Risk factors for noma disease: a 6-year, prospective, matched case-control study in Niger

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Summary

Background **Noma is a poorly studied disease that leads to severe facial tissue destruction in children in developing countries, but the cause remains unknown. We aimed to identify the epidemiological and microbiological risk factors associated with noma disease.**

Methods **We did a prospective, matched, case-control study in Niger between Aug 1, 2001, and Oct 31, 2006, in children younger than 12 years to assess risk factors for acute noma. All acute noma cases were included and four controls for each case were matched by age and home village. Epidemiological and clinical data were obtained at study inclusion. We undertook matched-paired analyses with conditional logistic regression models.**

Findings **We included 82 cases and 327 controls. Independent risk factors associated with noma were: severe stunting (odds ratio [OR] 4·87, 95% CI 2·35–10·09) or wasting (2·45, 1·25–4·83); a high number of previous pregnancies in the mother (1·16, 1·04–1·31); the presence of respiratory disease, diarrhoea, or fever in the past 3 months (2·70, 1·35–5·40); and the absence of chickens at home (1·90, 0·93–3·88). After inclusion of microbiological data, a reduced proportion of *Fusobacterium* (4·63, 1·61–13·35), *Capnocytophaga* (3·69, 1·48–9·17), *Neisseria* (3·24, 1·10–9·55), and *Spirochaeta* in the mouth (7·77, 2·12–28·42), and an increased proportion of *Prevotella* (2·53, 1·07–5·98), were associated with noma. We identified no specific single bacterial or viral pathogen in cases.**

Interpretation **Noma is associated with indicators of severe poverty and altered oral microbiota. The predominance of specific bacterial commensals is indicative of a modification of the oral microbiota associated with reduced bacterial diversity.**

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Introduction

Noma is a gangrenous disease causing high mortality and devastating facial lesions with severe functional and aesthetic consequences.[1] Hippocrates first described noma in the 5th century BC[2] and the disease continued to be reported throughout Europe until World War 2, when the last reports of noma concerned concentration camp and prison camp inmates.[3,4] In high-income countries, noma is now extremely rare and affects only patients with severe immunosuppression or blood dyscrasia.[5–8] By contrast, noma still persists in the poorest developing countries—mostly in Africa—and occurs mainly in children younger than 6 years. Cases have also been reported from Latin America and the Asia-Pacific region.[9,10] Although noma cannot be regarded as a major public health problem worldwide, the facial mutilations caused by the disease are intolerable and further research is needed to alleviate the suffering of affected underprivileged populations.

Noma disease develops from a gingival lesion that spreads to the underlying bone. The corresponding facial region develops oedema and becomes necrotic, leading to the destruction of large parts of the soft and hard facial tissues.[1] Findings from observational studies have suggested several possible risk factors for noma disease, including poverty,[11] stunting owing to malnutrition,[12] low birthweight,[13] absence of exclusive breastfeeding, poor sanitation, endemic infections (measles, malaria, and AIDS), poor oral hygiene, and proximity of livestock.[11–13] Although these factors might have a role in a cascade of events leading to a catastrophic derailment of a disorder that is essentially an opportunistic infection associated with poor health status, the cause of noma remains largely unknown.[1,11] We aimed to identify risk factors for noma, including epidemiological, biological, microbiological, and sociobehavioural variables.

Methods

Study design and participants

We did a prospective, matched, case-control study of all children diagnosed with acute noma between Aug 1, 2001, and Oct 31, 2006, in the Zinder region of southeast Niger, a landlocked country in western Africa. Cases were children younger than 12 years who presented with acute necrotising stomatitis with exposure of underlying bone, oedema, or initial facial necrosis (figures 1A and B).[1] Controls were children without any signs of noma or acute necrotising gingivitis at study inclusion and matched for age (within 3 months) and home village to cases recruited within 1 month of noma diagnosis. Children with sequelae

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from a previous episode of noma were excluded (figures 1C and D).

The study was done in accordance with the Declaration of Helsinki and in collaboration with Sentinelles, a Swiss non-governmental humanitarian organisation. Signed consent (usually by a cross or a fingerprint) by the parent or guardian of the child was obtained after oral information was given by local staff in the native language and translated from a French information leaflet. All consent forms were sent to the office of the Geneva Study Group on Noma (GESNOMA) in Geneva, Switzerland. The study was approved by the Ministry of Health of Niger and the WHO noma programme.

Clinical and biological data collection

We did structured interviews with the child's family members at the time of diagnosis for children with noma and at study inclusion for controls, followed by a clinical examination. We recorded demographic, clinical, and nutritional data, including environmental variables, and documented the presence and extent of facial and oral lesions.[14] We did viral sampling by gently rolling a cotton swab tip around the mouth mucosal surface; samples were then stored in dedicated tubes for virus shipment. Microbial samples were taken from the subgingival plaque of teeth with paper points and immersed immediately in guanidinium thiocyanate buffer (RLT-Buffer, Qiagen, Basel, Switzerland). In noma patients, one sample was taken from a diseased site and one from a healthy dentition site. In children presenting with substantial destruction of the gingival tissue with no existing gingival sulcus, the sample was collected at the edge of the lesion. For controls, only one sample was taken from a mandibular anterior tooth.

