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Neurolathyrism risk depends on type of grass pea preparation and on mixing with cereals and antioxidants

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Summary

OBJECTIVE To study an array of household individual and dietary risk factors for neurolathyrism.
METHOD Case–control study using recent cases in a district highly affected by the recent neurolathyrism epidemic in Ethiopia: 108 households with cases and 104 households with no cases; 170 neurolathyrism cases, 370 intra-family controls and 170 community controls frequency matched for age and sex.
RESULTS A history of acute illness was associated with a two- and threefold increased risk of paralysis in community and intra-family controls, respectively. Soaking grass pea in water before cooking roughly halved the risk of neurolathyrism but cooking in clay utensils more than quadrupled it. Consumption of grass pea in the green unripe and boiled forms increased the risk 10 times or more. Mixing the food with gravy that contains condiments with antioxidant activity reduced it by a factor of 4. The consumption of grass pea mixed with cereals rich in sulphur amino acids was also highly protective, but the magnitude of the effect depended on the grass pea preparation consumed.
CONCLUSION Consumption of pure grass pea, especially in the green unripe and boiled forms, should be avoided. Communities at risk of neurolathyrism during famine crises should be encouraged to combine and use grass pea with cereals before the household cereal stock is fully depleted. Breeding programmes, alongside traditional attempts to reduce the toxin content, should enhance the content of sulphur amino acids and antioxidants in grass pea.

keywords lathyrism, Ethiopia, grass pea, antioxidants

Introduction

Excessive consumption of grass pea (*Lathyrus sativus* L.) seeds causes neurolathyrism (Spencer & Schaumburg 1983), a disease characterized by symmetrical axonal degeneration of crossed and uncrossed pyramidal tracts in the thoracic, lumbar and sacral spinal cord (Streifler *et al.* 1977) and loss of pyramidal cells in the area of the cortex controlling the leg (Haimanot *et al.* 1990). The neurotoxin *β*-*N*-oxalyl-α,*β*-diaminopropionic acid (*β*-ODAP) present in grass pea seeds is proposed as the cause of the disease (Spencer *et al.* 1986) but the mechanism of its action has not been conclusively explained. Excitotoxicity action mediated through glutamate receptors (Ross *et al.* 1987), mitochondrial (Sriram *et al.* 1998) as well as enzymatic dysfunctions (Pai & Ravindranath 1993) have all been proposed to explain at least part of its neurotoxicity.

Neurolathyrism had been reported in many parts of the world long back, and nowadays it remains a disease of public health importance mainly in the Indian subcontinent and Ethiopia (Haimanot *et al.* 1990; Haque *et al.* 1996). Grass pea is still cultivated in all continents for human consumption or as an experimental feed crop. Recent reports showed that grass pea is used for human consumption in Asia (Haque *et al.* 1996), Africa (Getahun *et al.* 1999), Europe (Roldan *et al.* 1994; Falco & Pardo 2000; Milczak *et al.* 2001) and Latin America (Mera *et al.* 2000). A renewed interest for cultivating grass pea in Europe was evidenced by its increased production for family consumption by organic farmers in Italy (Falco & Pardo 2000) and its reintroduction in the Polish register of agricultural crops in 1997 (Milczak *et al.* 2001).

Neurolathyrism epidemics mainly occur during times of food shortages as in drought- and flood-related famine as grass pea is resistant to both water shortage and flooding. Outbreaks have occurred recently in Afghanistan in 2001 (Anonymous 2001), in Nepal in 1998 (ProMED 1999) and in Ethiopia in 1997 (Getahun *et al.* 2002).

While heavy consumption of the pulse is known to cause the disease, there exists an unexplained difference in

susceptibility among individuals and communities, despite apparently comparable dietary habits. Several factors were proposed to explain this variability including young age and male sex (Haimanot *et al.* 1990; Haque *et al.* 1996), heavy physical labour (Haimanot *et al.* 1990), diarrhoeal episode, febrile illness and zinc deficiency (Lambein *et al.* 1994). However, the lack of an appropriate animal model precludes exploring this experimentally and the evidence from most epidemiological studies stems from cases that had developed paralysis many years before the study. During the latest epidemic that occurred in Ethiopia we were able to study an array of household, individual and dietary risk factors for neurolathyrism among recent cases and in different control groups.

