48 was defined as mild anaemia, <90 g/L as moderate anaemia or severe anaemia. Cut-
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50 offs for elevated CRP and AGP were >5mg/L and >1mg/L, respectively²⁴. If one of
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52 these two indicators were elevated, the children surveyed were classified as infected
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54 children. Serum ferritin concentration <12ug/L and <30ug/L were used to define iron
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56 deficiency in non-infected children and infected children, respectively. The children
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58 with concurrent anaemia and iron deficiency were diagnosed with iron deficiency
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4 anaemia²⁵. A plasma folic acid concentration of <4ng/ml was used to define folic acid
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6 deficiency according to WHO guidelines²⁶. Vitamin B12 deficiency was defined as
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8 <197pg/ml. Cut-offs for elevated sTfR and HCY were 8.3mg/L²⁷ and 14umol/l,
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10 respectively²⁸.

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13 Body iron stores (BI) were estimated by applying Cook's formula as follows: body
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15 iron(mg/kg) =-[log(R/F ratio)-2.82290]/0.1207. The R/F ratio was soluble transferrin
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17 receptor(sTfR)/ serum ferritin (SF)²⁹. Among them, sTfR needs to be transformed by
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19 the following formula: Flowers sTfR=1.5 * Roche sTfR +0.35mg/L³⁰. The positive value
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21 means iron surplus in stores and the negative value means iron deficit in tissues.
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23 24 25 **Ethical considerations**

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27 The study was approved by the Ethics Committee of the Capital Institute of Pediatrics
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29 (reference no.2018017). All interviewees read the Information Sheet and provided
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31 written consent on behalf of the children involved in our study.
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33 34 35 **Participant and public involvement**

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37 The participants and the public were not involved in the design, recruitment and
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39 conduct of the study. There are no plans to disseminate the study findings to the study
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41 participants.
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43 44 45 **RESULTS**

46
47 The flowing chart of this study is shown in **Figure 1**. Among 912 children aged 6-23
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49 months on the name list, a total of 754 children and their caregivers were surveyed,
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51 with 444 being anaemic. There were 183 children aged 12-23 months agreed to draw
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53 venous blood for further laboratory tests, and 52 of them were found acute and/or
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55 chronic infections (CRP > 5 mg/L in blood routine test or AGP > 1 g/L in laboratory
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57 test). Besides, there was no statistical difference between 183 children aged 12-23
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months who were taken venous blood and 101 children who refused to draw venous blood (Supplementary Table 1).

Characteristics of 754 surveyed children and their caregivers are shown in **Table 1**. Nearly all main caregivers of the children surveyed were mothers and grandparents, and about 70% of mothers were Han nationality, followed by Tu and Tibetan nationality. More than 60% of mother attended junior high school, and the proportion of mothers who were illiterate was only 4.0%. More than half of grandparents were still illiterate. The main source of household income was working outside the county, followed by agriculture-related work such as growing crops, vegetables and animal husbandry.

Table1 Characteristics of surveyed children and their caregivers (N=754)

Characteristic	Percentage or median
Children	
Age, %(n)	
6-11months	32.8(247)
12-17months	32.6(246)
18-23months	34.6(261)
Sex, %(n)	
Boy	52.1(393)
Girl	47.9(361)
Main caregivers, %(n)	
Mother	48.7(367)
Grandparent	46.4(350)
Father	4.8(36)
Other	0.1(1)
Mothers	
Age in years (median (Q1, Q3))	29(26,31)
Nationality, %(n)	
Han	69.9(523)

Tu	22.8(171)
Tibetan	6.3(47)
Hui	0.3(2)
Others	0.7(5)
Education, %(n)	
Illiterate	4.0(30)
Primary school	13.8(103)
Junior high school	61.6(461)
Senior high school or above	17.4(130)
Do not know	3.2(24)
Grandparents	
Age in year (median (Q1, Q3))	54(50,59)
Education, %(n)	
Illiterate	60.3(211)
Primary school	22.6(79)
Junior high school	15.1(53)
Senior high school	1.1(4)
Do not know	0.9(3)
Household income, %(n)	
Working outside the county	89.2(673)
Agriculture-related work	6.4(48)
Self-employed	2.9(22)
Others	1.2(9)
Do not know	0.3(2)

Table 2 shows the prevalence of anaemia and hemoglobin levels by age and severity in this survey. Most of anaemic children were mildly anaemic, accounting for 76.9% of the total. The prevalence of anaemia in the 18 to 23-month group was significantly lower than that in the 6 to 11-month group ($p=0.0026$) and the 12 to 17-month group ($p=0.0261$). The median hemoglobin levels in the 6 to 11-month group and 12 to 17-month group were lower than that in the 18 to 23-month group (116g/L vs. 121 g/L, $p<0.0001$ and $p=0.0004$ respectively).

Table 2 Prevalence of anaemia and hemoglobin level by age and severity

	Anaemia prevalence (N=751) *			Hemoglobin level (median, (Q1, Q3))
	Mild anaemia	Moderate or severe	Total	
	(%, n)	anaemia (%, n)	(%, n)	
6-11m	49.8(123)	15.0(37)	64.8(160)	116(106,125)
12-17 m	51.2(126)	10.2(25)	61.4(151)	116(107,125)
18-23 m	43.4(112)	8.1(21)	51.6(133)	121(111,130)
Total	48.1(361)	11.1(83)	59.1(444)	117(108,127)

*Three children refused to measure hemoglobin.

Table 3 shows the results of a univariate logistic analysis of anaemia prevalence in this survey. Older children were less likely to suffer from anaemia than younger children ($p=0.0028$). The prevalence of anaemia in children with Tibetan nationality was significantly higher than those with Han nationality ($p=0.0016$). The prevalence of anaemia in children who consumed iron-rich or iron-fortified foods was significantly lower than that in children who did not consume these foods ($p=0.0150$). The prevalence of anaemia in children who were given meat was also significantly lower than that in children without meat ($p=0.0077$). Furthermore, the anaemia prevalence of children achieving minimum dietary diversity was significantly lower than that of children who did not meet the standard ($p=0.0163$).

Table 3 Univariate logistic analysis of the prevalence of anaemia

Factors	Sample	Case	Anaemia prevalence	p-value
Child's age				0.0028
Nationality				
Han	521	291	55.9	

Hui	2	0	0	0.9799
Tu	170	108	63.5	0.0836
Tibetan	47	38	80.9	0.0016
Other	5	3	60.0	0.8570
Children aged 6-23 months given iron-rich or iron-fortified food during the last 24 hours				
Yes	475	265	55.8	0.0150
No	276	179	64.9	
Children aged 6-23 months given meat during the last 24 hours				
Yes	210	108	51.4	0.0077
No	541	336	62.1	
Minimum dietary diversity during the last 24 hours				
Yes	364	199	54.7	0.0163
No	387	245	63.3	

Note:

¹ Children aged 6-23 months given iron-rich or iron-fortified foods during the last 24 hours: the proportion of children aged 6–23 months had been given iron-rich food or iron fortified food during the last 24 hours that was specially designed for infants and young children, or that was fortified in the home. Iron-rich or iron-fortified foods include flesh foods, commercially fortified foods specially designed for infants and young children which contain iron, or foods fortified in the home with a micronutrient powder containing iron or a liquid-based nutrient supplement containing iron, but not iron tablets.

² Children aged 6-23 months given meat during the last 24 hours: the proportion of children aged 6–23 months had been given meat during the last 24 hours that include beef, pork, lamb or other meat and liver, kidney, heart, or other organ meats, and fresh or dried fish, etc.

³ Minimum dietary diversity during the last 24 hours: the proportion of children aged 6-23 months who received foods from four or more food groups during last 24 hours. The food groups were: a) grains, root and tubers; b) legumes and nuts; c) dairy products (milk, yogurt, cheese); d) meat (meat, fish, poultry and liver/organ meat); e) eggs; f) vitamin-A rich fruits and green vegetables; g) other fruits and vegetables.

The results of multivariate logistic analysis of anaemia prevalence in surveyed children is shown in **Table 4**. Older children (OR=0.968, 95%CI 0.940, 0.998) and those consuming meat (OR=0.698, 95%CI 0.499,0.976) were associated with lower anaemia levels, whereas children of Tibetan nationality (OR=3.123, 95%CI 1.473,6.623) were more likely to be anaemic.

Table 4 Multivariate logistic analysis of anaemia prevalence

Independent variable	Influential factors of anaemia			
	β	Wald	<i>P</i>	OR (95%CI)
Child' age	-0.0321	4.4930	0.0340	0.968(0.940,0.998)
Nationality				
Hui –Han	-13.6385	0.0006	0.9797	<0.001
Tu-Han	0.3038	2.7371	0.0980	1.335(0.945,1.942)
Tibetan-Han	1.1388	8.8166	0.0030	3.123(1.473,6.623)
Other-Han	0.0294	0.0010	0.9745	1.030(0.169,6.260)
Introduction of meat	-0.3595	4.4118	0.0357	0.698(0.499,0.976)

Results of the laboratory tests for 183 children are shown in **Table 5**. There were 113 children with iron deficiency, accounting for 61.7%. The mean serum ferritin concentration in anaemic children was significantly lower than that in non-anaemic children ($10.2 \pm 9.6\text{ng/mL}$ vs. $22.6 \pm 15.9\text{ng/mL}$, $p < 0.0001$). The body iron store in anaemic children was significantly lower than that in non-anaemic children ($-3.1 \pm 4.4\text{mg/kg}$ vs. $2.1 \pm 3.3\text{mg/kg}$, $p < 0.0001$). 32.6% of children with anaemia had elevated sTfR, significantly higher than that in children without anaemia (4.3%, $p < 0.0001$). At the same time, the proportion of folic acid deficiency in anaemic children was also significantly higher than that in non-anaemic children (20.2% vs. 5.3%, $p = 0.0024$). There was no vitamin B12 deficiency either in anaemic or non-anaemic children.