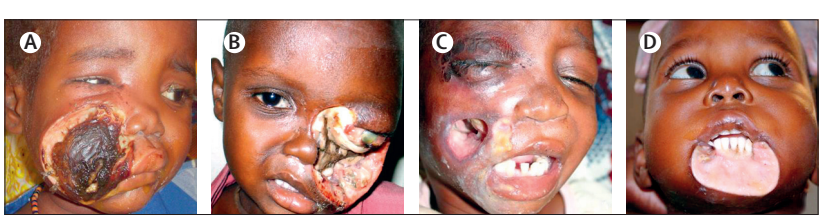

Figure 1: **Examples of noma lesions**
(A) A 3-year-old-girl with acute noma, showing oedema and necrosis. (B) A 3-year-old boy with acute noma, showing necrosis and important loss of tissue. (C and D) Children with more advanced noma stages showing a well-defined loss of substance.

We took blood samples from both groups. All aliquots of plasma, gingival fluid, and mucosal swabs were stored at –20°C until shipment and stored on delivery at –80°C at University of Geneva Hospitals. We assessed present (wasting) and past (stunting) nutritional status by anthropometric indicator estimates proposed by WHO and the US Centers for Diseases Control and Prevention (CDC).[15] Scores obtained were calculated and compared with the standard values of the US National Center for Health Statistics.

Biological analyses

We measured plasma concentrations of retinol (vitamin A) and α-tocopherol (vitamin E) with high-performance liquid chromatography (Beckman System Gold, 166 Detector, Beckman Instruments, Fullerton, CA, USA) according to standard techniques.[16] Serology for Epstein–Barr virus used indirect immunofluorescence assay and quantitative determination of IgM and IgG antibodies against viral capsid antigen in human serum (Abbott AG, Baar/Zug, Switzerland). For herpes simplex virus, varicella zoster virus, cytomegalovirus, and morbillivirus (measles), we used specific enzyme immunoassays (Siemens, Marburg, Germany) for the qualitative detection and the quantitative determination of IgM and IgG antibodies. Immunity to measles was defined as a positive IgG serology, a record of putative measles vaccination, or a history of disease when serology results were unavailable. Mucosal swabs were inoculated on human fibroblasts (primary culture strains) and three continuous cell lines (A549, Vero, and MDCK) to detect the possible presence of viruses. In a second step, cytomegalovirus early antigen from saliva and mucosal swabs was assessed with shell vial cell culture technique and by staining with monoclonal antibodies (Argene, Varilhes, France).

Microbiological procedures and microarrays

We extracted total genomic DNA with glass beads (diameter 212–300 µm; Sigma-Aldrich, Buchs, Switzerland) and a DNeasy kit (Qiagen), according to the manufacturer's instructions. We undertook asymmetrical PCR with biotinylated universal primers fD1 (0·4 µM) and rD1 (4 µM) as described previously.[17] We used spectrophotometry (NanoDrop ND-1000 Spectrophotometer, NanoDrop

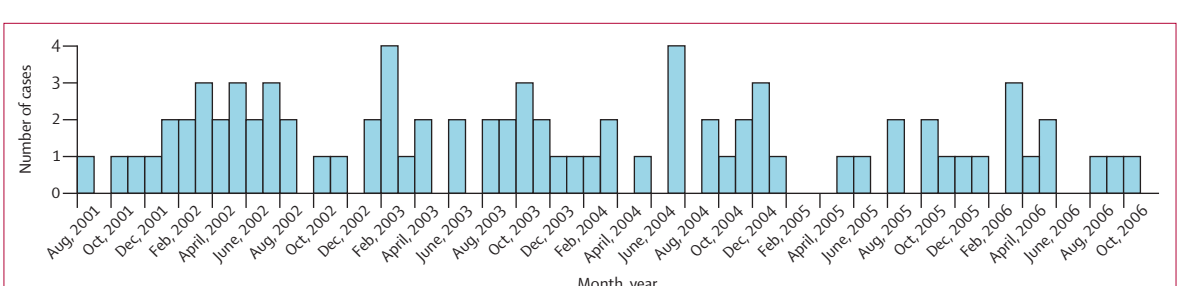

Figure 2: **Noma epidemic curve between Aug 1, 2001, and Oct 31, 2006, in the Zinder region of Niger**

Technologies Wilmington, DE, USA) for quality control and quantification of PCR products.

On the basis of the most relevant sequences identified in a previous pilot investigation,[18] we designed a low-density 16S rDNA array. Briefly, from a dataset containing 1237 partial 16S rRNA gene sequences representing 339 different phylotypes, we scanned 132 sequences showing an abundance of at least 1% for probes respecting defined physicochemical properties (Huyghe A, et al, University of Geneva, personal communication), leading