Subjects and methods

The study was conducted in May 2001 in Delanta Dawint, the district that was most severely hit by the recent neurolathyrism epidemic that started in 1995 in Ethiopia. The district has a total population of 165 000 and is administratively divided into 45 'Kebeles', which are the smallest administrative units. In the district health office, we put in place a neurolathyrism surveillance system (Getahun *et al.* 1999) that identified Asim-Elana, with 4983 inhabitants in 907 households, as the 'Kebele' with the highest number of neurolathyrism cases. All people living in Asim-Elana are from the Amhara ethnic group and they share the same traditions and culture.

Trained high school graduates administered a small screening questionnaire to all the inhabitants of this Kebele. They enumerated all people with walking difficulties and, subsequently, the principal investigator (H.G.), a medical doctor with extensive field experience in neurolathyrism, screened and examined them. The case definition used was symmetrical spastic leg weakness of subacute or insidious onset, with no sensory deficit and with a history of grass pea consumption prior to the onset of the paralysis. This case definition has a sensitivity of 95% and a specificity of 90%. The degree of disability of the patients was categorized into four stages following Haimanot *et al.* (1990).

A total of 170 neurolathyrism patients were thus identified in 108 households and they or their caretaker, if <10 years of age, were subsequently interviewed. All other members of these households who were not suffering from neurolathyrism were also examined and interviewed ($n = 370$). Additionally 104 households with no neurolathyrism patients (one in every eight) were randomly selected and the heads of household and females responsible for food preparation were interviewed. Furthermore, 170 controls, frequency-matched for age and sex, were randomly selected from those households with no cases, interviewed and examined.

Trained female enumerators used a pre-tested closed questionnaire to collect information on household and individual characteristics of the selected study units. At household level, besides socio-demographic characteristics, information on frequency of relief food aid received, habit of soaking grass pea in water before cooking and exclusive use of clay utensils for cooking grass pea preparations was collected. The terms 'family' and 'household' are used interchangeably in the manuscript to denote those living in the same house under the same roof regardless of the genetic relationship. At the individual level, we recorded age, sex, literacy and episodes of diarrhoea, febrile illness or cough during the epidemic (as a proxy for any acute illness). Information on each type of grass pea preparation consumed by each individual was collected for the period of 6 months prior to the detection of the first neurolathyrism case in the Kebele until the end of the epidemic. Grass pea can be consumed in a variety of preparations:

- Eshet (unripe green): grass pea is collected from farmsteads during the harvest season and directly consumed as snack without preparation.
- Kollo (roasted): grass pea is briefly soaked in boiling or cold water, after which excess water is decanted and the seed is roasted and consumed as it is.
- Nifiro (boiled): grass pea is washed with hot or cold water two or three times, then cold water is added and the pulse is cooked until it is soft enough to be eaten.
- Kitta (bread): grass pea is husked and possibly mixed with cereals of varying type and amount, and ground into flour; the flour is then mixed with water and a thick batter is prepared and baked into unleavened bread.
- Injera (pancake): grass pea is husked and possibly mixed with cereals of varying type and amount, and ground into flour; after mixing the flour with yeast and water a fairly thin batter is obtained which is kept at room temperature until it is fermented. The fermented dough is baked into injera (pancake).
- Shiro (gravy): grass pea is lightly toasted, then thoroughly washed in cold water and toasted lightly once more, subsequently husked and, after adding some spices and vegetables, ground into the flour that is used to prepare the Ethiopian gravy 'Shiro wot'. The gravy is exclusively consumed with Kitta or Injera.

The consumption of boiled, bread and pancake forms, which could possibly be mixed with cereals (mainly wheat and maize from relief food), were classified into three groups (no consumption, consumption mixed with at least

one third of cereals and consumption mixed with less than one-third cereal mixture). The gravy is consumed virtually by all inhabitants but the addition of spices and vegetables with known antioxidant effect (ginger, garlic or onion) was used to classify the consumption into two categories (with or without antioxidant).

Female members of the household who were responsible for preparing the food were asked to demonstrate the customary mixing of grass pea with cereals – if any used – during the period referred to above for each grass pea food type. Such a demonstration was observed on two occasions separated by at least 24 h. The woman was subsequently asked whether she thought the mixture used during the epidemic most of the time was at least 'Sisso' (a traditional notion meaning one part in a total of three parts). Possible discrepancies between this answer and the previous observations were resolved by further questioning before the enumerators classified the proportion of cereal–grass pea mixture as 'at least one-third of cereals' or 'less than one-third of cereals'. This information on the use of mixtures for particular grass pea preparation was extrapolated to every member of the household.