Table 5 Results of laboratory tests

	Anaemic children (N=89)	Non-anaemic children (N=94)	Total (N=183)	<i>p</i>
Serum ferritin(ug/L)				
Mean ± SD	10.2 ± 9.6	22.6 ± 15.9	16.6 ± 14.6	<0.0001
<12ug/L for non-infected children or <30ug/L for infected children (% , n)	80.9% (72)	43.6% (41)	61.7% (113)	<0.0001
sTfR (>8.3mg/L) (% , n)	32.6% (29)	4.3% (4)	18.0% (33)	<0.0001
Body iron store (mean ± SD)	-3.1 ± 4.4	2.1 ± 3.3	-0.45 ± 4.56	<0.0001
Folic acid (<4ng/mL) (% , n)	20.2% (18)	5.3% (5)	12.6% (23)	0.0024
Vitamin B12 (<197pg/mL) (% , n)	0.0% (0)	0.0% (0)	0.0% (0)	-
Homocysteine (>14umol/L) (% , n)	4.5% (4)	1.1% (1)	2.7% (5)	0.1425

Figure 2 shows biological causes of anaemia. 80.9% of children with anaemia were due to iron deficiency, and 20.2% of them had both iron and folic acid deficiencies. The biological causes of 19.1% anaemic children were unknown.

DISCUSSION

Main findings

The anaemia prevalence of children aged 6-23 months in Huzhu County was 59.1% and most of them were mildly anaemic. The prevalence of anaemia in the 18 to 23month group was significantly lower than that in the 6 to 11month group ($p=0.0026$) and the 12 to 17month group ($p=0.0261$). Meanwhile, children of younger age, Tibetan nationality and not introducing meat were more likely to be anaemic. 80.9% of children with anaemia were due to iron deficiency, and 20.2% suffered from both iron deficiency and folic acid deficiency. The prevalence of iron deficiency among all children was 61.7% and 43.6% of non-anaemic children also had iron deficiency. Body

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4 iron stores in all children tested averaged -0.45 ± 4.56 mg/kg. The proportion of
5 microcytic hypochromic anaemia (MCH, MCV and MCHC were lower than normal
6 value) was 13.1%. And the specificity of the combination of MCV + MCH + MCHC in
7 the diagnosis of iron deficiency anaemia was 100%, but its sensitivity was only 17.8%.
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13 **Influencing factors of anaemia**

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15 Children aged 6-11 months are in the transition period from exclusive breastfeeding
16 to complementary feeding, during which the storage iron from birth is depleted and
17 complementary foods become the main source of iron, and they were more likely to
18 suffer from anaemia. In our study, anaemia prevalence of children at this age is the
19 highest (64.8%).
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27 Our analysis showed that eating meat was a protective factor of child anaemia,
28 however, few caregivers gave meat to their children of this age due to the wrong
29 beliefs that they could not digest meat. In addition, some caregivers did not know how
30 to prepare meat for young infants, especially at the very beginning of complementary
31 feeding³¹, thus infant feeding counseling should include these specific issues to
32 provide caregivers accurate knowledge and help them solve problems, such as
33 demonstration of the preparation of meat instead of just giving information.
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43 Compared with other nationalities, children of Tibetan nationality, who accounted for
44 about 10% in Huzhu County, were more likely to be anaemic¹⁴, probably because their
45 special customs and dietary habits with the main complementary food for children
46 being zanba (a local ethnic food consisting mainly of carbohydrates) and porridge,
47 which contain very few irons^{32,33}. At the same time, poor family economic conditions
48 would also make it unaffordable to feed animal food to their children³⁴.
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56 **The biological causes of anaemia**

57 The biological causes of anaemia can generally be summarized into three categories:
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4 nutritional anaemia, infectious diseases and genetic hemoglobin disorders. Nutritional
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6 anaemia results from insufficient bioavailability of hemopoietic nutrients (iron,
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8 vitamin B12, vitamin A and folic acid) needed to meet the demands of hemoglobin
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10 and erythrocyte synthesis and decreased absorption enhancers such as vitamin C.
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12 Infectious diseases include soil-transmitted helminths, malaria and schistosomiasis.
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14 Genetic hemoglobin disorders include thalassemia and hemoglobin variants etc¹⁷.
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16 Many previous studies have found that iron deficiency may be the most common
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18 cause of anaemia ^{1, 6, 17, 35}. Our study confirmed that 80.9% of anaemic children aged
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20 12-23 months in Huzhu County were due to iron deficiency. The prevalence of iron
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22 deficiency among all children was 61.7% and body iron stores in all children tested
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24 averaged only -0.45 ± 4.56 mg/kg.

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27 Iron deficiency (ID) is a state in which iron is insufficient to maintain normal
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29 physiological functions of tissues such as blood, brain, and muscle. If iron deficiency
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31 lasts too long or is serious enough, it can result in iron deficiency anaemia (IDA)⁶. In
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33 addition to the important role of oxygen carrier in the heme group of hemoglobin,
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35 iron also exists in many key proteins in cells, such as cytochromes, myoglobin, neural
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37 transmitters, various enzymes and coenzymes³⁶. Therefore, iron deficiency not only
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39 causes anaemia, but also has many other adverse effects, especially on children in
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41 growth and development.

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44 Iron deficiency is often found in association with a deficiency of folic acid. Combined
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46 folic and iron deficiency may occur in preterm infants who are fed unfortified formula
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48 based on evaporated milk. Other study indicated that infants fed on goat's milk were
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50 also at risk³⁷. Our study found that 20.2% of children suffered from both iron
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52 deficiency and folic acid deficiency. Hence, attentions also need to be paid to the
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54 deficiency of folic acid. There was no vitamin B12 deficiency either in anaemic or non-
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56 anaemic children tested. However, biological causes of the remaining 19.1% of
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58 anaemic children were still unknowns and may need further explorations.
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Recommendations on reducing nutritional anaemia

Deficiencies of iron and folic acid were the main biological causes of children's anaemia in Huzhu County, therefore, feeding counseling and nutrients supplements, as biological interventions, are appropriate and should be further strengthened.

(1) Improving traditional delivery channels for infant and young child feeding (IYCF) nutrients supplements (YYB program).

IYCF is one of the key strategies to lower the risk of iron-deficiency anaemia in early infancy^{17, 38}. Previous studies found that inappropriate IYCF practices were common in many rural areas in China^{39,40}, for instance, complementary foods generally contained mainly carbohydrates and lacked protein and fat⁴¹, or were introduced to children too early or too late, or were given in too small amounts or not frequently enough^{42,43}.

Nutrients supplements have been commonly accepted as effective interventions in reducing child anaemia worldwide^{15, 44-49}. In China, a domestically produced multi-nutrient powders (MNPs) for infants and young children called Ying Yang Bao (YYB) was developed, and a study conducted in Gansu Province from 2001 to 2004 to test the effectiveness of this complementary food supplement, showed that the use of YYB could significantly reduce the anaemia prevalence⁴⁶.

The information-motivation-behavior skills (IMB) model indicates that information can be transformed into action that can motivate individuals and eventually influence their attitudes and behaviors^{50,51}. In China, information and knowledge about infant feeding was mainly disseminated through the traditional rural three-tier healthcare system (county-township-village). Village doctors were responsible to provide face-to-face infant and young child feeding counseling to caregivers^{14, 52}. We conducted a controlled interventional study in Huzhu and Guinan County from 2012 to 2014, training village doctors to provide IYCF counseling and disseminating YYB to caregivers,

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4 and the results showed that the anaemia prevalence decreased more in the
5 intervention county (receiving IYCF counseling and YYB) than in the control county(not
6 receiving IYCF counseling and YYB, 71.1% to 47.8% vs. 86.3% to 75.3%, respectively)¹⁵.
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8 We also found an improvement of caregivers' feeding practice¹⁵. Therefore, we should
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10 continue making use of the traditional health information dissemination system and
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12 measures need to be taken to further improve the quality of services, for example,
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14 conducting regular refresh training and supervision, providing monetary incentives to
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16 village doctors, and more importantly, tailoring IYCF information to the local feeding
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18 problems and special dietary habits instead of barely giving general knowledge.
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23 (2) Exploring new channels for delivering IYCF information and disseminating YYB.