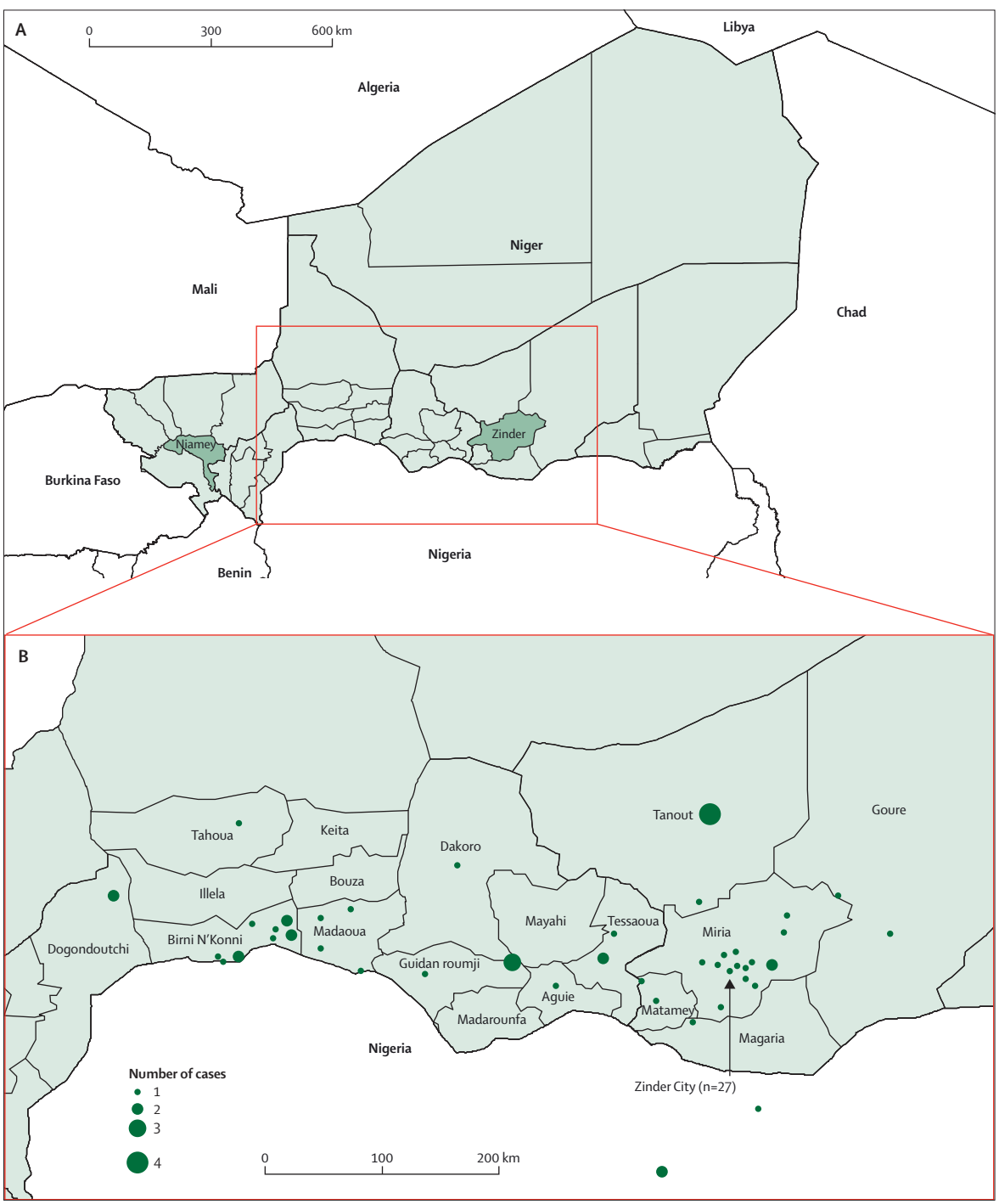

Figure 3: **Geographical localisation of the study population, Zinder region, Niger, Africa**
(A) Niger (capital: Niamey) and surrounding African countries. (B) Geographical distribution of noma cases between Aug 1, 2001, and Oct 31, 2006, in the Zinder region of Niger

	Cases (n=82)	Controls (n=327)	p value*
Sociodemographic variables			
Age at study entry (years)	3·7 (1·7)	4·0 (1·6)	0·002
Female sex	41 (51·9%)	160 (48·9%)	0·63
Mother's age (years)			0·36
<20	7 (8·6%)	46 (14·1%)	..
21–25	20 (24·7%)	87 (26·6%)	..
26–30	24 (29·6%)	95 (29·1%)	..
>30	31 (37·8%)	99 (30·3%)	..
Father's profession			0·42
Farmer	43 (53·1%)	192 (58·7%)	..
Storekeeper	20 (24·7%)	69 (21·1%)	..
Other	19 (23·2%)	66 (20·2%)	..
Number of people living in the household	7·3 (3·1)	6·2 (2·5)	0·001
Number of children per mother	5·3 (2·4)	4·3 (2·5)	0·001
Sibling chronological order			0·02
First position	8 (9·8%)	78 (23·8%)	..
Second position	15 (18·3%)	73 (22·3%)	..
Third position	15 (18·3%)	48 (14·7%)	..
Fourth–fifth position	19 (23·2%)	65 (19·9%)	..
>Fifth position	25 (30·4%)	63 (19·3%)	..
Clinical variables			
History of malaria			0·00026†
No	3 (3·7%)	45 (13·8%)	..
Yes (>3 months before)	79 (96·3%)	282 (86·2%)	..
Yes (in the past 3 months)	33 (43·4%)	76 (23·2%)	..
Natural immunity or vaccination against measles			0·08
No	52 (63·4%)	235 (71·9%)	..
Yes	30 (36·6%)	92 (28·1%)	..
History of measles in the past 6 months			0·81
No	80 (97·6%)	320 (97·9%)	..
Yes	2 (2·4%)	7 (2·1%)	..
Fever, respiratory disease, or diarrhoea in the past 3 months			<0·0001
No	38 (46·3%)	216 (66·1%)	..
Yes	44 (53·7%)	111 (33·9%)	..
Nutritional status			
Mid-upper-arm circumference (cm)*	12·9 (2·7)	15·1 (4·9)	<0·0001
Height-for-age *Z* score	–2·5 (1·8)	–0·9 (1·8)	<0·0001
Categories of height-for-age *Z* score			<0·0001
No stunting	29 (37·2%)	236 (72·2%)	..
Moderate stunting (between –3 SD and –2 SD)	19 (24·4%)	49 (15·0%)	<0·0001
Severe stunting (less than –3 SD)	30 (38·5%)	42 (12·8%)	<0·0001
Weight-for-age *Z* score	–5·2 (3·5)	–2·7 (1·9)	<0·0001
Weight-for-height *Z* score	–4·3 (3·2)	–3·5 (2·9)	<0·0005
Categories of weight-for-height *Z* score			<0·0001
No wasting	34 (41·5%)	151 (46·2%)	..
Moderate wasting (between –3 SD and –2 SD)	14 (17·1%)	101 (30·9%)	0·61
Severe wasting (less than –3 SD)	34 (41·5%)	75 (22·9%)	0·001

