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THE PREVALENCE OF VITAMIN A DEFICIENCY AND XEROPHTHALMIA
IN A DROUGHT STRICKEN AREA OF TANZANIA.
(SHINYANGA - RURAL)

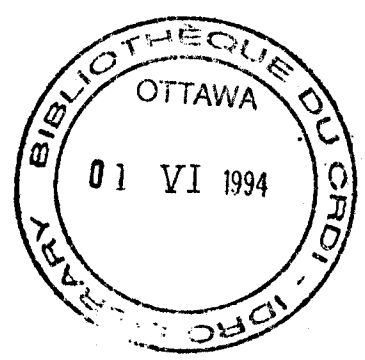
REPORT

A PRELIMINARY REPORT BY:

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THE PREVALENCE OF VITAMIN A DEFICIENCY AND XEROPHTHALMIA
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(SHINYANGA - RURAL)

I. ABSTRACT

In October/November 1988, Tanzania Food and Nutrition Centre conducted a survey in two divisions (Kishapu and Negezi) of Shinyanga Rural District to find out the prevalence and causes of vitamin A deficiency and xerophthalmia. The information collected included clinical, dietary, morbidity and biochemical parameters. The survey was funded by the International Development Research Centre (IDRC).

A total number of 3,518 children ages 6 months to 6 years were screened for clinical eye signs and biochemical parameters were conducted on a sub-sample of 400 children. The biochemical parameters included retinol, retinol binding and pre-albumin.

Clinical and biochemical results indicated that vitamin A deficiency and xerophthalmia are problems of public health significance in this part of the country. Based on corneal scars and serum retinol levels the problem is approximately 4 times the WHO criteria for corneal scars standing at $XS=0.22\%$ and low serum retinol level of less than $20\mu\text{g}/\text{dl}$ at 33% (1).

Main reasons for such a prevalence include inadequate intake of vitamin A rich foods, (dark green leafy vegetables, fruits, colostrum and animal products), high prevalence of diseases such as malaria, diarrhoea, fever, cough, and measles, etc.

II BACKGROUND INFORMATION:

In Tanzania the prevalence of blindness is estimated to be 1% and another 1% severely visually handicapped, representing 340,000 people. The major causes of blindness are cataract (50%), corneal scar (25%) and glaucoma (15%) with corneal scar due to vitamin A deficiency being responsible for 70% of childhood blindness (2).

Vitamin A deficiency ranks fourth to PEM, Anaemia and IDD in that ascending order. According to the Minister of Health in his opening speech of the National Workshop on the control of Vitamin A deficiency, "vitamin A deficiency has blinded 60,000 out of 200,000 blind people because of poor nutrition (3)".

It is estimated that every year 2,000 - 4,000 children lose their sight because the small amounts of vitamin A taken in their diet is insufficient to meet the body requirements which are increased by the many diseases especially measles. It is also estimated that in Tanzania at any one time there are about 10,000 children who are blind due to xerophthalmia, (3).

By using a simple xerophthalmia surveillance system which lasted 2 years since 1982 early xerophthalmia (night blindness and Bitot spots) was found in all 15 areas of the country studied. Nearly 300 cases of unilateral and bilateral corneal ulceration were documented; 46% and 79% respectively following recent measles infection. The single most important cause of bilateral corneal ulceration was found to be vitamin A deficiency. The hard hit regions are those in the dry zone of Tanzania, (3)

Prevalence surveys done in three regions (Tabora, Iringa and Kagera) suggested that vitamin A deficiency and xerophthalmia are problems of public health significance. Dietary survey accompanying such surveys have shown a low intake of vitamin A especially in infants and young children suggesting a causal relationship. (4,5,6)

first 5 year

The National Programme for the Control of Vitamin A Deficiency and Xerophthalmia features seven projects namely Vitamin A Capsules Distribution; Nutrition Education and Public Health Information; Laboratory Development and Analysis. The Use of Vitamin A rich foods in Alleviating vitamin A Deficiency; Monitoring and Evaluation; Logistical Support and Further Assessment of the Extent and Severity of the Problem.

It was under the last project that this study was proposed. The results of the survey have provided an indication of the extent and severity of the problem in drought stricken rural Shinyanga. It has also shed some light on the causal relationships between vitamin A deficiency and diseases, diet, social-economic aspects and other factors. All these will contribute to the effective implementation of the Second 5-year programme for the control of vitamin A deficiency and xerophthalmia in Tanzania which will start in 1990/91.