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25 Although traditional delivery channels (mainly by village doctors) proved to be
26
27 effective in our previous study, the key IYCF indicators were still low and the YYB
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29 compliance needed to be further improved⁵³ to increase the effectiveness of the
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31 interventional program. In addition, the sustainability of the YYB programs could also
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33 be an issue since the anaemia prevalence in Huzhu County went up from 47.8% in
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35 2014 to 59.1% in 2018. Therefore, we need to explore new channels for delivery these
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37 interventions.
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42 Nowadays, mobile phones and the Internet have spread to millions of households in
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44 China. Data showed that, by the end of 2017, there were 1417.49 million mobile
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46 phone users and 772 million Internet users, of which 753 million were smartphone
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48 Internet users. The Internet penetration rate reached 55.8%, of which 35.4% was in
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50 rural areas¹⁹. Social media and smartphones have become new channels for
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52 information acquisition, and these have been widely used in many health education
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54 researches⁵⁴⁻⁵⁶. A systematic review proved the feasibility of delivering eHealth
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56 interventions to improve health literacy skills among people with different health
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58 conditions, risk factors and socio-economic backgrounds⁵⁷. However, using eHealth or
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60 mHealth methods to deliver complementary feeding information in China is rarely

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4 reported. Therefore, further studies are needed to assess the feasibility and
5 effectiveness of such delivery channels in improving IYCF knowledge and practice as
6 well as YYB compliance in rural China.
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10 11 **CONCLUSIONS**

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14 The anaemia prevalence of children aged 6-23 months in Huzhu County was 59.1%
15 and children of younger age, Tibetan nationality and not introducing meat were more
16 likely to be anaemic. 80.9% of children with anaemia were due to iron deficiency, and
17 20.2% of them suffered from both iron and folic acid deficiencies. Therefore, previous
18 interventions of feeding counseling and nutrients supplements are appropriate and
19 should be further strengthened.
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28 **Strengths and limitations of this study**

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30 The first strength of our study was that we proved that iron deficiency was the leading
31 biological cause of anaemia for young children aged 12-23 months in Huzhu County,
32 and therefore confirm the appropriateness of the on-going interventional(YYB
33 supplementation). Secondly, we found specific age and ethnic groups of children who
34 were more vulnerable to be anaemic, and it implies that the future program
35 implementation should pay more attentions to these groups.
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41 Our study has several limitations. Firstly, the study took place only within one rural
42 Chinese county and caution is needed when generalizing the findings from this study
43 to other settings. Secondly, although we surveyed all the eligible children aged 6-23
44 months and their caregivers in each sampled village, the sample size was still small.
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53
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55 and Family Planning Service Center for coordination, logistic arrangements and blood
56 routine tests, and we want to thank all students from Qinghai Institute of Health
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4 Sciences for their hard work as interviewers. We are indebted to all the mothers and
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6 caregivers who participated in our survey.
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10 **Authorship statement**

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12 The study was initiated, conceptualized, and supervised by JSH, SYC and YFZ. LJW,
13
14 YWH, QW and WW collected and analyzed data. LJW conducted laboratory tests. YFZ,
15
16 YWH, and QW participated in the explanation and discussion of the results. The
17
18 manuscript was drafted by YWH, reviewed and revised by YFZ, QW, SYC and JSH. All
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20 authors read and approved the final manuscript.
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26
27 The survey was funded by the United Nations Children's Fund (UNICEF). The founder
28
29 was involved in study design, data interpretation, preparation of the manuscript, and
30
31 decision to publish.
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35 **Competing interests**

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38 None declared.
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42 **Data showing statement**

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44 Additional data can be accessed via the Dryad data repository at
45
46 <https://datadryad.org/> with the doi: 10.5061/dryad.57v2100
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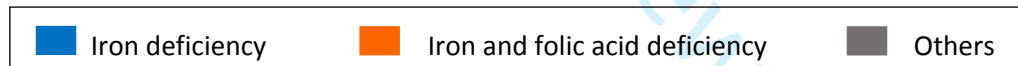
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Figure legends

Figure 1 Flowchart of study procedures

Figure 2 Biological causes of anaemia



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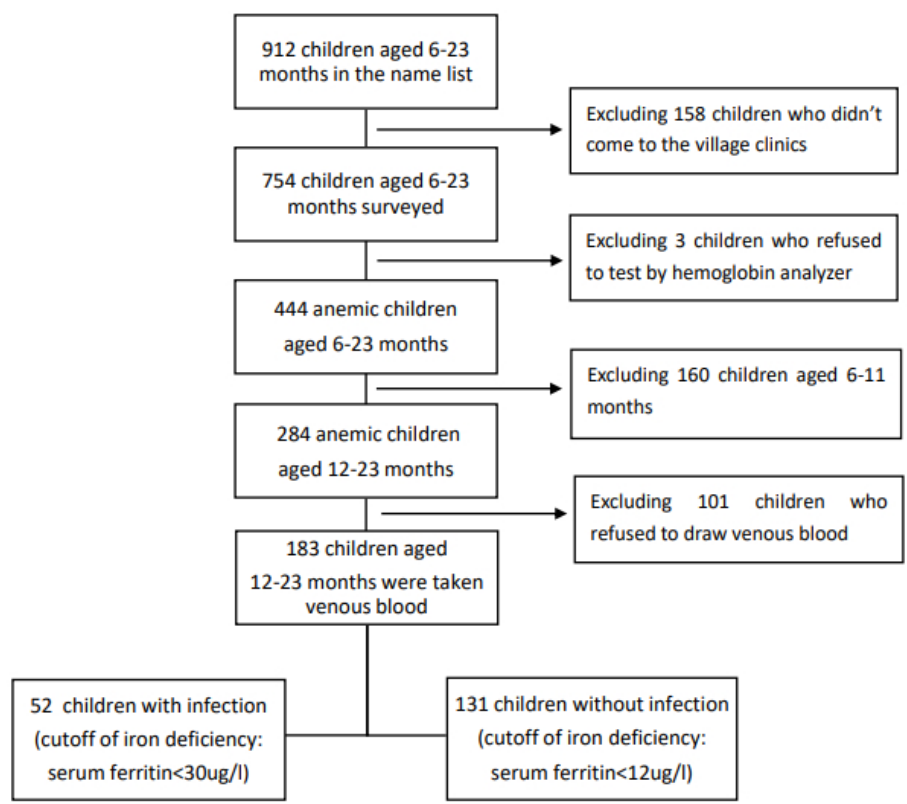
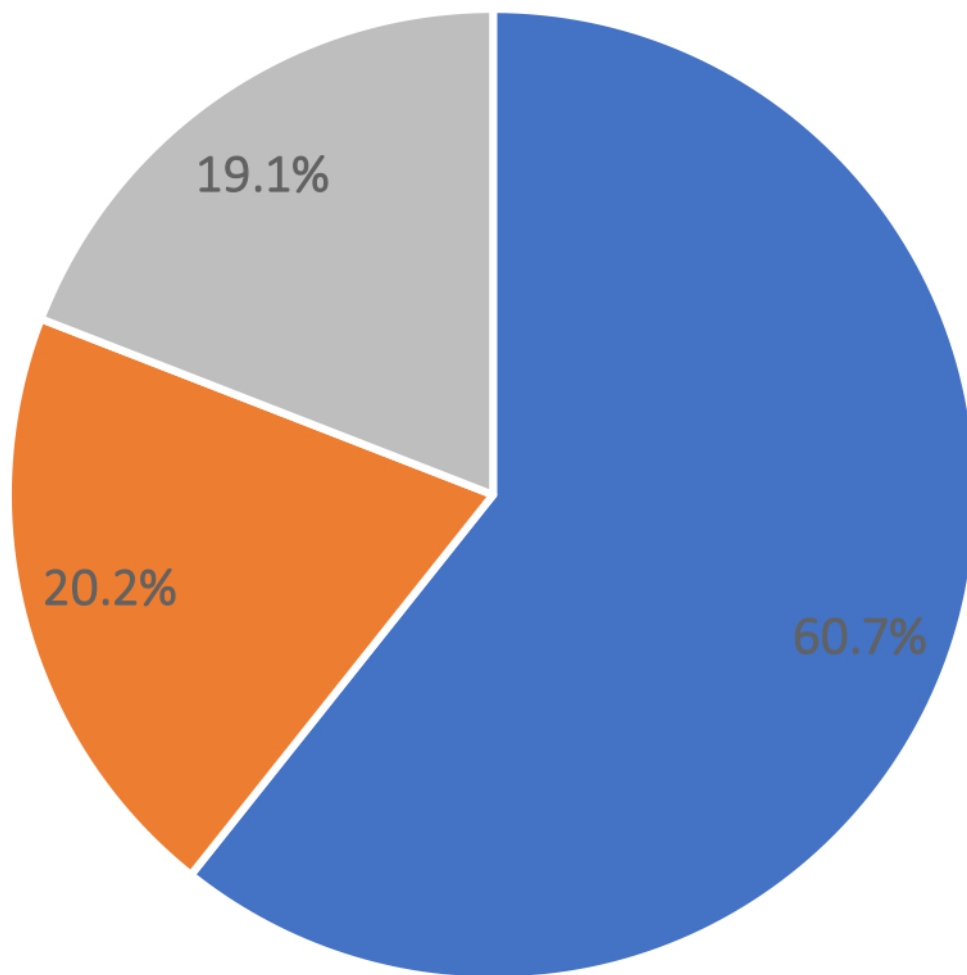


Figure 1 Flowchart of study procedures

Flowchart of study procedures



causes of anaemia

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Supplementary Table 1 Background characteristics of children with and without venous blood drawn

Characteristic	Children with venous blood drawn(N=183)	Children without venous blood drawn (N=101)	p-value
Children			
Age, %(n)			0.3486
12-17months	56.3(103)	50.5(51)	
18-23months	43.7(80)	49.5(50)	
Sex, %(n)			0.9184
Boy	54.1(99)	45.9(84)	
Girl	53.5(54)	46.5(47)	
Main caregivers, %(n)			
Mother	40.4(74)	49.5(50)	0.3213
Grandparent	53.6(98)	47.5(48)	
Father	5.5(10)	3.0(3)	
Other	0.6(1)	-	
Mothers			
Age in years (median (Q1, Q3))	29(26,32)	31(28,34)	0.0511
Nationality, %(n)			0.6858
Han	68.9(51)	62.0(31)	
Tu	24.3(18)	28.0(14)	
Tibetan	6.8(5)	10.0(5)	
Education, %(n)			0.2235
Illiterate	8.1(6)	2.0(1)	
Primary school	17.6(13)	26.0(13)	
Junior high school	58.1(43)	64.0(32)	
Senior high school or above	14.9(11)	8.0(4)	
Do not know	1.4(1)	-	
Grandparents			
Age in year (median (Q1, Q3))	54(50,60)	54(51,57.5)	0.6643
Education, %(n)			0.4168
Illiterate	62.2(61)	50.0(24)	
Primary school	20.4(20)	27.1(13)	
Junior high school	15.3(15)	18.8(9)	