Data were entered using EPI Info (version 6) and analysed using SPSS for windows version 11 (SPSS, Chicago, IL, USA). We conducted two matched case–control analyses at the individual level contrasting the cases with sex- and age-matched community controls and cases with intra-family controls, respectively. We also performed an unmatched case–control analysis at the household level, comparing households having cases with households having no cases.

In bivariate analysis, we used the chi-square and McNemar tests to compare categorical variables. The *t*-test was used to compare quantitative variables. In multivariate analyses, the potential risk factors and confounders, identified on the basis of *a priori* knowledge and the bivariate analysis, were considered for inclusion in the logistic regression model. The iterative modelling strategy recommended by Kleinbaum *et al.* (1998) was used to select the factors to be withheld in the final model.

Ethical clearance for the study was given by the Ethiopian Science and Technology Commission and by the Ethical Committee of the Faculty of Medicine, Ghent University. Informed oral consent was obtained from all study subjects.

Results

The current study found a higher female to male ratio (1:2) than previous studies and a higher proportion of cases in the most severe stages III (9%) and IV (11%) of the disease (Table 1). The age at onset of paralysis ranged between 2

Table 1 Profile of cases in the present and previous studies in Ethiopia

	Gebreab *et al.* (1978) (n = 371; %)	Haimanot *et al.* (1990) (n = 1792; %)	Getahun *et al.* 1998 (n = 333; %)	This study 2001 (n = 170) (%; 95% CI)
Sex				
Female	30	28	17	33 (28–38)
Male	70	72	83	67 (57–77)
Stage of disease				
I (use no walking stick)	53	52	46	37 (31–43)
II (use one walking stick)	35	42	45	43 (37–49)
III (use two walking sticks)	8	4	6	9 (8–10)
IV (crawler or bed-ridden)	4	3	3	11(9–13)
Age group at onset (years)				
0–10	27	26	37	44 (37–51)
11–20	27	27	45	24 (20–28)
21–30	15	16	11	10 (8–12)
31–40	16	15	5	10 (8–12)
40+	15	16	3	12 (10–14)
Number of cases per family*				
Only one	69	85	–	41 (35–47)
More than one	31	15	–	59 (50–68)

* Percentage calculated with patient as unit of observation.

and 58 years with a median of 11 years. There was no difference in age at onset of paralysis between males and females ($P = 0.11$). Unlike in previous studies, most (59%) of the cases had another afflicted family member. The relationship of risk of neurolathyrism with increasing family size was explored during the analysis and there was no monotonous relationship. However, a threshold was observed and the family size was dichotomized.

Table 2 shows the distribution of household level variables [with unadjusted odds ratios (OR)] in households with and without cases and Table 3 the distribution of selected individual variables in cases and controls. Use of clay utensils for cooking, illiteracy and large family size were associated with increased risk for neurolathyrism. The unadjusted ORs (for both individual and household level variables) for cases *vs.* community and intra-family controls are shown in Table 4. Remarkably, receiving relief food more than six times in a year and soaking the grass pea in water before preparing the foods were associated with reduced risk of neurolathyrism.

After controlling for confounding factors in logistic regression models, illiteracy, large family size and exclusive use of clay utensils remain significant household-level risk factors for neurolathyrism both in the individual and the household control analyses (Table 5). Reduced risk of paralysis was associated with soaking grass pea in water before preparation.