Data are mean (SD) or number (%). *Provided by conditional logistic regression. †Comparison between history of malaria in the past 3 months and no crisis or malaria more than 3 months before noma.

Table 1: **Sociodemographic and clinical characteristics of children with noma compared with controls**

to a set of 271 probes. Additionally, we included probes specific to various Archaebacteria and eight negative control probes, resulting in a final set of 335 probes. Oligonucleotide probes were synthesised with a C6-linker with free primary amine and spotted on ArrayStrip microarrays (Clondiag GmbH, Jena, Germany). We processed microarrays with proprietary reagents according to the manufacturer's instructions. We established signal intensities with IconoClust software (Clondiag GmbH). For every spot, the extinction signal of local background was subtracted from the intensity value. Array signals were first adjusted to normalise the DNA quantity. Each array was then normalised against the eight negative control probes and subjected to quantile normalisation with the Partek Genomic Suite 6.4.

Statistical analysis

The sample size was calculated to detect a 2·5-fold increase in the odds of development of noma in the presence of one specific predictor with 5% significance and 80% power. The calculation showed that 80 cases, each with four matched controls, would be necessary to show such an association.[19] We assumed that the main risk factor would be present in 60% of controls and that the correlation coefficient for exposure between matched cases and controls would be 0·45.

To assess independent risk factors, we included all variables associated with an increased likelihood of noma in univariate analysis at a significance level of a p=0·25 in a multivariate, conditional logistic regression model with a forward stepwise procedure. We estimated the percentage of variance in the outcome explained by the model by calculation of a pseudo-R^2 value.

Microarray data were also analysed by conditional logistic regression. We compared diseased with healthy sites in cases, healthy sites in cases with controls, and diseased sites in cases with controls. We corrected for multiple testing with the false discovery rate method;[20] all p values less than 0·0238 were regarded as significant. The 132 phylotypes with significant p values led to 396 conditional logistic regression models. Bacterial genera that were significantly associated with case-control status at conditional logistic regression were analysed and tested for log linearity. Since log linearity was not respected, we used categorical variables, based on the quintiles of their distribution for each bacteria. Variables retained in the two previous steps were included in the final conditional logistic regression model. Because of the low number of events per variable,[21] we presented a parsimonious model including only significant variables associated with the case status plus age that was kept in the model. All clinical and epidemiological variables and microbiological data from the healthy site of noma cases significantly associated with noma status were kept in the final model. All tests were two-tailed and p values less than 0·05 were regarded as significant. We used Stata (version intercooled 12.0) for all analyses.

