PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Prevalence and causes of anaemia in children aged 6-23 months in rural Qinghai, China: findings from a cross-sectional study
AUTHORS	Huang, Yiwen; Wang, Lijuan; Huo, Junsheng; Wu, Qiong; Wang, Wei; Chang, Suying; Zhang, Yanfeng

VERSION 1 - REVIEW

REVIEWER	John Maluccio Middlebury College, USA
REVIEW RETURNED	03-May-2019

GENERAL COMMENTS	Main Comments 1. The sample size calculation discussion not sufficiently clear – eg what exactly is meant by 856 being “sufficient in this study.” Additionally, because this sample size is not achieved, does it mean the insignificant relationships estimated may be insignificant because of low statistical power? 2. While 754 children were interviewed, is there an estimate of the universe of children in the pps selected villages (presumably less than 100% agreed to participate, including those who did not show up to the clinic)? Related, it seems likely that the third of the 284 children 12-23 who were determined anemic but refused blood draw is not a random subsample. These two layers of “selective” sampling call into question how representative the final sample is. Some assessment of background characteristics of those lost/attrited at the blood draw point would be valuable. 3. Variable definitions need to be further specified/clarified – for example children given iron-rich food – what is the reference period or question for this statement. The same is true for some variables that appear to be summarized in the discussion such as caregiver knowledge. 4. Why was education not considered in the logistic analyses. It may, for example, be correlated with things like ethnicity that are included and provide richer insight into correlates of anemia. Was rural location considered? 5. While the recommendations in the paper all seem plausible, and consistent with what is found in this paper, it may be important to consider/recognize that this study in and of itself does not directly demonstrate these recommendations would be effective. Taking a provocative stance for the purpose of this review (to which I expect the authors likely have a good response), the feature that there is an intervention occurring that has elements of the recommendations, and yet there is still anemia (and even

	apparently increasing since last study), could be taken as evidence that these are not the appropriate interventions (and certainly not sufficient) and we need to consider something else. Minor comments 6. In the final pages I think I understood that the intervention in Huzhu was on-going (but page 6 line 13 suggests it had ended with “after the intervention”; this should be clarified if on-going and underscored earlier since it affects how we might think about this population in the broader context, including external validity – presumably caution needs to be taken regarding how representative it is not only because of being only a single county but also because of this intervention. Apologies if I have misunderstood the setting. 7. Page 17 final line: The “therefore” here seems to suggest a causal link that is not substantiated by this particular analysis (though may be true). Is “consistent with”, perhaps. 8. Would it be a useful distinction to frame the causes subsection – page 18 line 39 – as the “biological causes” since that appears to be what the blood analysis yields. Further back on the pathway there are other “causes” including the behaviors described prior to this subsection. Of course the authors are clear in that they do not treat estimates of those other relationships as “causal” in this cross-sectional setting.

REVIEWER	Rune J. Ulvik Department of Clinical Science, Faculty of Medicine, University of Bergen, Norway
REVIEW RETURNED	31-May-2019

GENERAL COMMENTS	A well done and solid study ! Some minor points. Table 3: - Tibetan, p-value (0,0016) differ from that given in the text (< 0,0001) - Iron-fortified food … what is it ? addition of elemental iron to certain foods ? supplementation of iron tablets, micronutrients with iron, YingYangBao ? Table 5: They used sTfR which is a (very reliable) indicator of insuffient iron uptake during erythropoiesis. How do they interpret the disparity that 80,9 % of anaemic children had iron deficiency, but only 32,6 % of these children had increased sTfR ?

VERSION 1 – AUTHOR RESPONSE

Reviewer(s)’s Comments to Author:

Reviewer: 1

Reviewer Name: John Maluccio

Institution and Country: Middlebury College, USA

Please state any competing interests or state ‘None declared’: None

Please leave your comments for the authors below

Main Comments

1. The sample size calculation discussion not sufficiently clear – eg what exactly is meant by 856 being "sufficient in this study." Additionally, because this sample size is not achieved, does it mean the insignificant relationships estimated may be insignificant because of low statistical power?

Response:

We would like to thank the reviewer for the thoughtful comments. Actually, when we designed the study, we used the sample size calculation for proportion in single cross-sectional survey to estimate the sample for our study [1]. 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. Therefore, our actual sample of 754 children was sufficient for the cross-sectional study. We added this to the 'Sample size and Sampling' part of methods in the manuscript to make it clearer.