III SHINYANGA REGION

1.0 Demography and Health Status

For the location of Shinyanga region in Tanzania see figure 1 and for the details of the region see fig. 2.

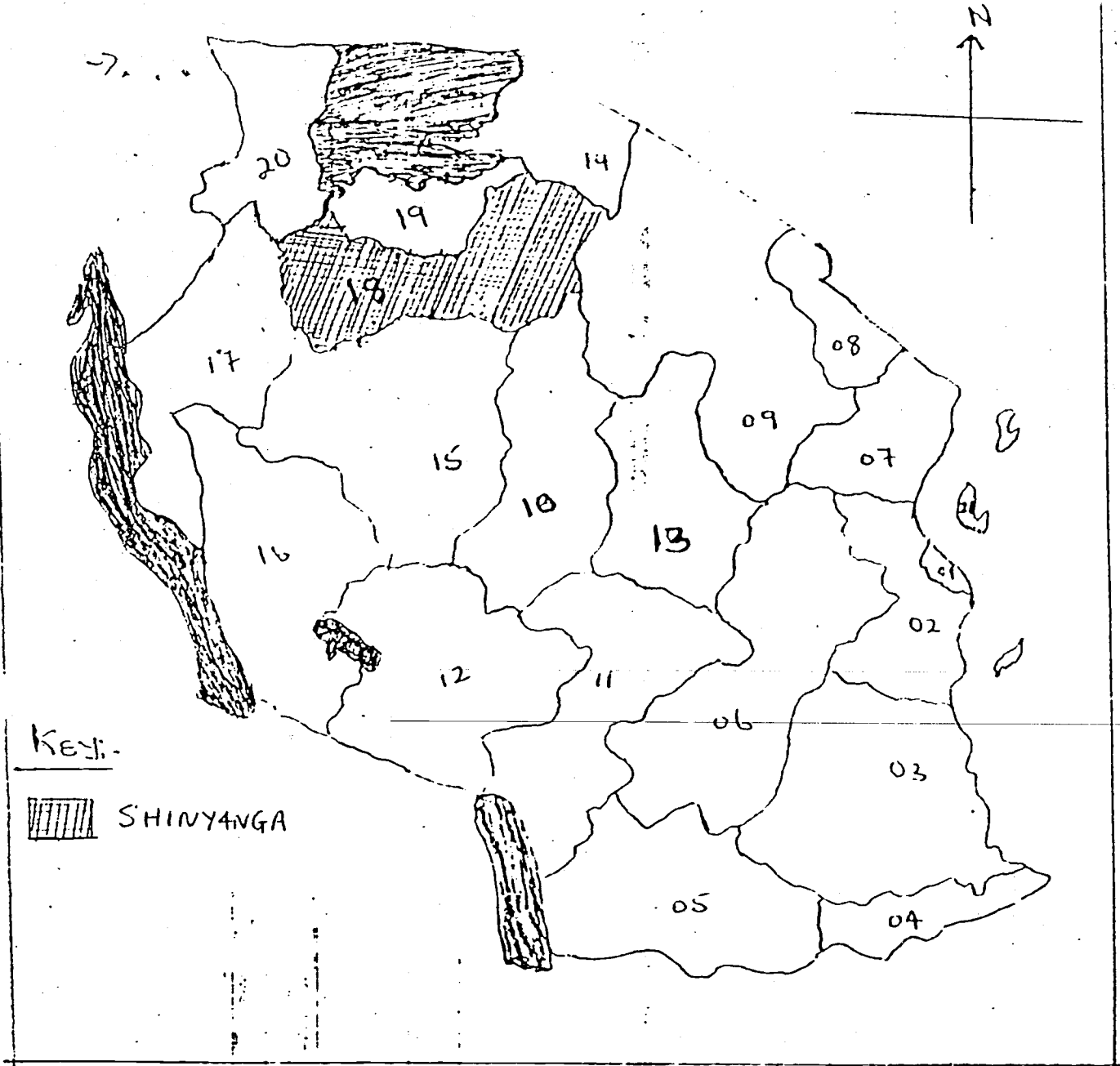


Fig 1. REGIONS

- 01 - DAR ES SALAAM
- 02 - COAST
- 03 - LINDI
- 04 - MTWARA
- 05 - RUVUMA
- 06 - MOROGORO