The independent effects of the individual level risk factors in cases contrasted with community and intra-family controls were also assessed in logistic regression models (Table 6). There were important differences in the consumption pattern of grass pea preparations even among members of the same family, except for gravy. The green unripe and roasted seeds were mostly consumed by young members of the family (data not shown). After control for confounding factors, the community control and family control models yielded consistent results. Increased risk of paralysis was associated with the consumption of grass pea in green unripe or boiled forms. The consumption of bread and pancake prepared from grass pea provided a relative protective effect, particularly if mixed with more than one-third of cereal. Likewise, the consumption of gravy with antioxidants had a protective effect. As both the pancake and bread form were frequently consumed in conjunction

Table 2 Characteristics of households with and without neurolathyrism cases, Delanta Dawint, Ethiopia 2001

Variable	Number of households (%)		Unadjusted OR (95% CI)	*P*-value
	With case ($n = 108$)	Without case ($n = 104$)		
Sex of head of household				0.23
Female	16 (14.8)	22 (21.2)	1.0	
Male	92 (85.2)	82 (78.8)	1.54 (0.72–3.33)	
Education of family				0.01
Literate	51 (47.2)	67 (64.4)	1.0	
Illiterate	57 (52.8)	37 (35.6)	2.02 (1.16–3.51)	
Farmland ownership				0.09
No	2 (1.9)	7 (6.7)	1.0	
Yes	106 (98.1)	97 (93.3)	3.82 (0.70–27.35)	
Religion				0.09
Christian	106 (98.1)	96 (92.3)	1.0	
Moslem	2 (1.9)	8 (7.7)	0.23 (0.03–1.19)	
Family size				0.02
1–7	88 (81.5)	96 (92.3)	1.0	
8+	20 (18.5)	8 (7.7)	2.72 (1.14–6.50)	
Cattle ownership				0.13
No	73 (67.6)	60 (57.7)	1.0	
Yes	35 (32.4)	44 (42.3)	0.65 (0.37–1.14)	
Relief food (annual frequency)				0.29
≤6 times	25 (23.1)	18 (17.3)	1.0	
7+ times	83 (76.9)	86 (82.7)	0.69 (0.35–1.36)	
Exclusive clay utensils for cooking				<0.005
No	80 (74.1)	98 (94.2)	1.0	
Yes	28 (25.9)	6 (5.8)	5.71 (2.25–14.48)	
Soaking before cooking				0.63
No	17 (15.7)	14 (13.5)	1.0	
Yes	91 (84.3)	90 (86.5)	0.83 (0.36–1.91)	

Table 3 Characteristics of individual study subjects (neurolathyrism cases, community controls and intra-family controls), Delanta Dawint District 2001

Variable	Number of cases (%) (n = 170)	Number of controls (%)	
		Community* (n = 170)	Intra-family† (n = 370)
Sex			
Male	114 (67.1)	114 (67.1)	162 (43.8)
Female	56 (32.9)	56 (32.9)	208 (56.2)
Age (years)			
0–9	13 (7.6)	13 (7.6)	92 (24.9)
10–19	88 (51.8)	88 (51.8)	96 (25.9)
20–29	23 (13.5)	23 (13.5)	30 (8.1)
30–39	19 (11.2)	19 (11.2)	32 (8.6)
40–49	14 (8.2)	14 (8.2)	51 (13.8)
50–59	9 (5.3)	9 (5.3)	35 (79.5)
60+	4 (2.4)	4 (2.4)	34 (9.2)
Education of individual			
Illiterate	118 (69.4)	100 (58.8)	280 (75.7)
Literate	52 (30.6)	70 (41.2)	90 (24.3)
History of illness			
No	145 (85.3)	163 (95.9)	330 (89.2)
Yes	25 (14.7)	7 (4.1)	40 (10.8)
Grass pea food consumption			
Green unripe			
No	55 (32.4)	110 (64.7)	196 (53.0)
Yes	115 (67.6)	60 (35.3)	174 (47.0)
Roasted			
No	53 (31.2)	105 (61.8)	173 (46.8)
Yes	117 (68.8)	65 (38.2)	197 (53.2)
Boiled			
No consumption	52 (30.6)	109 (64.1)	187 (50.5)
Consumption with ≥1/3 cereal	52 (30.6)	38 (22.4)	101 (27.3)
Consumption with <1/3 cereal	66 (38.8)	23 (13.5)	82 (22.2)
Bread			
No consumption	80 (47.1)	94 (55.3)	225 (60.8)
Consumption with ≥1/3 cereal	44 (25.9)	41 (24.1)	92 (24.9)
Consumption with <1/3 cereal	46 (27.1)	35 (20.6)	53 (14.3)
Pancake			
No consumption	60 (35.3)	70 (41.2)	173 (46.8)
Consumption with ≥1/3 cereal	41 (24.1)	48 (28.2)	110 (29.7)
Consumption with <1/3 cereal	69 (40.6)	52 (30.6)	87 (23.5)
Gravy			
With antioxidants	74 (43.5)	101 (59.4)	160 (43.2)
With no antioxidants	96 (56.5)	69 (40.6)	210 (56.8)

* Matched for age and sex; † de facto matched for gravy consumption (see text).

with the gravy form, interaction between these variables was tested but it turned out to be non-significant ($P > 0.05$). Finally, a history of acute illness before the onset of symptoms was significantly associated with higher risk of neurolathyrism.