Senior high school	2.0(2)	2.1(1)	
Do not know	-	2.1(1)	
Household income, %(n)			0.1215
Working outside the county	91.3(167)	91.1(92)	
Agriculture-related work	7.1(13)	3.9(4)	
Self-employed	1.6(3)	3.0(3)	
Others	-	2.0(2)	

For peer review only

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of cross-sectional studies

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Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study’s design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	3-4
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	6-7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants	7
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	11-12
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	9-10
Bias	9	Describe any efforts to address potential sources of bias	8-10
Study size	10	Explain how the study size was arrived at	8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	N/A
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	10-11
		(b) Describe any methods used to examine subgroups and interactions	N/A
		(c) Explain how missing data were addressed	10-11
		(d) If applicable, describe analytical methods taking account of sampling strategy	10-11
		(e) Describe any sensitivity analyses	N/A
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	12;27
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	29
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	12-13
		(b) Indicate number of participants with missing data for each variable of interest	29
Outcome data	15*	Report numbers of outcome events or summary measures	14-18
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	N/A
		(b) Report category boundaries when continuous variables were categorized	18
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarise key results with reference to study objectives	18
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	23
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	18-20
Generalisability	21	Discuss the generalisability (external validity) of the study results	23
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	24

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Prevalence and causes of anaemia in children aged 6-23 months in rural Qinghai, China: findings from a cross-sectional study

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Secondary Subject Heading:	Nutrition and metabolism, Public health, Epidemiology
Keywords:	Anaemia < HAEMATOLOGY, Nutrition < TROPICAL MEDICINE, iron deficiency, children, China

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Manuscripts

ORIGINAL ARTICLE**Title page****Prevalence and causes of anaemia in children aged 6-23 months in rural Qinghai,****China: findings from a cross-sectional study**

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24 **Key words:**
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26 Anaemia; iron deficiency; nutrition; children; China
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29 **Word count:**
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31 4607
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ABSTRACT

Objective: To investigate the current situation of anaemia among children aged 6-23 months in a rural county in China, and to explore the influencing factors and the main causes of anaemia.

Design: A cross-sectional study.

Setting: Huzhu County in Qinghai Province, China

Participants: We selected 38 sampled villages using Proportional to Population Size sampling method. We obtained the name list of children aged 6-23 months in each sampled village and planned to survey all the eligible children aged 6-23 months and their caregivers.

Primary and secondary outcomes measures: The prevalence of anaemia, the influencing factors of anaemia, the laboratory tests for biological causes of anaemia, including serum ferritin, sTfR, folic acid, Homocysteine and Vitamin B12.

Results: A total of 754 children aged 6-23 months and their caregivers were surveyed, and 183 anaemic children aged 12-23 months were collected venous blood sample. The anaemia prevalence of children aged 6-23 months in Huzhu County was 59.1%. Children of younger age (OR=0.968, 95%CI 0.940, 0.998), Tibetan nationality (OR=3.123, 95%CI 1.473,6.623) and not introducing meat (OR=0.698, 95%CI 0.499,0.976) were more likely to be anaemic. More than 80% of children with anaemia were due to iron deficiency, and 20.2% of them had both iron and folic acid deficiencies.

Conclusions: The anaemia prevalence of children aged 6-23 months in Huzhu County was high and children of younger age, Tibetan nationality and not introducing meat were more likely to be anaemic. The main cause of anaemia was nutritional anaemia, with the vast majority being iron deficiency. Interventions of feeding counseling and nutrients supplements are appropriate and should be further strengthened.

Strengths and limitations of this study:

- In addition to the prevalence of anemia for young children, we also investigated the influencing factors and biological causes of anaemia through statistical analysis and laboratory tests to provide guidance for future program implementation.
- The study took place only within one rural Chinese county and caution is needed when generalizing the findings from this study to other settings.
- The sample size was relatively small.

Trial registration number: ChiCTRPRC12002444 (Feb 15th, 2016; Version1).

Main text

INTRODUCTION

Childhood anaemia has long been a major public health problem worldwide. A systematic analysis of population-representative data suggested that anaemia prevalence in children younger than 5 years was 43% and there were 273 million children with anaemia globally in 2011¹. The national anaemia prevalence in children under 5 in China fluctuated between 12% and 23% between 1990 and 2005 and then decreased from 19.3% in 2005 to 12.6% in 2010². National Nutrition and Health Surveillance in 2013 showed that the prevalence of anaemia among children aged 0-5 years was 11.6% across the country, 10.6% in urban areas and 12.4% in rural areas

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4 respectively. However, huge regional differences exist, with rural Qinghai province
5 being the highest (27.5%)³. In some rural areas, anaemia prevalence in children aged
6 6-23 months was higher than 30%^{4,5}.
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11 In the public health perspective, anaemia is defined by the World Health Organization
12 as a hemoglobin concentration 2 SDs below the mean hemoglobin concentration for
13 a normal population of the same gender and the same age group⁶. Anaemia has
14 irreversible adverse effects on childhood growth and development, even their
15 working abilities in adulthood⁷. Many studies suggested an association between
16 anaemia and impaired psychomotor development; impaired cognitive functions such
17 as concentration, intellectual status, memory and scholastic skills; psychological and
18 behavioral disorders such as attention-deficit/hyperactivity disorder (ADHD)⁸ and
19 autism spectrum disorder (ASD)⁹; decreased physical activity¹⁰. Meanwhile, anaemia
20 has been confirmed to be associated with impaired renal function, increased
21 absorption of lead, and impaired immunity¹¹. The Global Burden of Disease (GBD)
22 2000 report estimated that anaemia resulted in 68.4 million years lived with disability
23 (YLD), accounting for 8.8% the total number of all cases of disability¹², and the GBD
24 2004 update had similar findings, which exerted a substantial economic burden¹³.
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41 Our previous study in Qinghai Province found that the prevalence of anaemia in rural
42 areas was much higher than the national average level. In 2012, the prevalence of
43 anaemia among children aged 6-23 months in Huzhu, Minhe and Guinan County was
44 71.1%, 56.1% and 86.3%, respectively¹⁴. We carried out a controlled interventional
45 study in Huzhu and Guinan county from 2012 to 2014. And all children aged 6-23
46 months in the intervention county, Huzhu County, received YingYangBao (a
47 domestically produced multiple micronutrient powders for infants and young children)
48 and their caregivers received infant feeding counseling from trained village doctors.
49 The study found that the anaemia prevalence significantly decreased from 71.1% to
50 47.8% in Huzhu County¹⁵. WHO defines the prevalence of anaemia in the population
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4 less than 20% as a mild public health problem, 20% to 40% as a moderate public health
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6 problem, and the prevalence of anaemia in the population \geq 40% as a serious public
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8 health problem¹⁶. Therefore, childhood anaemia in Huzhu County is still a serious
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10 public health problem in spite of the dramatic reduction of anaemia prevalence after
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12 the study, and more efforts are needed to further decrease anaemia prevalence and
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14 improve the nutrition status of children.

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17 There are many reasons for anaemia: acute and chronic infections that result in
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19 inflammation (including chronic blood loss caused by hookworm infection or
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21 schistosomiasis); nutritional anaemia caused by iron deficiency and other
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23 micronutrient deficiencies, especially of folic acid, vitamin B12 and vitamin A; and
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25 genetically inherited traits such as thalassemia¹⁷. Although it is generally accepted
26
27 that nutrients deficiency is the leading cause of child anaemia, proportions of anaemic
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29 causes in specific areas are usually unknown. Since interventions based on
30
31 improvement of child feeding and nutrients supplements are only effective for
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33 nutritional anaemia, understanding the status of anaemia-related nutrients (iron, folic
34
35 acid and vitamin B12) is crucial to estimate the potential effectiveness of nutritional
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37 interventions. The purpose of this study is to investigate the current situation of
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39 anaemia among children aged 6-23 months in Huzhu County, and to explore the
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41 influencing factors and the main causes of anaemia, so as to develop more appropriate
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43 strategies for combating this intractable public health issue.