- 07 - TANGA

- 08 - KILIMANJARO
- 09 - ARUSHA
- 10 - SINGIDA
- 11 - IRINGA
- 12 - MBEZI
- 13 - DODOMA
- 14 - MARA

- 15 - TABORA
- 16 - RUKWA
- 17 - KIGOMA
- 18 - SHINYANGA
- 19 - MWANZA
- 20 - KAGERA
- 21 - ZANZIBAR

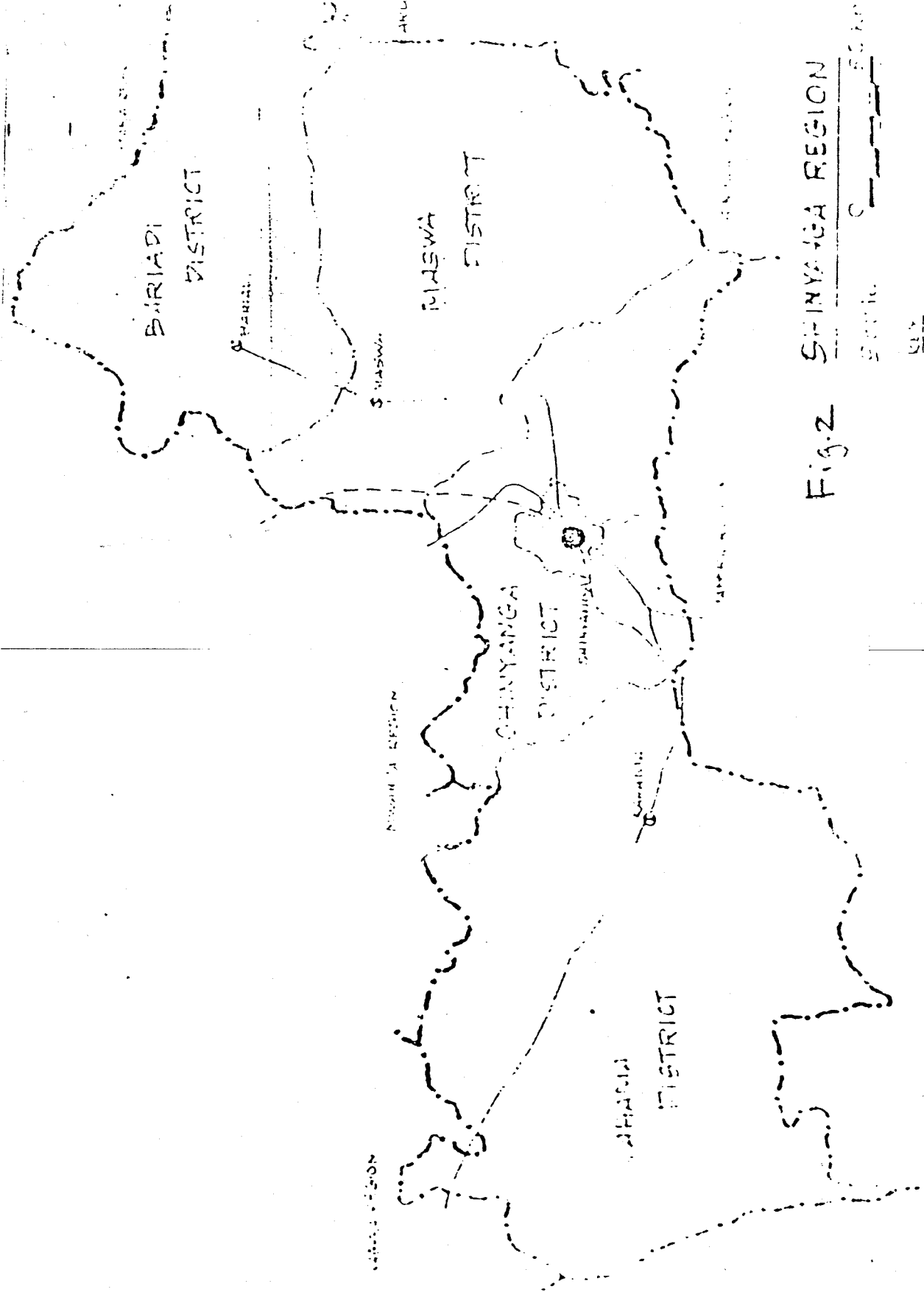
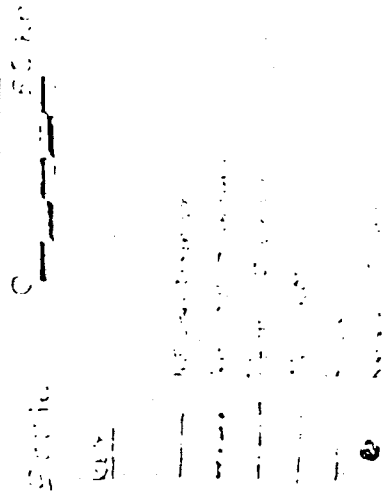