Discussion

Several studies have documented that grass pea is consumed in a variety of forms (Roldan *et al.* 1994; Haque *et al.* 1996; Getahun *et al.* 1999) but very few have attempted to associate the type of grass pea preparation with the risk of neurolathyrism. Lacking a good animal model for the disease, our approach was to look into the food basket of a community that recently suffered from an epidemic precipitated by drought and food shortage. The data we collected might not convey the same impression of accuracy as the information from a strictly quantitative 24-h food recall, and memory bias cannot be entirely excluded. However, the effects we observed are

Table 4 Unadjusted OR for individual risk factors for neurolathyrism, Delanta Dawint, 2001

	Cases *vs.* community controls*		Cases *vs.* intra-family controls†	
Variable	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Individual variables				
Sex		–		<0.005
Male	–		1.0	
Female	–		0.38 (0.26–0.56)	
Age (years)		–		<0.005
0–9	–		1.20 (0.36–3.93)	
10–19	–		7.79 (2.65–22.84)	
20–29	–		6.51 (2.02–20.99)	
30–39	–		5.04 (1.54–16.44)	
40–49	–		2.33 (0.70–7.69)	
50–59	–		2.18 (0.61–7.77)	
60+	–		1.0	
History of acute illness		0.002		0.25
No	1.0		1.0	
Yes	4.01 (1.68–9.55)		1.42 (0.83–2.43)	
Grass pea food consumption				
Green unripe		<0.005		<0.005
No	1.0		1.0	
Yes	3.83 (2.44–6.01)		2.35 (1.61–3.44)	
Roasted		<0.005		0.001
No	1.0		1.0	
Yes	3.56 (2.27–5.58)		1.93 (1.32–2.84)	
Boiled		<0.005		<0.005
No boiled at all	1.0		1.0	
With ≥1/3 cereal	2.86 (1.68–4.88)		1.85 (1.17–2.91)	
With <1/3 cereal	6.01 (3.37–10.72)		2.89 (1.85–4.52)	
Bread		0.25		0.001
No bread at all	1.0		1.0	
With ≥1/3 cereal	1.26 (0.75–2.12)		1.34 (0.86–2.09)	
With <1/3 cereal	1.54 (0.90–2.62)		2.44 (1.52–3.90)	
Pancake		0.15		<0.005
No pancake at all	1.0		1.0	
With ≥1/3 cereal	0.99 (0.58–1.71)		1.07 (0.67–1.70)	
With<1/3 cereal	1.54 (0.94–2.54)		2.28 (1.48–3.52)	
Gravy		0.004		–
With antioxidants	1.0		–	
With no antioxidants	1.89 (1.23–2.92)		–	
Household variables				
Sex of head of household		0.63		–
Male	1.0		–	
Female	1.16 (0.62–2.18)		–	
Education of family		<0.005		–
Literate	1.0		–	
Illiterate	2.30 (1.49–3.56)		–	
Farmland ownership		0.21		–
No	1.0		–	
Yes	2.39 (0.60–9.39)		–	
Family size		0.002		–
1–7	1.0		–	
8+	2.37 (1.36–4.12)		–	
Cattle ownership		0.13		–
No	1.0		–	
Yes	0.68 (0.41–1.12)		–	

Table 4 Continued

	Cases *vs.* community controls*		Cases *vs.* intra-family controls†	
Variable	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Relief food (annual frequency)		0.01		–
≤6 times	1.0		–	
7+ times	0.57 (0.35–0.91)		–	
Only clay utensils for cooking		0.007		–
No	1.0		–	
Yes	1.81 (1.17–2.79)		–	
Soaking before cooking		0.02		–
No	1.0		–	
Yes	0.59 (0.38–0.93)		–	

* Frequency-matched for age and sex; † matched (by design) for family characteristics including gravy consumption.