Meanwhile, we also expected to draw 200 venous blood samples of anaemic children aged 12-23 months. Based on 35% anaemia prevalence, we calculated the sample size of 571 for children aged 12-23 months needed to be surveyed. 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 285 children aged 6-11 months needed. Therefore, we used 856 children aged 6-23 months as our final sample size.

2. While 754 children were interviewed, is there an estimate of the universe of children in the pps selected villages (presumably less than 100% agreed to participate, including those who did not show up to the clinic)? Related, it seems likely that the third of the 284 children 12-23 who were determined anemic but refused blood draw is not a random subsample. These two layers of "selective" sampling call into question how representative the final sample is. Some assessment of background characteristics of those lost/attrited at the blood draw point would be valuable.

Response:

The first comment:

Before conducting the survey, we got the name list of all the PPS selected villages from the Huzhu County Maternal and Child Health Family Planning Service Centre. There were a total of 912 children aged 6-23 months in the name list, and we assumed 912 was the universe of children in the PPS selected villages. We asked village doctors in those villages to inform all the children in the name list to come to village clinics to participate in our survey, however, only 754 eligible children aged 6-23 months and their caregivers came and be surveyed. We have added this point to the manuscript.

The second comment:

This is a valuable suggestion! A total of 284 children with anemia were identified by HemoCue Hb 301 analyzer in this study, however, the venous blood samples were only taken from 183 children, and

101 children refused to take venous blood samples for various reasons. According to the reviewer's suggestion, we compared the background characteristics of these two groups. We found that there was no statistical difference between the two groups in background characteristics (Supplementary Table 1). We have described this point in the manuscript and referred to the table as a supplementary file.

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)			0.3213
Mother	40.4(74)	49.5(50)	
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)	

3. Variable definitions need to be further specified/clarified – for example children given iron-rich food – what is the reference period or question for this statement. The same is true for some variables that appear to be summarized in the discussion such as caregiver knowledge.

Response:

We would like to thank the reviewer for the thoughtful comment. We calculated the feeding practice indicators based on the WHO guideline “Indicators for Assessing Infant and Young Child Feeding Practices” [2], which based on the 24 hour recall. We added this point to the ‘data management and statistical analysis’ section of methods in the manuscript. We also added “during the last 24 hours” in the following indicators in Table 3:

- Children aged 6-23 months given iron-rich or iron-fortified food during the last 24 hours
- Children aged 6-23 months given meat during the last 24 hours
- Minimum dietary diversity during the last 24 hours

We also added footnotes on the definition of these three indicators in the manuscript.

[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.

[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 groupsduring 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.

4. Why was education not considered in the logistic analyses. It may, for example, be correlated with things like ethnicity that are included and provide richer insight into correlates of anemia. Was rural location considered?

Response:

Thank you for the comment. Actually, we considered parents' education in univariate logistic analysis, but it was not significant. The following relevant factors were first selected by univariate logistic analysis: child's age, child's sex, parents' age, parents' nationality, parents' education, parents' job, whether parent worked outside the county, whether children aged 6-23 months had been given iron-rich or iron-fortified foods during last 24 hours, whether children aged 6-23 months had been given meat during last 24 hours, minimum dietary diversity, whether child had coughed, fever or diarrhea in the past two weeks, and whether children aged 6-23 months had been given YYB 5 bags or more. Only those that were significant were presented in Table 3. We added all these factors in the 'data management and statistical analysis' part of methods in the manuscript.

All the children we surveyed came from rural areas, so we did not need to consider rural location issues. We highlighted this point in the manuscript.

5. While the recommendations in the paper all seem plausible, and consistent with what is found in this paper, it may be important to consider/recognize that this study in and of itself does not directly demonstrate these recommendations would be effective. Taking a provocative stance for the purpose of this review (to which I expect the authors likely have a good response), the feature that there is an intervention occurring that has elements of the recommendations, and yet there is still anemia (and even apparently increasing since last study), could be taken as evidence that these are not the appropriate interventions (and certainly not sufficient) and we need to consider something else.

Response:

Response:

Thank you for the comment. Our study demonstrated that the iron deficiency was the main biological causes of anaemia in this county, therefore, YYB and infant feeding counseling, as biological interventions, should be appropriate for most of children. In our recommendation part, we aimed to

focus mainly on the delivery channels for these interventions. We are actually in agreement with the reviewer since we discussed that new delivery channels were needed to be explored to further improve the effectiveness, such as mHealth approach. We rearranged the ‘recommendations on reducing nutritional anaemia’ part and highlighted these points.