Fig. 2 SHINYANGA REGION



The region is situated in North West Tanzania. It is semi arid with an annual rainfall of about 700 mm. The rains are erratic and the region has been hit by severe drought in recent years. The drought was most severe in Kishapu and Negezi divisions of Shinyanga Rural, district. Hence the selection of these areas for the survey. There are two main seasons: the rainy seasons - a short one (October-December) and a long one (February-May); the dry season (June-October).

Administratively, Shinyanga is divided into 6 districts with further divisions into wards and villages as shown in table I below.

Table 1:

District	No. of Divisions	No. of Wards	No. of Villages	Total Population (1978)
Shinyanga Urban	1	4	23	68,759
Shinyanga Rural	6	36	194	362,170
Kahama	6	20	167	291,622
Maswa	4	33	132	302,079
Bariadi	3	20	116	296,931
Meatu	?	?	?	?
TOTAL	20	113	632	1,321,541

Source: Adapted from Unicef programmes for child survival and development Shinyanga 1985.

It is estimated that the number of underfive children in the survey divisions of Kishapu and Negezi is 19,439. The infant mortality rate in Shinyanga is estimated as 150/1000 which is higher than the national average of 130/1000 (1984-86).

2.0 FOOD AND NUTRITION.

The economy of Shinyanga is agricultural based, sorghum, millet and cassava are the major food crops. Beans, groundnuts, maize, paddy and sweet potatoes are also grown in the region. Cotton is the predominant cash crop providing edible oil as source of fat as a by product. Shinyanga has a large livestock population estimated at 2,000,000 heads (cattle, goats and sheep). Milk production is inadequate and range poultry keeping is common.

Although there is a large number of livestock and poultry, meat hardly features in the normal diet of the inhabitants. Most families have only one or two meals a day. Breastfeeding continues for 2 years in most cases and weaning foods are mainly thin porridge of maize or sorghum. It is obvious that these diets do not meet the needs of a growing child with respect to energy and other nutrients including vitamins and minerals. Malnutrition, manifesting itself as PEM and other nutrient deficiencies affects about 30% of children under 5 years of age (7). Other diseases affecting children include diarrhoea, measles gastrointestinal infections and malaria.

3.0 FOOD STATUION: 1983/84 - 1984/85

Shinyanga is one of the regions which experienced severe food shortage during this period. The region reached a stage whereby they had to seek food aid. A large percentage of the Shinyanga population

was affected by famine (48%). The problem was more acute in three out of the five districts of the region, namely Shinyanga Rural, Maswa and partly Bariadi. Food production for the planting season 1983/84 was below planned levels due to shortened duration of the rainy season and the severity of the drought during the dry season. This resulted in severe hunger, forcing peasant families to consume their crop seeds when food was no longer available. This resulted in the increased levels of malnutrition, especially in children. Whatever food that was obtained was usually starch and contained little protein and vitamins.

4.0 VITAMIN A DEFICIENCY

Evidence of clinical xerophthalmia have been reported in Shinyanga (3). During the AMOO xerophthalmia surveillance lasting 9 months and covering ages 0-10 years in Bariadi, it was found that 15 children had corneal ulcers. This gives a prevalence figure of 1.80%. Compared to the WHO criteria of 0.01%, xerophthalmia in Bariadi was found to be a problem of public health significance. The other parts of Shinyanga especially the drought stricken ones could be worse.

IV. THE STUDY

1.0 Objectives of the study

General Objective: To assess the presence and severity of vitamin A deficiency and xerophthalmia in Shinyanga Region, Tanzania.