Table 5 Independent family-level risk factors for neurolathyrism, Delanta Dawint 2001

	Individual cases *vs.* community controls		Households with *vs.* without cases	
Variable	Adjusted OR (95% CI)*	*P*-value	Adjusted OR (95% CI)†	*P*-value
Education of family		0.03		0.01
Literate	1.0		1.0	
Illiterate	1.85 (1.15–2.98)		2.12 (1.29–3.48)	
Family size		0.01		0.02
1–7	1.0		1.0	
8+	2.45 (1.35–4.43)		2.87 (1.34–6.15)	
Soaking of grass pea before cooking		0.005		0.14
No	1.0		1.0	
Yes	0.20 (0.11–0.36)		0.55 (0.27–1.07)	
Only clay utensils for cooking		<0.005		<0.005
No	1.0		1.0	
Yes	4.62 (2.59–8.24)		6.39 (2.86–14.27)	

* Adjusted in logistic regression for the other household variables listed in Table 2 and for the individual variables listed in Table 3.
† Adjusted in logistic regression for the other household variables listed in Table 2.

biologically plausible and the consistency of the OR in both individual and household level analyses give additional strength to our findings.

Methionine and cysteine are deficient in grass pea, as in other legumes. Methionine is an essential sulphur-containing amino acid that has a large number of metabolic functions, including in the central nervous system (Amara *et al.* 1995). Cereals such as wheat and maize, which were distributed to the community as relief food during the food crisis, are rich in sulphur amino acids (McCracken *et al.* 2002) and are possibly consumed together with grass pea. The better balance of essential amino acids (Spencer & Schaumburg 1983; Getahun *et al.* 1999) hand-in-hand with lower levels of the toxin intake can explain the reduced risk of paralysis observed with the consumption of grass pea mixed with more than one-third of cereals. In a historic, dramatic situation during the Second World War in a Ukrainian prisoner of war camp, inmates receiving 200 g of boiled grass pea seed and 200 g of barley bread developed signs of undernutrition but no neurolathyrism. When the ration of grass pea seeds was doubled with unchanged ration of barley bread, the worst epidemic of neurolathyrism on record developed within 6 weeks to 4 months, until grass pea was removed from the diet (Kessler 1947). This underlines the importance of the ratio of grass pea to cereals in the susceptibility to neurolathyrism. It could thus be advised that during food shortages and grass pea abundance, communities at risk of neurolathyrism should be encouraged to combine grass pea with cereals well before the household cereal stock is fully

Table 6 Independent individual level risk factors for neurolathyrism, Delanta Dawint 2001

Variable	Cases *vs.* community controls†		Cases *vs.* intra-family controls‡	
	Adjusted OR (90% CI)*	*P*-value	Adjusted OR (90% CI)§	*P*-value
Grass pea food consumption				
Green unripe		0.04		0.002
No	1.0		1.0	
Yes	2.13 (1.14–3.97)		10.31 (2.98–35.65)	
Roasted		0.17		0.93
No	1.0		1.0	
Yes	2.47 (0.84–7.35)		1.12 (0.11–11.31)	
Boiled		<0.005		0.006
No boiled at all	1.0		1.0	
With ≥1/3 cereal	7.67 (2.65–22.16)		16.0 (2.96–86.29)	
With <1/3 cereal	19.94 (6.61–60.08)		26.30 (4.71–146.85)	
Bread		0.004		0.12
No bread at all	1.0		1.0	
With ≥1/3 cereal	0.22 (0.10–0.48)		0.66 (0.16–2.86)	
With <1/3 cereal	0.32 (0.15–0.67)		2.89 (0.70–11.83)	
Pancake		0.10		0.01
No pancake at all	1.0		1.0	
With ≥1/3 cereal	0.36 (0.16–0.83)		0.05 (0.004–0.60)	
With <1/3 cereal	0.42 (0.20–0.92)		1.23 (0.09–16.93)	
Gravy		<0.005		–
With antioxidants	1.0		–	
With no antioxidants	3.81 (2.18–6.66)		–	
History of illness		0.04		0.03
No	1.0		1.0	
Yes	1.85 (1.21–7.07)		3.34 (1.30–8.61)	
Sex				<0.005
Male	–		1.0	
Female	–		0.20 (0.12–0.32)	
Age (years)				<0.005
0–9	–		9.01 (4.24–19.13)	
10–19	–		13.21 (4.90–35.59)	
20–29	–		6.12 (2.37–15.77)	
30–39	–		1.62 (0.61–4.28)	
40–49	–		1.14 (0.37–3.54)	
50–59	–		0.44 (0.11–1.77)	
60+	–		1.0	

* Adjusted for household variables listed on Table 4 and for individual education.
† Frequency-matched for age and sex.
‡ Matched by design for household characteristics including gravy consumption.
§ Adjusted for individual education.

depleted. Consumption of the grass pea in non-mixed forms and especially in the green unripe and boiled forms should be discouraged.