44 45 46 47 **METHODS**

48 49 **Study design**

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51 This study was conducted as a cross-sectional survey of children aged 6-23 months
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53 and their caregivers in Huzhu County, Qinghai province. Proportional to Population
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55 Size (PPS) sampling method was used to select sampled villages in the county. We first
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57 obtained the name list of children aged 6-23 months in each sampled village and
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59 aimed to survey all the eligible children aged 6-23 months and their caregivers. The
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4 HemoCue Hb 301 analyzer (HemoCue, Lake Forest, CA, USA) was used to collect
5 children's fingertip peripheral blood by using microcuvettes (for blood samples) to
6 detect hemoglobin levels of children in the all sampled villages. After 4-7 days,
7 anaemic children screened out by HemoCue Hb 301 analyzer were called to Huzhu
8 Maternal and Child Health and Family Planning Service Center to draw venous blood
9 sample for further blood routine test and laboratory tests.
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17 **Study setting**

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19 Qinghai Province lies in northwest China, with an area of around 720,000 km². By the
20 end of 2017, the resident population of the province was 5,838,000, of which the
21 resident population of rural areas was 2,808,400, accounting for 46.9%, and the ethnic
22 minority population was 2,854,900, accounting for 47.7%. Qinghai Province has 34
23 counties and 439 townships. The Qinghai resident per capita disposable income in
24 2017 was ¥9,462 (US\$1363.13)¹⁸ for rural people, which was far lower than the
25 national level (¥13,432(US\$1935.06))¹⁹.
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35 Huzhu County is located in the northeastern part of Qinghai Province and 27.9% of its
36 population is part of an ethnic minority, which includes Tu, Tibetan, Hui and other 28
37 ethnic minorities²⁰. Huzhu County covers an area of 3,424 km². The county governs
38 19 townships with 294 villages, with a total population of 401,540, of which the rural
39 population accounted for 76.0%. By the end of 2017, the resident per capita
40 disposable income in the rural area was ¥9810 (US\$1414.91)²¹.
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49 **Participants**

50 Children aged 6-23 months and their caregivers were invited to participate in this
51 survey. We excluded children with a structural or genetic birth defect such as neural
52 tube defects, congenital heart disease or phenylketonuria or caregivers who refused
53 to participate.
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Survey instrument

We used the adapted WHO Maternal, Newborn and Child Health Household Survey (World Health Organization, 2009) to collect household information and infant feeding practices in each sampled village.

Sample size and Sampling

The sample size required were calculated based upon estimated anaemia prevalence in Huzhu County. We used the sample size calculation for proportion in single cross-sectional survey to estimate the sample for our study. Based on 35% of expected anaemia prevalence for children aged 6-23 months in Huzhu County, 5% of desired absolute precision, and 2 of design effect, we calculated the sample size of 699. Meanwhile, we also expected to draw 200 venous blood sample of anaemic children aged 12-23 months. According to the estimated anaemia prevalence of 35% in Huzhu County, we calculated that the total number of children aged 12-23 months needed was 571. We assumed that the number of children equally distributed in three age groups (6-11 months, 12-17 months, and 18-23 months), and calculated that 285 children aged 6-11 months were needed. Hence a sample size of 856 children aged 6-23 months was used in this study.

We knew the average number of children aged 6-23 months in each villages of Huzhu County from the local Maternal and Child Health Family Planning Service Centre. Proportional to Population Size (PPS) sampling method was used to select 38 sampled villages to meet our sample size requirements in the county. We obtained the name list of children aged 6-23 months in each sampled village and planned to survey all the eligible children and their caregivers.

Training of Interviewers

Staff from the Capital Institute of Pediatrics in Beijing were supervisors for this survey, and 25 students were recruited from Qinghai Institute of Health Sciences as

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4 interviewers. We provided them training for two days before fieldwork, which
5 included communication skills, explanation of questionnaires, demonstration, role
6 plays, field practice, and group discussions. In addition, 4 of them were trained on
7 measuring hemoglobin levels with a HemoCue Hb 301 analyzer. After the training, a
8 half-day field practice was held in a village clinic. Any problems arising during the field
9 practice were discussed and solved directly.
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17 **Data collection**

18 We carried out the survey from July 23th to 27th 2018. In every surveyed township,
19 staff notified the village doctors of the sample villages in advance, then the village
20 doctor called the caregivers to take their children to the village clinic for investigation.
21 Firstly, interviewers introduced the aim of the survey to the mothers or other
22 caregivers and obtained written informed consent from them. Then the interviewers
23 questioned them following the instructions.
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33 We used smartphones with the household survey questionnaire set up in specially
34 developed software to record data ²². Four teams of interviewers carried out the
35 survey, with 6 surveyors and 1 supervisor in each team. Data for each questionnaire
36 were uploaded into an Excel database via the Internet server. Once the interview was
37 completed, the special surveyor in each team measured hemoglobin with a HemoCue
38 Hb 301 analyzer (HemoCue, Lake Forest, CA) by drawing around 10 ul finger blood.
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46 Children aged 12-23 months screened as anaemia by HemoCue Hb 301 analyzer were
47 informed to go to the Huzhu County Maternal and Child Health Family Planning Service
48 Centre for further tests from July 31 to August 1 2018. About 4-5ml venous blood of
49 each children were collected by experienced nurses and placed into two tubes. The
50 first 1-2ml blood sample was collected into a labeled EDTA-K2 coated tube for blood
51 routine test using whole blood. And the second 3ml blood sample was collected
52 (without removing the needle) into a labeled vacuum separating tube for serum
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4 ferritin(SF), soluble transferrin receptor(sTfR), C-reactive protein(CRP), α -1 acid
5 glycoprotein(AGP), vitamin B12, Homocysteine(HCY), and folic acid concentration
6 using serum.
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12 Blood routine test and blood centrifugation were completed in the local laboratory.
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14 The blood sample in labeled vacuum separating tubes were placed for 30 minutes,
15 then were centrifuged at 1500 turn/minute for 15 minutes. The serum was separated
16 into 1, 2 and 3 cryotubes using disposable pipettes.
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22 After the field work, all the blood samples were immediately stored at -70°C and
23 transported as soon as possible to Nutritional Institute for Institute for Nutrition and
24 Health, China Center for Disease Control and Prevention for further laboratory testing.
25 Repeated freezing and thawing were strictly avoided during transportation and
26 storage.
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31 32 33 **Laboratory analysis**

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35 The blood routine test was conducted using automatic blood cell analyzer (Horiba,
36 ABX Micros 60-OT, France) in local laboratory. SF, vitamin B12 and serum folic acid
37 were analyzed by Roche Cobas e601 analyzer (Germany) using
38 electrochemiluminescence immunoassay (ECLIA). sTfR, CRP, AGP were analyzed by
39 Hitachi 7600-110 chemistry autoanalyzer (Japan) using immunoturbidimetry and HCY
40 was analyzed by Hitachi 7600-110 chemistry autoanalyzer (Japan) using enzymatic
41 cycling assay.
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51 **Data management and statistical analysis**

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53 Data of questionnaires were automatically transformed and pooled into a Microsoft
54 Excel sheet. After the data cleaning, we converted the database into a database file
55 (dbf) for the final analysis.
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4 We carried out statistical analysis with SAS 9.2 for Windows. The median (Q1, Q3) was
5 used to describe the age in years of mothers and grandparents of children. Mean and
6 standard deviation (SD) was used to describe the values of serum ferritin and body
7 iron. Percentages were presented in binary or categorical variables. We used the
8 Pearson χ^2 -test and Fisher exact test to compare binary and categorical variables.
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15 Moreover, we carried out a logistic regression analysis to identify factors associated
16 with children's anaemia in this survey. All relevant factors were first selected by
17 univariate logistic analysis, including child's age, child's sex, parents' age, parents'
18 nationality, parents' education, parents' job, whether parent worked outside the
19 county, whether children aged 6-23 months had been given iron-rich or iron-fortified
20 foods during last 24 hours, whether children aged 6-23 months had been given meat
21 during last 24 hours, minimum dietary diversity, whether child had coughed, fever or
22 diarrhea in the past two weeks, and whether children aged 6-23 months had been
23 given YYB 5 bags or more. Only those that were significant in the final multivariate
24 model are presented. We present Odds Ratios (OR) and 95% confidence intervals (CI)
25 and considered two-tailed P-values of <0.05 for a significant difference.
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39 We calculated the feeding practice indicators based on the WHO guideline "Indicators
40 for Assessing Infant and Young Child Feeding Practices"²³, which based on the 24 hour
41 recall method. And all individual hemoglobin values were adjusted using WHO
42 recommendations based on the altitude of the surveyed villages where children lived.
43 An adjusted hemoglobin lower than 110g/L¹⁶ was defined as anaemia and was used
44 to calculate the prevalence of anaemia. A hemoglobin concentration of 90–110 g/L
45 was defined as mild anaemia, <90 g/L as moderate anaemia or severe anaemia. Cut-
46 offs for elevated CRP and AGP were >5mg/L and >1mg/L, respectively²⁴. If one of
47 these two indicators were elevated, the children surveyed were classified as infected
48 children. Serum ferritin concentration <12ug/L and <30ug/L were used to define iron
49 deficiency in non-infected children and infected children, respectively. The children
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4 with concurrent anaemia and iron deficiency were diagnosed with iron deficiency
5 anaemia²⁵. A plasma folic acid concentration of <4ng/ml was used to define folic acid
6 deficiency according to WHO guidelines²⁶. Vitamin B12 deficiency was defined as
7 <197pg/ml. Cut-offs for elevated sTfR and HCY were 8.3mg/L²⁷ and 14umol/l,
8 respectively²⁸.
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15 Body iron stores (BI) were estimated by applying Cook's formula as follows: body
16 iron(mg/kg) =-[log(R/F ratio)-2.82290]/0.1207. The R/F ratio was soluble transferrin
17 receptor(sTfR)/ serum ferritin (SF)²⁹. Among them, sTfR needs to be transformed by
18 the following formula: Flowers sTfR=1.5 * Roche sTfR +0.35mg/L³⁰. The positive value
19 means iron surplus in stores and the negative value means iron deficit in tissues.
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26 27 **Ethical considerations**

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29 The study was approved by the Ethics Committee of the Capital Institute of Pediatrics
30 (reference no.2018017). All interviewees read the Information Sheet and provided
31 written consent on behalf of the children involved in our study.
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36 37 **Participant and public involvement**

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39 The participants and the public were not involved in the design, recruitment and
40 conduct of the study. There are no plans to disseminate the study findings to the study
41 participants.
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46 47 **RESULTS**

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49 The flowing chart of this study is shown in **Figure 1**. Among 912 children aged 6-23
50 months on the name list, a total of 754 children and their caregivers were surveyed,
51 with 444 being anaemic. There were 183 children aged 12-23 months agreed to draw
52 venous blood for further laboratory tests, and 52 of them were found acute and/or
53 chronic infections (CRP > 5 mg/L in blood routine test or AGP > 1 g/L in laboratory
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test). Besides, there was no statistical difference between 183 children aged 12-23 months who were taken venous blood and 101 children who refused to draw venous blood (Supplementary Table 1).