	Cases (n=82)	Controls (n=327)	p value*
Nutritional habits			
Breastfeeding duration (months)	20·2 (2·6)	20·7 (2·4)	0·01
Children still breastfed			0·34
Yes	3 (3·7%)	7 (2·1%)	..
No	79 (96·3%)	320 (97·9%)	..
Daily consumption of millet	82 (100·0%)	327 (100·0%)	0·99
Daily consumption of sorghum	80 (97·6%)	324 (99·1%)	0·21
Daily consumption of rice	79 (96·3%)	323 (98·8%)	0·99
Daily consumption of beans	79 (96·3%)	327 (100·0%)	0·99
Daily consumption of milk (animal origin)	16 (19·5%)	94 (28·8%)	0·04
Meat consumption			0·38
Daily†	9 (11·1%)	29 (8·9%)	..
Weekly	52 (64·2%)	227 (69·9%)	0·45
Monthly	20 (24·7%)	69 (21·2%)	0·76
Fish consumption			0·02
Daily or weekly†	33 (40·3%)	102 (31·3%)	..
Monthly	24 (29·3%)	140 (42·9%)	0·01
Never	25 (30·5%)	84 (25·8%)	0·63
Fruit consumption			0·35
Daily†	13 (16·5%)	46 (14·3%)	..
Weekly	49 (62·0%)	214 (66·7%)	0·50
Monthly	17 (21·5%)	61 (19·0%)	0·73
Vegetable consumption			0·37
Daily†	16 (20·0%)	62 (19·2%)	..
Weekly	54 (67·5%)	201 (62·2%)	0·84
Monthly	10 (12·5%)	60 (18·6%)	0·27
Recent nutritional habits (within the past month)			
Number of meals per day	3·72 (0·58)	3·52 (0·52)	0·004
Number of meals per day within the past month	2·57 (0·89)	3·50 (0·56)	<0·0001
Direct contact with animals			
Donkey			0·89
Yes	16 (19·5%)	62 (19·0%)	..
No	66 (80·5%)	264 (81·0%)	..
Goat			0·84
Yes	63 (76·8%)	247 (75·8%)	..
No	19 (23·2%)	79 (24·2%)	..
Cow			0·45
Yes	27 (32·9%)	118 (36·2%)	..
No	55 (67·1%)	208 (63·8%)	..
Sheep			0·04
Yes	26 (31·7%)	139 (42·6%)	..
No	56 (68·3%)	187 (57·4%)	..
Horse			0·15
Yes	8 (9·8%)	45 (13·8%)	..
No	74 (90·2%)	281 (86·2%)	..
Dog			0·48
Yes	2 (2·4%)	11 (3·4%)	..
No	80 (97·6%)	315 (96·6%)	..
Cat			0·11
Yes	2 (2·4%)	20 (6·1%)	..
No	80 (97·6%)	306 (93·9%)	..
Chicken			0·005
Yes	27 (32·9%)	154 (47·2%)	..
No	55 (67·1%)	172 (52·8%)	..
Guinea fowl			0·99
Yes	1 (1·2%)	19 (5·8%)	..
No	81 (98·8%)	307 (94·2%)	..
Duck			0·57
Yes	1 (1·2%)	2 (0·6%)	..
No	81 (98·8%)	324 (99·4%)	..
Camel			0·99
Yes	0 (0·0%)	4 (1·2%)	..
No	82 (100·0%)	322 (98·8%)	..
Rat			0·16
Yes	5 (6·1%)	12 (3·7%)	..
No	77 (93·9%)	314 (96·3%)	..

Data are mean (SD) or number (%). *Provided by conditional logistic regression. †Denotes reference groups in conditional logistic regression.

Table 2: **Nutritional habits and environmental life conditions of children with noma compared with controls**

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

We recruited 85 noma cases and 327 controls. Three cases were excluded for administrative reasons, resulting in 82 eligible cases. For one case, only three controls were matched. Overall, seven deaths (8·5%) were attributable to noma; one other child died 1 year after noma diagnosis because of malaria complications. All children received adequate antibiotics (amoxicillin plus metronidazole) immediately after diagnosis and biological specimen sampling. Figure 2 shows the epidemic curve of new noma cases during the study period. Cases were identified from all areas of the Zinder region (figure 3).

Table 1 shows the baseline characteristics of the study population. Most cases were recruited at the Sentinelles centre for noma care after referral from a dispensary (n=28, 34·1%) or a hospital (n=22, 26·8%), or were brought in by their family (n=26, 31·7%). Six (7·3%) cases were identified in remote villages during follow-up visits to children who had previously been operated on. Mean delay to consultation at the centre was 8·1 days (SD 12·6; median 3 days, range 0–50). 20 patients (24·4%) had already received antibiotics for more than 24 h, and

	Cases	Controls	p value*
Viral serology	n=61–69	n=239–317	
Serology for cytomegalovirus, IgM			0·53
Positive	17/61 (27·9%)	50/239 (20·9%)	..
Negative	44/61 (72·1%)	189/239 (79·1%)	..
Serology for cytomegalovirus, IgG			0·91
Positive	60/61 (98·4%)	246/250 (98·4%)	..
Negative	1/61 (1·6%)	4/250 (1·6%)	..
Serology for Epstein–Barr virus, IgM			0·74
Positive	4/62 (6·6%)	12/266 (4·5%)	..
Negative	58/62 (93·6%)	254/266 (95·5%)	..
Serology for Epstein–Barr virus, IgG			0·11
Positive	58/63 (92·1%)	263/276 (95·3%)	..
Negative	5/63 (7·9%)	13/276 (4·7%)	..
Serology for herpes simplex virus, IgM			0·54
Positive	1/62 (1·6%)	3/317 (1·0%)	..
Negative	61/62 (98·4%)	314/317 (99·0%)	..
Serology for herpes simplex virus, IgG			0·93
Positive	54/69 (81·8%)	229/292 (78·5%)	..
Negative	12/69 (18·8%)	63/292 (21·5%)	..
Serology for varicella zoster virus, IgM			0·97
Positive	2/65 (3·1%)	8/283 (2·8%)	..
Negative	63/65 (96·9%)	275/283 (97·2%)	..
Serology for varicella zoster virus, IgG			0·60
Positive	7/62 (11·3%)	39/278 (14·0%)	..
Negative	55/62 (88·7%)	239/278 (86·0%)	..
Serology for measles, IgM			0·23
Positive	3/61 (4·9%)	5/288 (1·7%)	..
Negative	58/61 (95·1%)	283/288 (98·3%)	..
Serology for measles, IgG			0·19
Positive	39/62 (62·9%)	145/252 (57·5%)	..
Negative	23/62 (37·1%)	107/252 (42·5%)	..
Mucosal swabs	n=78	n=265	
Cytomegalovirus PCR			0·99/0·99
Oral presence of cytomegalovirus (healthy:lesion side)	1:0	0	..
Absence of oral presence of cytomegalovirus (healthy:lesion side)	78:77	265	..
Other viruses PCR			0·99/0·19
Oral presence (healthy:lesion side)	0:3†	2‡	..
Absence of oral presence (healthy:lesion side)	77:75	261	..
Vitamins	n=20	n=114	
Vitamin A concentration (µmol/L)	0·82 (0·53)	0·51 (0·38)	0·005
Vitamin E concentration (µmol/L)	0·80 (2·57)	0·41 (1·71)	0·50