Besides the mixing of cereals, the fermentation of grass pea in the pancake and the use of condiments in gravy appear to be protective and reduce the risk of neurolathyrism. Fermentation has the established dual effect of reducing the content of β-ODAP and improving the balance of essential amino acids (Kuo *et al.* 1995). The condiments added to the grass pea-based gravy (ginger, garlic and onion) have a well-established antioxidant activity (Chu *et al.* 2002). It has been suggested that oxidative stress contributes towards the excitotoxicity of β-ODAP through the generation of oxygen radicals and the promotion of cell injury by triggering glutamate release (Sriram *et al.* 1998). The reduced risk associated with the consumption of gravy could thus be explained by the antioxidant effect of the added condiments and the

complex preparation process that includes soaking of the grass pea seeds, which may wash out part of the water-soluble toxin.

The findings of our study corroborate those of a previous one indicating that the exclusive use of traditional clay utensils for the preparation of grass pea foods increases the risk of paralysis (Getahun *et al.* 2002). β-ODAP forms chelates with bivalent cations such as zinc, manganese, copper and iron that are essential for the activity of superoxide dismutase and peroxidase. These enzymes protect against the abrasive effects of reactive oxygen species (ROS) that play a role in oxidative stress and are produced by any reaction involving nitric oxide such as physical labour and neuronal excitation to name a few. In the plant, β-ODAP was proposed to play a role as carrier molecule for zinc ions (Lambein *et al.* 1994). The uninterrupted daily intake of gram amounts of β-ODAP from foods prepared in traditional clay pots that release bivalent cation micronutrients might contribute towards disturbing the balance of these nutrients and hence to oxidative stress. Although these findings warrant further biochemical studies, it seems worthwhile, from a theoretical point of view, to encourage rural communities not to use hand made traditional clay pots to prepare grass pea foods. From a public health perspective, however, the feasibility and cost-effectiveness of such interventions could be questionable.

The higher incidence among males of the younger age group and the lack of association between age at onset and gender have been documented before (Gebreab *et al.* 1978; Haimanot *et al.* 1990; Haque *et al.* 1996). However, the average number of cases per family and the relative proportion of more severely affected patients is higher in our study than in previous studies (Gebreab *et al.* 1978; Haimanot *et al.* 1990; Getahun *et al.* 2002). This can be explained by the much shorter time gap between the onset of paralysis and time of the study. The deaths of the more severely affected patients may have been more frequent with the longer time gap in previous studies.

The interesting link of family illiteracy with risk of neurolathyrism cannot be adequately explained within the scope of the current study. We hypothesize that literate people have better access to information and could be less influenced by myths and misconceptions. Our literacy campaign enabled more than half of the subjects in our study to acquire basic reading and writing skills. Revitalizing the literacy campaign that was discontinued, could, as a side-effect, enhance the reception of preventive neurolathyrism information, education and communication (IEC) to communities at risk.

Since the discovery of the neurotoxin in grass pea seeds in 1964, research to solve the public health problem of neurolathyrism has focused on toxicity mechanisms and β-ODAP elimination from the plant. While both lines achieved definite, albeit incomplete successes, it has been overlooked that grass pea is deficient in the sulphur-containing amino acids methionine and cysteine. This study provides a strong indication of a neutralization of the toxic β-ODAP effect upon mixing grass pea with cereals rich in these essential amino acids and with antioxidants. It could imply the need for a paradigm shift in breeding programmes aimed at improving the nutritional quality of this ancient 'survival crop'. Enhancing the content of sulphur amino acids and antioxidants may also be possible through plant genetics, alongside attempts to reduce the neurotoxin content that have been ongoing almost for four decades.

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