Characteristics of 754 surveyed children and their caregivers are shown in **Table 1**. Nearly all main caregivers of the children surveyed were mothers and grandparents, and about 70% of mothers were Han nationality, followed by Tu and Tibetan nationality. More than 60% of mother attended junior high school, and the proportion of mothers who were illiterate was only 4.0%. More than half of grandparents were still illiterate. The main source of household income was working outside the county, followed by agriculture-related work such as growing crops, vegetables and animal husbandry.

Table1 Characteristics of surveyed children and their caregivers (N=754)

Characteristic	Percentage or median
Children	
Age, %(n)	
6-11months	32.8(247)
12-17months	32.6(246)
18-23months	34.6(261)
Sex, %(n)	
Boy	52.1(393)
Girl	47.9(361)
Main caregivers, %(n)	
Mother	48.7(367)
Grandparent	46.4(350)
Father	4.8(36)
Other	0.1(1)
Mothers	
Age in years (median (Q1, Q3))	29(26,31)
Nationality, %(n)	
Han	69.9(523)
Tu	22.8(171)

Tibetan	6.3(47)
Hui	0.3(2)
Others	0.7(5)
Education, %(n)	
Illiterate	4.0(30)
Primary school	13.8(103)
Junior high school	61.6(461)
Senior high school or above	17.4(130)
Do not know	3.2(24)
Grandparents	
Age in year (median (Q1, Q3))	54(50,59)
Education, %(n)	
Illiterate	60.3(211)
Primary school	22.6(79)
Junior high school	15.1(53)
Senior high school	1.1(4)
Do not know	0.9(3)
Household income, %(n)	
Working outside the county	89.2(673)
Agriculture-related work	6.4(48)
Self-employed	2.9(22)
Others	1.2(9)
Do not know	0.3(2)

Table 2 shows the prevalence of anaemia and hemoglobin levels by age and severity in this survey. Most of anaemic children were mildly anaemic, accounting for 76.9% of the total. The prevalence of anaemia in the 18 to 23-month group was significantly lower than that in the 6 to 11-month group ($p=0.0026$) and the 12 to 17-month group ($p=0.0261$). The median hemoglobin levels in the 6 to 11-month group and 12 to 17-month group were lower than that in the 18 to 23-month group (116g/L vs. 121 g/L, $p<0.0001$ and $p=0.0004$ respectively).

Table 2 Prevalence of anaemia and hemoglobin level by age and severity

	Anaemia prevalence (N=751) *			Hemoglobin level
	Mild anaemia	Moderate or severe	Total	(median, (Q1, Q3))
	(%, n)	anaemia (%, n)	(%, n)	
6-11m	49.8(123)	15.0(37)	64.8(160)	116(106,125)
12-17 m	51.2(126)	10.2(25)	61.4(151)	116(107,125)
18-23 m	43.4(112)	8.1(21)	51.6(133)	121(111,130)
Total	48.1(361)	11.1(83)	59.1(444)	117(108,127)

*Three children refused to measure hemoglobin.

Table 3 shows the results of a univariate logistic analysis of anaemia prevalence in this survey. Older children were less likely to suffer from anaemia than younger children ($p=0.0028$). The prevalence of anaemia in children with Tibetan nationality was significantly higher than those with Han nationality ($p=0.0016$). The prevalence of anaemia in children who consumed iron-rich or iron-fortified foods was significantly lower than that in children who did not consume these foods ($p=0.0150$). The prevalence of anaemia in children who were given meat was also significantly lower than that in children without meat ($p=0.0077$). Furthermore, the anaemia prevalence of children achieving minimum dietary diversity was significantly lower than that of children who did not meet the standard ($p=0.0163$).

Table3 Univariate logistic analysis of the prevalence of anaemia

Factors	Sample	Case	Anaemia prevalence	<i>p</i> -value
Child's age				0.0028
Nationality				
Han	521	291	55.9	
Hui	2	0	0	0.9799
Tu	170	108	63.5	0.0836
Tibetan	47	38	80.9	0.0016
Other	5	3	60.0	0.8570
Children aged 6-23 months given iron-rich or				

iron-fortified food during the last 24 hours				
Yes	475	265	55.8	0.0150
No	276	179	64.9	
Children aged 6-23 months				
given meat during the last 24 hours				
Yes	210	108	51.4	0.0077
No	541	336	62.1	
Minimum dietary diversity during the last 24 hours				
Yes	364	199	54.7	0.0163
No	387	245	63.3	

Note:

¹ Children aged 6-23 months given iron-rich or iron-fortified foods during the last 24 hours: the proportion of children aged 6–23 months had been given iron-rich food or iron fortified food during the last 24 hours that was specially designed for infants and young children, or that was fortified in the home. Iron-rich or iron-fortified foods include flesh foods, commercially fortified foods specially designed for infants and young children which contain iron, or foods fortified in the home with a micronutrient powder containing iron or a liquid-based nutrient supplement containing iron, but not iron tablets.

² Children aged 6-23 months given meat during the last 24 hours: the proportion of children aged 6–23 months had been given meat during the last 24 hours that include beef, pork, lamb or other meat and liver, kidney, heart, or other organ meats, and fresh or dried fish, etc.

³ Minimum dietary diversity during the last 24 hours: the proportion of children aged 6-23 months who received foods from four or more food groups during last 24 hours. The food groups were: a) grains, root and tubers; b) legumes and nuts; c) dairy products (milk, yogurt, cheese); d) meat (meat, fish, poultry and liver/organ meat); e) eggs; f) vitamin-A rich fruits and green vegetables; g) other fruits and vegetables.

The results of multivariate logistic analysis of anaemia prevalence in surveyed children is shown in **Table 4**. Older children (OR=0.968, 95%CI 0.940, 0.998) and those consuming meat (OR=0.698, 95%CI 0.499,0.976) were associated with lower anaemia

levels, whereas children of Tibetan nationality (OR=3.123, 95%CI 1.473,6.623) were more likely to be anaemic.

Table 4 Multivariate logistic analysis of anaemia prevalence

Independent variable	Influential factors of anaemia			
	β	Wald	<i>P</i>	OR (95%CI)
Child' age	-0.0321	4.4930	0.0340	0.968(0.940,0.998)
Nationality				
Hui –Han	-13.6385	0.0006	0.9797	<0.001
Tu-Han	0.3038	2.7371	0.0980	1.335(0.945,1.942)
Tibetan-Han	1.1388	8.8166	0.0030	3.123(1.473,6.623)
Other-Han	0.0294	0.0010	0.9745	1.030(0.169,6.260)
Introduction of meat	-0.3595	4.4118	0.0357	0.698(0.499,0.976)

Results of the laboratory tests for 183 children are shown in **Table 5**. There were 113 children with iron deficiency, accounting for 61.7%. The mean serum ferritin concentration in anaemic children was significantly lower than that in non-anaemic children ($10.2 \pm 9.6\text{ng/mL}$ vs. $22.6 \pm 15.9\text{ng/mL}$, $p < 0.0001$). The body iron store in anaemic children was significantly lower than that in non-anaemic children ($-3.1 \pm 4.4\text{mg/kg}$ vs. $2.1 \pm 3.3\text{mg/kg}$, $p < 0.0001$). 32.6% of children with anaemia had elevated sTfR, significantly higher than that in children without anaemia (4.3%, $p < 0.0001$). At the same time, the proportion of folic acid deficiency in anaemic children was also significantly higher than that in non-anaemic children (20.2% vs. 5.3%, $p = 0.0024$). There was no vitamin B12 deficiency either in anaemic or non-anaemic children.

Table 5 Results of laboratory tests

	Anaemic children (N=89)	Non-anaemic children (N=94)	Total (N=183)	<i>p</i>
Serum ferritin(ug/L)				

Mean \pm SD	10.2 \pm 9.6	22.6 \pm 15.9	16.6 \pm 14.6	<0.0001
<12ug/L for non-infected children or <30ug/L for infected children (% , n)	80.9% (72)	43.6% (41)	61.7% (113)	<0.0001
sTfR (>8.3mg/L) (% , n)	32.6% (29)	4.3% (4)	18.0% (33)	<0.0001
Body iron store (mean \pm SD)	-3.1 \pm 4.4	2.1 \pm 3.3	-0.45 \pm 4.56	<0.0001
Folic acid (<4ng/mL) (% , n)	20.2% (18)	5.3% (5)	12.6% (23)	0.0024
Vitamin B12 (<197pg/mL) (% , n)	0.0% (0)	0.0% (0)	0.0% (0)	-
Homocysteine (>14umol/L) (% , n)	4.5% (4)	1.1% (1)	2.7% (5)	0.1425

Figure 2 shows biological causes of anaemia. 80.9% of children with anaemia were due to iron deficiency, and 20.2% of them had both iron and folic acid deficiencies. The biological causes of 19.1% anaemic children were unknown.