Data are n/N (%) or mean (SD) unless otherwise indicated. *Provided by conditional logistic regression. †Adenovirus (n=1), herpes simplex virus 1 (n=1), and Coxsackie virus (n=1). ‡Herpes simplex virus 1 (n=1) and poliovirus 1 (n=1).

Table 3: **Biological and viral variables in children with noma compared with controls**

33 (40·2%) had been exposed to traditional medicine procedures before seeking medical attention.

At clinical presentation, 24 cases (29·3%) had a body temperature of 38·5°C or higher, 73 (89·0%) enlarged submandibular lymph nodes, six (7·3%) general oedema, and five (6·1%) ascites. Locally, cases presented with facial oedema (n=45, 54·9%) or initial necrosis (n=38, 46·3%), with external (n=22, 26·8%) or buccal (n=68, 82·9%) bone exposure, or both. 75 cases (91·5%) had local pain. At oral assessment, 77 cases (93·9%) presented with halitosis and 61 (75·4%) with pseudomembranes. 68 (82·9%) showed signs of gingivitis, including 45 (54·9%) with spontaneous gingival bleeding. 60 cases (73·1%) showed loss of tips of interdental gingival papilla and 35 (42·7%) presented with other oral lesions. Table 2 shows a comparison of the nutritional habits and environmental life conditions between cases and controls.

Because of poor health conditions and dehydration, blood samples from only 20 cases (24·4%) and 114 controls (34·9%) were available for measurement of vitamin concentrations (table 3). We recorded very low serum concentrations of vitamins A and E (<1 µmol/L) in both groups. Antiviral antibody concentrations for different viruses could be measured in samples for 61–69 cases (74·4–84·1%) and 239–317 controls (73·1–96·9%; table 3). We recorded no significant difference between cases and controls in terms of recent or past immunisation against measles (IgG positivity <65% in both groups) or other viral diseases (table 3). Mucosal swab testing did not identify notable acute viral illness in cases or controls (table 3).

On the basis of complete clinical epidemiological data available for 407 children (table 4), severe wasting and stunting, a high number of past pregnancies in the mother, the presence of febrile illness in the past 3 months, and the absence of chickens at home were significant risk factors for noma disease. The proportion of variance explained by the model was 27%.

After exclusion of 20 cases who received antibiotics or whose specimens deteriorated, we processed 117 microbial samples from noma cases (59 from diseased sites and 58 from healthy sites) and 235 from controls. Each specimen yielded a maximum of 132 different results corresponding to the number of phylotypes sought by low-density 16s rDNA array.[22] Of 396 comparisons, 185 (46·7%) provided significant differences with use of the predetermined false discovery rate cutoff. We used data from the healthy sites of cases and obtained complete data for 291 observations (59 cases and 232 controls), which were similar in all characteristics to the original dataset of 409 children (82 cases and 327 controls; data not shown). By multivariate analysis, we identified five bacterial genera that were significantly associated with case status compared with controls: *Capnocytophaga*, *Fusobacterium*, *Neisseria*, *Prevotella*, and *Spirochaeta* (table 5). When we introduced microbiological variables into the clinicoepidemiological model (table 6), severe stunting and a higher number of past pregnancies in the mother were independently associated with noma, together with microbiological predictors of disease: a reduced proportion of *Spirochaeta*, *Fusobacterium*, *Capnocytophaga*, and *Neisseria* in the oral microbiota, but an increased proportion of *Prevotella*. The proportion of variance explained by the model was 42%.

Discussion

Our findings provide new insight into the understanding of the risk factors for noma disease and take into account sociodemographic, clinical, biological, and microbiological factors that are potentially involved in its pathogenesis (panel).[1,11,23] Disease predictors include severe malnutrition, recent respiratory or diarrhoeal syndrome, the number of previous pregnancies in the mother, the absence of chickens at home, and an altered oral microbiota compared with controls.