Specific Objectives

- a) To determine the prevalence of xerophthalmia and vitamin A deficiency in underfive children in Shinyanga region.
- b) To identify the main causes of vitamin A deficiency and xerophthalmia
- c) To document the existance of feeding practices which are known to influence vitamin A status (e.g. colostrum, duration of breastfeeding and the use of dark green leafy vegetables and fruits)

2.0 RESEARCH METHODOLOGY

A cross-sectional survey was conducted in 22 villages in Negezi and Kishapu divisions in Shinyanga region, the same divisions as for the Child Survival and Development Programme (CSD). 3518 children between the ages of 6 months and 6 years were screened. In estimating the sample size of children to be screened, it was assumed that the prevalence of corneal ulcer, X3, to be 0.02% and a marginal error of 0.05%. The number of children to be screened is given by the formula.

$$n = t^2 \frac{p(1-p)}{d^2}$$

where n= number of children to be screened
 p= prevalence of corneal ulcer (X3) of 0.02%
 t= Std. deviation at which value will be 0.05%
 d= marginal error
 $n = (1.96)^2 (0.02)(0.98)/(0.005)^2$
 = 3012 - 3500 (giving allowance for non attendance)

• Presenting the Questionnaire

The questionnaire was presented in a village not included in the survey. The necessary adjustments were made in the questionnaire and the corrected version was used in the study.

• Selection of the Villages

• Equal number of villages (i.e. 11 villages) was selected from each division. At the division level, the percentage of children in the grey and red zone (underweight) of the MCH card was calculated. Two villages were selected from the wards with a relatively high percentage of underweight children. Those with a smaller percentage contributed one village. For details see tables II and III below.

• Examination and Flow of Taking Measurements

The examination consisted of 6 stations and the description of each station is given below: The stations ^{were} located at a dispensary, health centre or school whichever was convenient.

• Registration

Registration included the following parameters:-

- name of the child
- names of the parents
- name of ten cell leader
- name of the village, ward and division.

TABLE II PERCENTAGE OF UNDERWEIGHT CHILDREN IN NEGEZI APRIL-MARCH 1988

WARD	VILLAGES	CHILDREN / 5 YRS X	CHILDREN WEIGHED Y	CHILDREN IN GREY AND RED ZONES Z	% UNDERWEIGHT (Z X 100) Y	VILLAGES SELECTED FOR SURVEY	NAMES OF VILLAGES
WAMACHELE	4	2652	534	9	1.60	1	
LAKINA	4	1141	962	142	14.8	2	
WILIMA	8	940	852	128	15.8	2	
KILODKI	5	492	492	63	12.8	1	
URUMENGO	4	968	915	50	5.5	1	
NGOFILA	4	1707	945	158	16.7	2	
FALAGA	7	529	529	109	20.6	2	
TOTAL	34	8409	5209	659		11	

CONTINUED

TABLE III PERCENTAGE OF UNDERWEIGHT CHILDREN IN KISHAFU APRIL-JUNE 1988

WARD	NO. OF VILLAGES	CHILDREN / 5YRS		CHILDREN WEIGHED	CHILDREN IN GREY AND RED ZONES	% UNDERWEIGHT (Z _x 100) Y	VILLAGES SELECTED FOR SURVEY	NAME OF VII
		X	Y					
MJAKIPOYA	3	1061	949	104	10.95	1		
UCHUNGA	6	1510	1333	550	24.75	2		
SOMAGEDI	3	1112	925	172	18.59	2		
MWALALASA	5	1721	1631	269	16.49	2		
KISHAFU	5	1812	1663	255	15.33	1		
SACILEJEU	5	1833	1670	208	12.45	1		
MALANGA	2	1978	1766	225	12.74	2		
TOTAL	29	11,030	9937	2463		11		

COMMENTS

Dietary Information

- . The 24 hour dietary (history) recall was used. The collected information was both qualitative and quantitative.
- . Information on the food distribution situation in the community was collected.

Information on history of breastfeeding

- a) How soon was the child put on the breast after delivery (c.g. immediately, one, two, three etc. hours, 2,3,4 - days etc).
- b) Duration of breastfeeding, how long has the child been breastfed.
- c) Weaning period and types of foods given
- d) If the child gets any type of multivitamins at the dispensary or health centre and if the child has received vitamin A capsules and if so how many and for what duration.