DISCUSSION

Main findings

The anaemia prevalence of children aged 6-23 months in Huzhu County was 59.1% and most of them were mildly anaemic. The prevalence of anaemia in the 18 to 23month group was significantly lower than that in the 6 to 11month group ($p=0.0026$) and the 12 to 17month group ($p=0.0261$). Meanwhile, children of younger age, Tibetan nationality and not introducing meat were more likely to be anaemic. 80.9% of children with anaemia were due to iron deficiency, and 20.2% suffered from both iron deficiency and folic acid deficiency. The prevalence of iron deficiency among all children was 61.7% and 43.6% of non-anaemic children also had iron deficiency. Body iron stores in all children tested averaged -0.45 ± 4.56 mg/kg. The proportion of microcytic hypochromic anaemia (MCH, MCV and MCHC were lower than normal value) was 13.1%. And the specificity of the combination of MCV + MCH + MCHC in the diagnosis of iron deficiency anaemia was 100%, but its sensitivity was only 17.8%.

Influencing factors of anaemia

Children aged 6-11 months are in the transition period from exclusive breastfeeding to complementary feeding, during which the storage iron from birth is depleted and complementary foods become the main source of iron, and they were more likely to suffer from anaemia. In our study, anaemia prevalence of children at this age is the highest (64.8%).

Our analysis showed that eating meat was a protective factor of child anaemia, however, few caregivers gave meat to their children of this age due to the wrong beliefs that they could not digest meat. In addition, some caregivers did not know how to prepare meat for young infants, especially at the very beginning of complementary feeding³¹, thus infant feeding counseling should include these specific issues to provide caregivers accurate knowledge and help them solve problems, such as demonstration of the preparation of meat instead of just giving information.

Compared with other nationalities, children of Tibetan nationality, who accounted for about 10% in Huzhu County, were more likely to be anaemic¹⁴, probably because their special customs and dietary habits with the main complementary food for children being zanba (a local ethnic food consisting mainly of carbohydrates) and porridge, which contain very few irons^{32,33}. At the same time, poor family economic conditions would also make it unaffordable to feed animal food to their children³⁴.

Laboratory indicators for assessment of iron status

Our study found that 80.9 % of anaemic children had iron deficiency assessed by serum ferritin, but only 32.6% of these children had increased sTfR, which indicated the inconsistency of these two laboratory tests.

The World Health Organization issued guidelines on serum ferritin cut-off for the

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4 assessment of iron status²⁵, which are widely used in nutrition surveys and researches
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6 in different countries. However, using sTfR to assess iron status is still controversial,
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8 as there is no internationally agreed cut-off for sTfR at present. Therefore, we mainly
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10 used ferritin to assess iron deficiency in our study.

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13 Literatures showed that the cut-off for sTfR used in the age group of children under
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15 three years old are varied, such as 3.3mg/L, 4.6mg/L and 8.3mg/L^{35-36,27}. A study
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17 conducted in Kenya showed that the threshold of 8.3mg/L was better to assess the
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19 prevalence of iron deficiency in children aged 6-35 months (sensitivity 92.0%,
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21 specificity 96.0%). Therefore, we used cut-off 8.3mg/L in our study.

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25 Ferritin and sTfR reflect different stage of iron deficiency. We found that the results of
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27 serum ferritin and sTfR were not consistent in children aged 12-23 months. Further
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29 studies should be conducted to clarify the exact relationship between serum ferritin
30
31 and sTfR in children and to explore a more effective combination of indicators to
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33 assess population iron status.

34 35 36 37 **The biological causes of anaemia**

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39 The biological causes of anaemia can generally be summarized into three categories:
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41 nutritional anaemia, infectious diseases and genetic hemoglobin disorders. Nutritional
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43 anaemia results from insufficient bioavailability of hemopoietic nutrients (iron,
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45 vitamin B12, vitamin A and folic acid) needed to meet the demands of hemoglobin
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47 and erythrocyte synthesis and decreased absorption enhancers such as vitamin C.
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49 Infectious diseases include soil-transmitted helminths, malaria and schistosomiasis.
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51 Genetic hemoglobin disorders include thalassemia and hemoglobin variants etc¹⁷.
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53 Many previous studies have found that iron deficiency may be the most common
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55 cause of anaemia ^{1, 6, 17, 37}. Our study confirmed that 80.9% of anaemic children aged
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57 12-23 months in Huzhu County were due to iron deficiency. The prevalence of iron
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59 deficiency among all children was 61.7% and body iron stores in all children tested
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4 averaged only -0.45 ± 4.56 mg/kg.
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8 Iron deficiency (ID) is a state in which iron is insufficient to maintain normal
9 physiological functions of tissues such as blood, brain, and muscle. If iron deficiency
10 lasts too long or is serious enough, it can result in iron deficiency anaemia (IDA)⁶. In
11 addition to the important role of oxygen carrier in the heme group of hemoglobin,
12 iron also exists in many key proteins in cells, such as cytochromes, myoglobin, neural
13 transmitters, various enzymes and coenzymes³⁸. Therefore, iron deficiency not only
14 causes anaemia, but also has many other adverse effects, especially on children in
15 growth and development.
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25 Iron deficiency is often found in association with a deficiency of folic acid. Combined
26 folic and iron deficiency may occur in preterm infants who are fed unfortified formula
27 based on evaporated milk. Other study indicated that infants fed on goat's milk were
28 also at risk³⁹. Our study found that 20.2% of children suffered from both iron
29 deficiency and folic acid deficiency. Hence, attentions also need to be paid to the
30 deficiency of folic acid. There was no vitamin B12 deficiency either in anaemic or non-
31 anaemic children tested. However, biological causes of the remaining 19.1% of
32 anaemic children were still unknowns and may need further explorations.
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43 **Recommendations on reducing nutritional anaemia**

44 Deficiencies of iron and folic acid were the main biological causes of children's
45 anaemia in Huzhu County, therefore, feeding counseling and nutrients supplements,
46 as biological interventions, are appropriate and should be further strengthened.
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53 (1) Improving traditional delivery channels for infant and young child feeding (IYCF)
54 nutrients supplements (YYB program).
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56 IYCF is one of the key strategies to lower the risk of iron-deficiency anaemia in early
57 infancy^{17, 40}. Previous studies found that inappropriate IYCF practices were common in
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4 many rural areas in China^{41,42}, for instance, complementary foods generally contained
5 mainly carbohydrates and lacked protein and fat⁴³, or were introduced to children too
6 early or too late, or were given in too small amounts or not frequently enough^{44,45}.
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11 Nutrients supplements have been commonly accepted as effective interventions in
12 reducing child anaemia worldwide^{15, 46-51}. In China, a domestically produced multi-
13 nutrient powders (MNPs) for infants and young children called Ying Yang Bao (YYB)
14 was developed, and a study conducted in Gansu Province from 2001 to 2004 to test
15 the effectiveness of this complementary food supplement, showed that the use of YYB
16 could significantly reduce the anaemia prevalence⁴⁸.
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25 The information-motivation-behavior skills (IMB) model indicates that information
26 can be transformed into action that can motivate individuals and eventually influence
27 their attitudes and behaviors^{52,53}. In China, information and knowledge about infant
28 feeding was mainly disseminated through the traditional rural three-tier healthcare
29 system (county-township-village). Village doctors were responsible to provide face-to-
30 face infant and young child feeding counseling to caregivers^{14, 54}. We conducted a
31 controlled interventional study in Huzhu and Guinan County from 2012 to 2014,
32 training village doctors to provide IYCF counseling and disseminating YYB to caregivers,
33 and the results showed that the anaemia prevalence decreased more in the
34 intervention county (receiving IYCF counseling and YYB) than in the control county(not
35 receiving IYCF counseling and YYB, 71.1% to 47.8% vs. 86.3% to 75.3%, respectively)¹⁵.
36 We also found an improvement of caregivers' feeding practice¹⁵. Therefore, we should
37 continue making use of the traditional health information dissemination system and
38 measures need to be taken to further improve the quality of services, for example,
39 conducting regular refresh training and supervision, providing monetary incentives to
40 village doctors, and more importantly, tailoring IYCF information to the local feeding
41 problems and special dietary habits instead of barely giving general knowledge.
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4 (2) Exploring new channels for delivering IYCF information and disseminating YYB.
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6 Although traditional delivery channels (mainly by village doctors) proved to be
7 effective in our previous study, the key IYCF indicators were still low and the YYB
8 compliance needed to be further improved⁵⁵ to increase the effectiveness of the
9 interventional program. In addition, the sustainability of the YYB programs could also
10 be an issue since the anaemia prevalence in Huzhu County went up from 47.8% in
11 2014 to 59.1% in 2018. Therefore, we need to explore new channels for delivery these
12 interventions.
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21 Nowadays, mobile phones and the Internet have spread to millions of households in
22 China. Data showed that, by the end of 2017, there were 1417.49 million mobile
23 phone users and 772 million Internet users, of which 753 million were smartphone
24 Internet users. The Internet penetration rate reached 55.8%, of which 35.4% was in
25 rural areas¹⁹. Social media and smartphones have become new channels for
26 information acquisition, and these have been widely used in many health education
27 researches⁵⁶⁻⁵⁸. A systematic review proved the feasibility of delivering eHealth
28 interventions to improve health literacy skills among people with different health
29 conditions, risk factors and socio-economic backgrounds⁵⁹. However, using eHealth or
30 mHealth methods to deliver complementary feeding information in China is rarely
31 reported. Therefore, further studies are needed to assess the feasibility and
32 effectiveness of such delivery channels in improving IYCF knowledge and practice as
33 well as YYB compliance in rural China.
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50 CONCLUSIONS