Malnutrition was very apparent in our study population. Importantly, both present and past malnutrition was a risk factor for noma.[24] By comparing each case to four matched controls, we showed that affected children were more undernourished than were controls. In Niger, chickens are a sign of wealth and their absence in the case environment and the presence of a higher number of people in the household are surrogate indicators of poverty that might explain the degree of acute and chronic malnutrition of these children. Investigators of some studies[13,25] have reported that noma could be related to a higher number of previous pregnancies in the mother. In sub-Saharan Africa, pregnant and lactating women are especially at risk of maternal malnutrition related to poor nutritional quality and short interpregnancy intervals.[26] Frequent pregnancies often cause maternal anaemia, resulting in an increased risk of low-birthweight babies.[11,13]

Debilitating diseases, especially malaria and measles, have been described as possible contributory factors for noma.[4,24] We noted that the risk of noma increased in the presence of a recent febrile respiratory or digestive illness, which again suggests that affected children had a worse health status than did controls. Measles could be one of the most important risk factors for noma because of the accompanying immunosuppression.[24,25] Our data showed that immunity against measles, either through vaccination or previous disease, was insufficient in the overall study population, and exposure to measles was not associated with an increased risk of disease. Herpes viruses, particularly cytomegalovirus or Epstein-Barr virus, could trigger the evolution of a pre-existing gingivitis towards acute necrotising gingivitis and noma.[11,12,27] Although we tested for the presence of herpes in mucosal swabs, our results seemed to exclude this hypothesis, but we could not exclude a type 2 error or the existence of a viral pathway that we did not explore in our study.

In addition to the many epidemiological facets of the disease, noma has always been regarded as a disease of bacterial origin because of the smell of the lesion, its rapid evolution, and possible response to broad-spectrum antibiotic treatment.[1,11,12,28] Apart from findings from early studies[29,30] that reported the presence of spirochaetes and fusiform organisms via dark field microscopy, recent efforts to prove a primary microbiological cause have been unsuccessful because of difficulties in accessing and assessing acute cases[31] and the unavailability of modern laboratory techniques in the countries where noma is present. Microbiota associated with noma lesions were studied in a few patients with anaerobic culturing techniques.[32] Microorganisms identified as *Fusobacterium necrophorum* and *Prevotella intermedia* on the basis of phenotypic criteria were recovered from seven and six of the eight sampled noma lesions, respectively. In our study, we used advanced molecular techniques and included only acute noma cases to investigate potential causative microorganisms. Microarrays offer the dual advantage of assessment of the possible presence of a

	Univariate model		Multivariate model*	
	Odds ratio (95% CI)	p value	Odds ratio (95% CI)	p value
Severe stunting (HAZ<–3 SD)	5·22 (2·73–9·97)	<0·0001	4·87 (2·35–10·09)	<0·0001
Severe wasting (WHZ<–3 SD)	3·01 (1·66–5·43)	0·00027	2·45 (1·25–4·83)	0·009
Number of mother's past pregnancies	1·19 (1·08–1·32)	0·0005	1·16 (1·04–1·31)	0·01
Respiratory disease, diarrhoea, or fever in the past 3 months	3·52 (1·89–6·54)	<0·0001	2·70 (1·35–5·40)	0·005
Absence of chickens at home	2·53 (1·32–4·82)	0·005	1·90 (0·93–3·88)	0·08
Age at diagnosis (years)	0·46 (0·29–0·72)	0·001	0·54 (0·34–0·87)	0·01

HAZ=height-for-age *Z* score. WHZ=weight-for-height *Z* score. *Odds ratio from a multivariate conditional logistic regression model (n=407). The pseudo-R^2 of the multivariate model was 0·27.

Table 4: **Risk factors for noma disease identified in children included in the study (univariate and multivariate models without microbiological data)**

	Odds ratio* (95% CI)	p value
Capnocytophaga genus		0·002
≥25th percentile	1·00	..
<25th percentile	4·02 (1·68–9·64)	..
Fusobacterium genus		0·003
≥75th percentile	1·00	..
<75th percentile	4·52 (1·69–12·10)	..
Neisseria genus		0·005
≥75th percentile	1·00	..
<75th percentile	4·50 (1·57–12·89)	..
Prevotella genus		0·007
<50th percentile	1·00	..
≥50th percentile	3·15 (1·37–7·20)	..
Spirochaeta genus		<0·000385
≥75th percentile	1·00	..
<75th percentile	10·61 (2·88–39·07)	..

Percentiles refer to the quantile of distribution of the signal obtained from the microarrays, which provide the relative quantity of each specific genus in the sample tested. *Odds ratio from a multivariate conditional logistic regression model (n=291). The pseudo-R^2 of the model was 0·35.

Table 5: **Bacterial genera associated with noma in children enrolled in the study**

	Univariate model		Multivariate model*	
	Odds ratio (95% CI)	p value	Odds ratio (95% CI)	p value
Severe stunting (HAZ< –3 SD)	6·28 (2·79–14·15)	<0·0001	2·86 (1·05–7·76)	0·04
Number of mother's past pregnancies	1·18 (1·05–1·33)	0·004	1·21 (1·04–1·40)	0·01
Age at diagnosis (years)	0·41 (0·23–0·71)	0·002	0·72 (0·38–1·37)	0·317
Capnocytophaga genus				
<25th percentile (*vs* ≥25th percentile)	6·42 (3·19–12·92)	<0·0001	3·69 (1·48–9·17)	0·005
Fusobacterium genus				
<75th percentile (*vs* ≥75th percentile)	1·97 (0·91–4·27)	0·084	4·63 (1·61–13·35)	0·005
Prevotella genus				
≥50th percentile (*vs* <50th percentile)	3·35 (1·78–6·27)	0·000169	2·53 (1·07–5·98)	0·035
Neisseria genus				
<75th percentile (*vs* ≥75th percentile)	5·55 (2·54–12·13)	<0·0001	3·24 (1·10–9·55)	0·033
Spirochaeta genus				
<75th percentile (*vs* ≥75th percentile)	3·20 (1·20–8·54)	0·02	7·77 (2·12–28·42)	0·002