Anthropometry Measurements

3548 children had their weight, height and age determined. The weight was taken to the nearest 0.1 kg. and height to the nearest 1.0 cm. Also birthweight from ICH cards were recorded.

Children under 2 years were weighed by using salter scale and a lengthboard for height whereas those above 2 years, a bathroom scale for weight and a height rod for height were used.

Clinical Examination

The children were examined for any signs of malnutrition, measles or history of measles, fever, respiratory infections, diarrhoea, etc.

Assessment of Vitamin A Deficiency by

- . Clinical eye signs, specifically signs of xerophthalmia. The attache questionnaire was used to record clinical findings (appendix 2) (total = 400 children).
- . Also at this station, impression cytology was done on a subsample of every 20th child (total - 100 children).

Biochemical Measurements

- . At this station venous blood was collected from a subsample of 400 children (i.e. every 10th child). Two millilitres of blood enough to give 400-600 ul. of serum was collected using 2 ml syringes by an experienced technician.

The blood was centrifuged, and serum stored in a deep freezer at the shinyanga regional hospital unit after the survey when the serum was shipped to Dar es Salaam for analysis.

At Dar es Salaam the 600ul, of serum was divided into 2 portions. One portion was retained at TFHC for analysis of Retinol, Retinol Binding Protein (RBP) and Pre-albumin. The other portion of 300ul was be airfreighted to Sweden for analysis of C-Reactive Protein, Seruloplasmin and Serosomucoid.

From the same venous blood, drops were taken for blood slide (BS), and Haemoglobin (Hb).

Also at this station stool was collected for examination of Giardia, Amoebiasis, Hookworm, ova and Ascaris.

RESULTS AND DISCUSSION

The presentation of results is divided into clinical and biochemical findings and a brief discussion on the possible causes of vitamin A deficiency in this part of the country.

Clinical Findings

Table IV below contains a summary of clinical findings by divisions and by villages.

Night blindness (KN)

Out of the 3,518 children examined, only 5 (0.14%) children had night blindness. This figure is below the WHO criteria of 1% for public significance based on this parameter. This could mean that all the time of the survey, there were no cases with active xerophthalmia or that the mothers could not easily recognise the problem in their children.

Bitot spots (NIB)

This parameter is another indicator of active xerophthalmia. At the time of the survey only 0.02% (1/3518) of the children examined had bitots spots. Again this is below the WHO criteria (0.5%) for public health significance.

SUMMARY OF CLINICAL FINDINGS

DIVISION	VILLAGE	CHILDREN SCREENED	POSITIVE CASES					
			XN	XIB	XIA	A2	X3A	XS
NEGEZI	LAGANA	210			5			
	MWAMANOTA	85			3			
	NGOFIJA	224		1	11			
	KILOLENI	201			5			
	NHOBOLA	152			4			
	KINDILO	90	1		2			1
	MWAJIGEMA B	84			1			2
	BUBINZA	75						1
	IKONDA	277	1		1			1
	NWAWEJA	143	1		3			2
MINYUGUYU	198			1				
SUB TOTAL	11	1739	3	1	36	-	-	7
% POSITIVE			0.17%	0.12%	2.1%	-	-	0.40%
KISHAFU	BUPIGI	190	-	-				
	BUNYAIYEMBE	151	-		3			
	LUBAGA	156			2			
	KISHAFU	105			-			
	GIMAGI	91			1			
	NWANGONGO	150			5			
	NGEME	247	1		2			
	KINAMPANDA	212			2			
	BUBINZA	206	1		1		1	1
	SANJO	128	-		-			
MIGUNGA	142							
SUB TOTAL	11	1779	2		17		1	1
% POSITIVE			0.11%	-	0.9%		0.05%	0.05%
GRAND TOTAL	22	3518	5	1	53	-	1	8
PERCENTAGE POSITIVE			0.14%	0.02%	1.5%	-	0.02%	0.22%
WHO CRITERIA			1%	0.5%	-	-	0.01%	0.05%

- XN = Night Blindness
- XIB = Bitot Spots
- XIA = Conjunctival Xerosis
- X2 = Corneal Xerosis
- X3A = Corneal Ulcer
- XS = Corneal Scar

Corneal and Conjunctival Xerosis - These parameters were not used in the assessment due to the inherent problems associated with interpretation. In most cases xerosis in the tropics could be due to environmental factors (e.g. smoke in the house, dry weather, etc) rather than pure vitamin A deficiency.