Minor comments

6. In the final pages I think I understood that the intervention in Huzhu was on-going (but page 6 line 13 suggests it had ended with “after the intervention”; this should be clarified if on-going and underscored earlier since it affects how we might think about this population in the broader context, including external validity – presumably caution needs to be taken regarding how representative it is not only because of being only a single county but also because of this intervention. Apologies if I have misunderstood the setting.

Response:

We would like to apologize for the confusion. We carried out a controlled interventional study in Huzhu and Guinan county from 2012 to 2014 to evaluate the effectiveness of YYB on improving children’s health status. Since the end of this evaluation study in 2014, the intervention of YYB and infant feeding counseling has been on-going in this county. We have changed “after the intervention” to “after the study”.

7. Page 17 final line: The “therefore” here seems to suggest a causal link that is not substantiated by this particular analysis (though may be true). Is “consistent with”, perhaps.

Response:

We agree with the reviewer. We revised the last sentence in page 17: “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.

8. Would it be a useful distinction to frame the causes subsection – page 18 line 39 – as the “biological causes” since that appears to be what the blood analysis yields. Further back on the pathway there are other “causes” including the behaviors described prior to this subsection. Of course the authors are clear in that they do not treat estimates of those other relationships as “causal” in this cross-sectional setting.

Response:

We agree with the reviewer. We replaced “the causes of anaemia” with “the biological causes of anaemia” in page 18 line 39. In addition, all the relevant expressions in this text are replaced by "biological causes".

Reviewer: 2

Reviewer Name: Rune J. Ulvik

Institution and Country: Department of Clinical Science, Faculty of Medicine, University of Bergen, Norway

Please state any competing interests or state ‘None declared’: None declared

Please leave your comments for the authors below

A well done and solid study !

Some minor points.

1. Table 3:

- Tibetan, p-value (0,0016) differ from that given in the text

(< 0,0001)

Response:

We apologized for this mistake. We have carefully checked the original data and statistical analysis results, 0.0016 should be the correct p-value result. We have revised it in the text.

2. Iron-fortified food … what is it? addition of elemental iron to certain foods? supplementation of iron tablets, micronutrients with iron, YingYangBao ?

Response:

Thank you for the comments. Iron-rich or iron-fortified foods in our study 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. Therefore, they include addition of elemental iron to certain foods, micronutrients with iron and YingYangBao, but not Iron tablets. We added a footnote in the manuscript to explain it.

3. Table 5: They used sTfR which is a (very reliable) indicator of insuffient iron uptake during erythropoiesis. How do they interpret the disparity that 80,9 % of anaemic children had iron deficiency, but only 32,6 % of these children had increased sTfR ?

Response:

Thank you for your valuable comment. Firstly, using ferritin to identify iron deficiency has been well reviewed by The World Health Organization. WHO has also issued guidelines on serum ferritin cutoffs for the assessment of iron status, which are widely used in nutrition surveys and researches in different countries. However, using sTfR to assess iron status is still controversial, as there is no internationally agreed cut off for sTfR at present. Therefore, we mainly used ferritin to assess iron deficiency in our study, while used sTfR only as a reference.

We reviewed literatures and found the cut off for sTfR used in the age group of children under three years old are varied, such as 3.3mg/L, 4.6mg/L and 8.3mg/L. If we use the threshold of 4.6mg/L in our results, the proportion of elevated sTfR in anaemic children is 85.4%, which is in good agreement with the results of low ferritin (80.9%). However, a study conducted in Kenya showed that the threshold of 8.3mg/L was better to assess the prevalence of iron deficiency in children aged 6-35 months (the sensitivity was 92.0%, specificity was 96.0%). That's why we chose this threshold.

In addition, ferritin and sTfR reflect different stage of iron deficiency, and there is no literature showed the proportion of decreased ferritin must be consistent with the proportion of elevated sTfR in anaemic children. We obtained our results through rigorous experimental design and blood drawing criteria, and found that the results of serum ferritin and sTfR were not consistent in children aged 12-23 months. The inconsistent issue we found in this study should also be a good point for future studies to clarify the exact relationship between serum ferritin and sTfR in children and to explore a more effective combination of indicators to assess population iron status.

VERSION 2 – REVIEW

REVIEWER	John Maluccio Professor of Economics, Middlebury College
REVIEW RETURNED	12-Jul-2019

GENERAL COMMENTS	Good careful revisions