51 The anaemia prevalence of children aged 6-23 months in Huzhu County was 59.1%
52 and children of younger age, Tibetan nationality and not introducing meat were more
53 likely to be anaemic. 80.9% of children with anaemia were due to iron deficiency, and
54 20.2% of them suffered from both iron and folic acid deficiencies. Therefore, previous
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4 interventions of feeding counseling and nutrients supplements are appropriate and
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6 should be further strengthened.
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11
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13 and Family Planning Service Center for coordination, logistic arrangements and blood
14 routine tests, and we want to thank all students from Qinghai Institute of Health
15 Sciences for their hard work as interviewers. We are indebted to all the mothers and
16 caregivers who participated in our survey.
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25 **Authorship statement**

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27 The study was initiated, conceptualized, and supervised by JSH, SYC and YFZ. LJW,
28 YWH, QW and WW collected and analyzed data. LJW conducted laboratory tests. YFZ,
29 YWH, and QW participated in the explanation and discussion of the results. The
30 manuscript was drafted by YWH, reviewed and revised by YFZ, QW, SYC and JSH. All
31 authors read and approved the final manuscript.
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41
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43 was involved in study design, data interpretation, preparation of the manuscript, and
44 decision to publish.
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50 **Competing interests**

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52 None declared.
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56 **Data showing statement**

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58 Extra data can be accessed via the Dryad data repository at <http://datadryad.org/> with
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the doi:10.5061/dryad.57v2100

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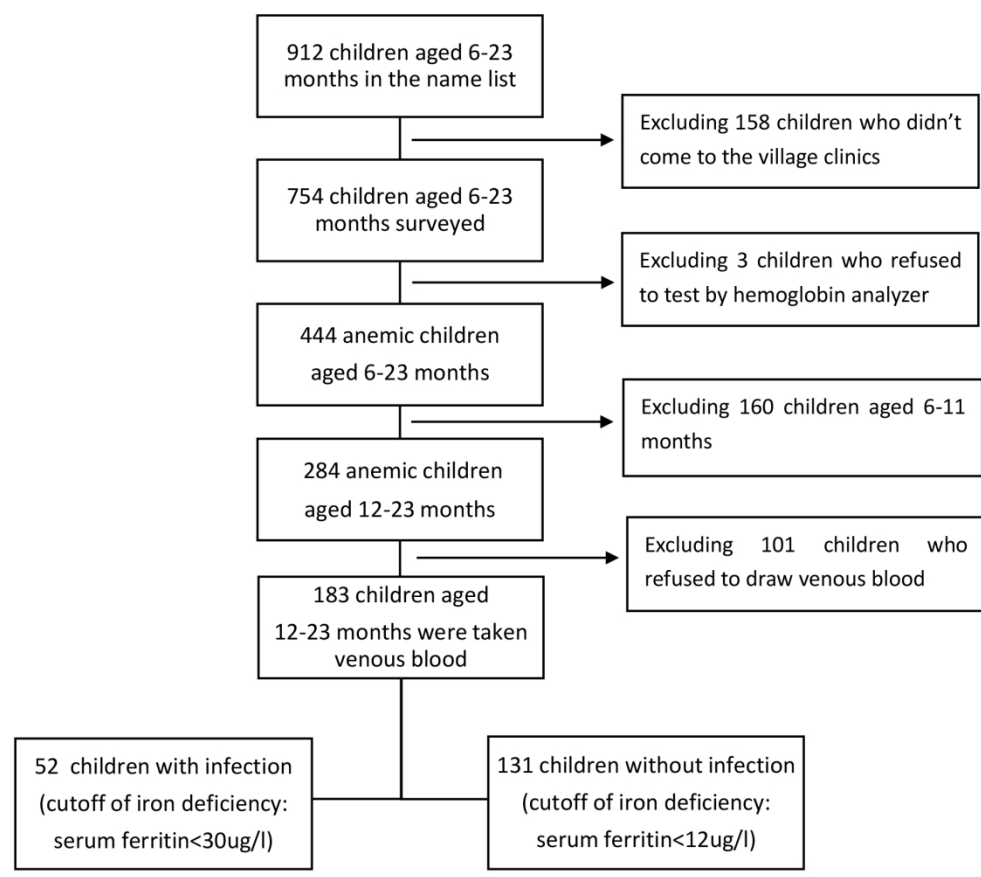
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Figure legends

Figure 1 Flowchart of study procedures

Figure 2 Causes of anaemia

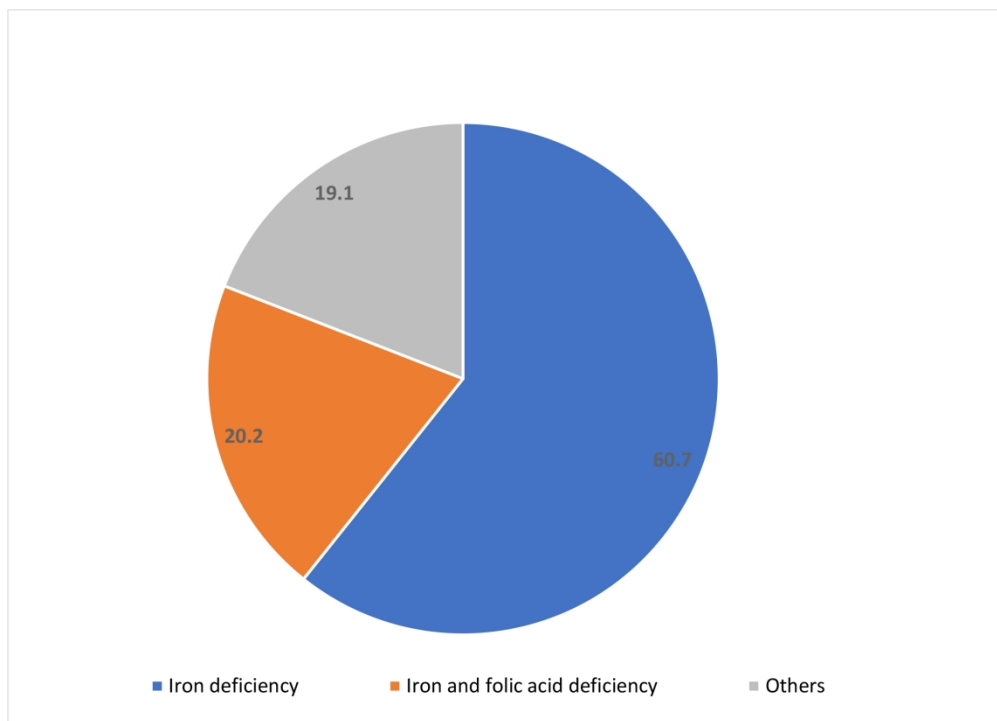
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Flowchart of study procedures

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Biological causes of anaemia

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Supplementary Table 1 Background characteristics of children with and without venous blood drawn

Characteristic	Children with venous blood drawn(N=183)	Children without venous blood drawn (N=101)	<i>p</i> -value
Children			
Age, %(n)			0.3486
12-17months	56.3(103)	50.5(51)	
18-23months	43.7(80)	49.5(50)	
Sex, %(n)			0.9184
Boy	54.1(99)	45.9(84)	
Girl	53.5(54)	46.5(47)	
Main caregivers, %(n)			
Mother	40.4(74)	49.5(50)	0.3213
Grandparent	53.6(98)	47.5(48)	
Father	5.5(10)	3.0(3)	
Other	0.6(1)	-	
Mothers			
Age in years (median (Q1, Q3))	29(26,32)	31(28,34)	0.0511
Nationality, %(n)			0.6858
Han	68.9(51)	62.0(31)	
Tu	24.3(18)	28.0(14)	
Tibetan	6.8(5)	10.0(5)	
Education, %(n)			0.2235
Illiterate	8.1(6)	2.0(1)	
Primary school	17.6(13)	26.0(13)	
Junior high school	58.1(43)	64.0(32)	
Senior high school or above	14.9(11)	8.0(4)	
Do not know	1.4(1)	-	
Grandparents			
Age in year (median (Q1, Q3))	54(50,60)	54(51,57.5)	0.6643
Education, %(n)			0.4168
Illiterate	62.2(61)	50.0(24)	
Primary school	20.4(20)	27.1(13)	
Junior high school	15.3(15)	18.8(9)	

Senior high school	2.0(2)	2.1(1)	
Do not know	-	2.1(1)	
Household income, %(n)			0.1215
Working outside the county	91.3(167)	91.1(92)	
Agriculture-related work	7.1(13)	3.9(4)	
Self-employed	1.6(3)	3.0(3)	
Others	-	2.0(2)	

For peer review only

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	3-4
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	6-7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants	7
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	10-12
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	9-10
Bias	9	Describe any efforts to address potential sources of bias	8-10
Study size	10	Explain how the study size was arrived at	8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	N/A
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	10-11
		(b) Describe any methods used to examine subgroups and interactions	N/A
		(c) Explain how missing data were addressed	10-11
		(d) If applicable, describe analytical methods taking account of sampling strategy	10-11
		(e) Describe any sensitivity analyses	N/A
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	12;27
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	29
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	12-13
		(b) Indicate number of participants with missing data for each variable of interest	29
Outcome data	15*	Report numbers of outcome events or summary measures	12-18
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	N/A
		(b) Report category boundaries when continuous variables were categorized	18
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarise key results with reference to study objectives	18
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	4
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	18-21
Generalisability	21	Discuss the generalisability (external validity) of the study results	23
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	24

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.