Corneal Ulceration (X3A) and Corneal scar (XS)

These two parameters are indicators of post xerophthalmia. Only 1 (0.02%) child had a corneal ulcer indicating that on the basis of this parameter xerophthalmia was a problem of public health significance by WHO criteria (0.01%).

On the basis of scarring (XS), a total of 8 (0.22%) children had corneal scars indicating that xerophthalmia is a problem of public health significance by WHO criteria of 0.05% for corneal scars.

On the basis of the above clinical findings, it was also clear that of the two divisions, Negezi had more severe problem than Kishapu. Negezi is relatively drier than Kishapu which also has more rivers which enable the inhabitants to grow more food which might include some vegetables.

Biochemical Findings

Table V and VI contain summaries of biochemical findings by divisions.

TABLE V : THE PERCENTAGE OF CHILDREN UNDER THE DIFFERENT RETINOL CUTT-OFF POINTS: HEGEZI DIVISION (*n* = 143 children)

<u>RETINOL LEVEL</u>	<u>DEFICIENT</u>	<u>LOW</u>	<u>NORMAL</u>	<u>HIGH</u>
<u>VILLAGES</u>	<u>< 10ug/dl</u>	<u>10-19ug/dl</u>	<u>20-50ug/dl</u>	<u>50ug/dl</u>
LAGANA	6.3	25.0	68.7	0.0
MWAMANOTA	20.0	10.0	70.0	0.0
NGHOFILA	28.6	28.6	42.8	0.0
KILOLENI	18.2	27.3	54.5	0.0
NHOBOIA	28.0	16.0	44.0	12.0
LILINDILO	20.0	20.0	60.0	
MWAJIGINYA	50.0	25.0	25.0	
IKONDA	10.0	10.0	75.0	5.0
BUBINZA	28.6	28.6	28.5	14.3
HIYUGUWUGU	44.4	22.2	33.4	
NWAVEJA	23.1	15.4	53.8	7.7
TOTAL	277.2	228.1	555.7	39
X	25.2	20.74	50.52	3.55
WHO CRITERIA	5%	15%		

THE PERCENTAGE OF CHILDREN UNDER THE DIFFERENT RETINOL
 CUT-OFF POINTS: KISHAPU DIVISION. (n= 158 children)

RETINOL LEVEL VILLAGES	< 10ug/dl	10-19ug/dl	20-50ug/dl	50ug/dl
BUPIGI	26.7	26.7	33.3	13.3
BUNYANYEMBE	26.7	20.0	46.6	6.7
LUBAGA	14.5	35.7	42.9	7.1
KISHAPU	16.7	41.7	33.3	8.3
GIMAJI	22.2	22.2	55.6	-
MWANGONGO	4.8	23.8	61.9	9.5
MGEME	29.4	5.9	58.8	5.9
KINAMPANDA	7.7	15.3	46.2	30.8
BUZINZA	37.5	6.3	43.7	12.5
SANJO	0.0	25.0	58.3	16.7
MIGUNGA	7.1	14.3	57.1	21.4
TOTAL	195.1	236.9	537.7	132.2
X	17.55	21.54	48.88	12.02
WHO CRITERIA	5%	15%		

The cut off-points for serum retinol are less than 10ug/dl for severe deficiency and less than 20ug/dl for low levels. Vitamin A deficiency is a public health significance if more than 5% of the children have retinol levels of less than 10ug/dl (SEVERE) or 15% of the children have less than 20ug/dl (LOW)

On the basis of this classification, the percentages of children with retinol less than 20ug/dl were 45.9 and 39 level for Negezi and Kishapu respectively indicating that the problem was 3 times the WHO criteria of 15% for Negezi and 2.6 times for Kishapu.

These biochemical findings further confirm the clinical findings that vitamin A deficiency and xerophthalmia are problems of public health significance warranting short term (capsule distribution) and long term (increased intake of vitamin A rich foods and its conservation by controlling diseases) interventions.

Possible Causes of Vitamin A Deficiency in Kishapu and Negezi Divisions

Careful analysis of the information obtained from questionnaires the following factors have been pointed out as possible causes of vitamin A deficiency resulting in xerophthalmia and nutritional blindness.

1) Food Availability

In general food availability in Shinyanga is seasonal experiencing severe shortage during the dry season. This means that a balanced diet necessary for vitamin A absorption and metabolism is difficult to come by. Food unavailability affects underfives more than other members of the family.

Vitamin A rich fruits and vegetables are produced in limited quantity especially along rivers and in valleys. The study has shown that the available fruits and vegetables are normally available to adults rather than the vulnerable underfives.

Milk and fats and oils are available in limited quantity also whereby the underfives receive a minimal share of these foods.

2. Childfeeding Practices:

Surveyed children received very little dietary vitamin A as well as other essential nutrients as shown below.

Use of Colostrum:

In both divisions, few infants were given colostrum immediately after birth. Of the children surveyed, those given colostrum were 80.8% and 84.9% for Negezi and Kishapu respectively. It is known that colostrum is a rich source of vitamin A for new borns and that if taken, it will build up vitamin A liver reserves to meet body requirements for the first 6 months of the child's life. The majority of the mothers claimed that colostrum was dirty milk unfit for human consumption and that if given to the child it would cause diarrhoea. Because of this the majority of the mothers had not even tried to give it to their infants and its avoidance was based on heresy.

Weaning practices:

Most of the children were weaned between 3-5 months which is an acceptable practice. The problem was found to be with weaning foods. They were found to be both qualitatively and quantitatively deficient in vitamin A and other nutrients. Fruits and green leafy vegetables were rarely included in the foods. The maize porridge used as the main weaning food is a watery liquid low in essential nutrients. Additional foods were also given only in small quantities.

The frequency of feeding the children was found to be low compared to the recommended five or more a day. The average was found to be three times a day and in certain cases one to two times a day. Low feeding frequency results in low food intake leading to low nutrient intake. This is because children have small stomachs and thus cannot eat large amount of food at one time; they need to eat small quantities at short intervals to cover their needs.

3. Disease prevalence:

The most prevalent diseases which possibly contributed to vitamin A deficiency are fever, cough, diarrhoea, malaria (34.5%) and parasitic infections (ascaris = 5.7%; hookworm ova = 5.7%; Giardia = 8.6%; Amoeba 8.6%).

At the time of the survey, immunisation coverage was about 50% which was below the national average of 73%. This is regarded as inadequate thus increasing the chance of children being attacked by killer diseases (measles, polio, tuberculosis, whooping cough and diphtheria).

4. Nutritional Status:

A total number of 3,300 children in the two divisions were assessed for nutritional status by using the WHO classification. (8) On the basis of this classification using weight for Age (W/A) as an indicator, 20.2% of the children surveyed were moderately malnourished whereas 7.6% were severe. The national average for severe cases is 5.0% and this implies that children in Kishapu and Negezi are above the national average of severely malnourished children by 2.6%. This does to a great extent, precipitate vitamin A deficiency, xerophthalmia and nutritional blindness.

VI. CONCLUSIONS AND RECOMMENDATIONS

This study indicates that vitamin A deficiency and xerophthalmia are problems of public health significance in drought stricken Kishapu and Negezi:

On the basis of corneal scars, the problem is 0.22% which is 4 times the WHO criteria cut off point of 0.05%; and on the basis of serum retinol levels, 33% of the children have levels below 20ug/dl. which is 2.2% the WHO cut off point of 15% for low retinol levels.

The main causes of vitamin A deficiency are inadequate food intake, (including vitamin A rich foods) poor child feeding practices, diseases (fever, diarrhoea, cough and probably immunizable diseases e.g. measles) and malnutrition.

To combat this problem, the following measures should be taken:

1. short term:
 - Distribution of vitamin A capsules:
 - Mass distribution of Vitamin A capsules (200,000 I.U. to all children under six years,
 - strengthen of distribution of the 50,000, I.U. capsules through the Essential Drug Programme. (EDP)
2. Long term:
 - promote the production and consumption of vitamin A rich foods (fruits, green leafy vegetables, and red palm oil,)
 - strengthen health services (immunization and sanitation)
 - control malnutrition by improving child feeding practices.

VII. A C K N O W L E D G E M E N T S

We wish to extend our thanks to the International Development Research Centre (IDRC) of Canada for funding the study, the Shinyanga Regional and District authorities for their support, Tanzania Food and Nutrition Centre for organising and conducting the study and all those who have in one way or another contributed to the success of this study.

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