HAZ=height-for-age *Z* score. *Odds ratio from a multivariate conditional logistic regression model (n=291). Age was kept in the model (even if non-significant). The pseudo-R^2 of the multivariate model was 0·42.

Table 6: **Risk factors for noma disease identified in children included in the study (univariate and multivariate models including microbiological data)**

Panel: Research in context

Systematic review

We searched PubMed and Medline for papers published in French and English between Jan 1, 1969 and Oct 1, 2012, with the terms "noma", "cancrum oris", "acute necrotizing gingivitis/acute necrotizing ulcerative gingivitis and noma", "acute necrotizing gingivitis/acute necrotizing ulcerative gingivitis and malnutrition", "acute necrotizing gingivitis/acute necrotizing ulcerative gingivitis and herpes viruses/herpesviridae", and "Fusobacterium necrophorum". We also searched the reference lists of identified articles for further relevant papers and the personal libraries of the authors (DB-M, BP-C, AM, DM, JS, and DP). Previous studies of risk factors for noma reported the importance of malnutrition and debilitating diseases, such as malaria or measles. However, no investigators have done a case-control study that includes only acute cases and control children from the same region and at the same time. Moreover, none of the previous studies presented a formal sample size estimation, which suggests that these studies might have had inadequate power to detect a specific association with some bacteria or other risk factors. Of note, early studies suggested that bacteria had a crucial role in noma pathogenesis, but most had a small sample size and included patients with non-acute noma. Finally, previous analyses used only microscopy and culture techniques that were standard at the time of the study.

Interpretation

To the best of our knowledge, this study is the first to assess both epidemiological risk factors and microbiology with a case-control design. From a practical perspective, our data confirm the importance of pre-existing malnutrition and the role of poverty in children with noma. Our results showed no involvement of specific bacteria in the cause of the disease. Overall, these data suggest new areas of research and draw attention to potential new primary preventive strategies to be developed for the disease.

large panel of phylotypes and quantification of their relative proportion in the subgingival microbiota. Until now, conventional microbiological techniques could have led to an underestimation of the amount of microbiological diversity.

We noted significant independent associations between noma and microbiological species, including a trend towards an increased risk of disease when the *Fusobacterium* genus was under-represented. *F necrophorum*, a commensal found in the gut of herbivores and a cause of necrotising infections in animals, might contaminate livestock and potentially infect children.[11,32] *F necrophorum* was found in seven of eight advanced noma lesions in a previous report[31] and has long been regarded as one of the putative pathogens of noma.[31,32] However, the ten phylotypes of *Fusobacterium* genus recorded in our study belong to the *Fusobacterium nucleatum* complex (data not shown). Other representatives of the Fusobacteriales family tended to be associated with healthy sites. Thus, the intriguing hypothesis that *F necrophorum* could act as a trigger organism was not confirmed in our study with molecular techniques. Rather, we noted an alteration of the oral microbiota of noma cases, with a reduced amount of *Fusobacterium* genus. By contrast, *P intermedia*, a pathogen identified frequently in adult periodontitis,[32] was clearly associated with noma. In previous studies, investigators have reported its presence and its putative role in noma lesions;[11,32,33] it might play a part by promoting tissue destruction through lipid and protein degradation.[31] *P intermedia* produces IgA1 proteases that could be crucial in changing mouth mucosal immunity,[34,35] but this species is not recognised as a mono-infecting agent.[36,37] Noma cases also had a lower proportion of *Capnocytophaga* spp, *Neisseria* spp, and *Spirochaeta* spp than did controls. In a study in which investigators compared the microbial composition of supragingival and subgingival plaque of patients with periodontitis, supragingival samples had statistically significantly higher counts of *Neisseria mucosa*, *Capnocytophaga ochracea*, and *Capnocytophaga sputigena* than did the subgingival samples, whereas subgingival samples, taken from the same teeth, had statistically significantly higher counts of *Prevotella nigrescens* and *P intermedia* than did supragingival samples.[38] This difference might represent an imbalance in the microbiota, with a composition more closely related to the subgingival microbiota in periodontal diseases that promotes the overgrowth of opportunistic pathogens. Importantly, we did not identify any specific causative pathogen for noma disease with low-density microarrays. However, for technical reasons and density issues, the microarray analysis is dedicated only to bacteria and some Archaea, but did not detect fungi or parasites. Of note, neither fungi nor parasites have been suspected in the pathogenesis of noma disease.[1,11]

Our study has some limitations. First, we did not strictly respect age matching between cases and controls (ie, we attempted to match controls to their cases in terms of age, but globally we found that matched controls were significantly older than cases)

but we adjusted for this problem in all multivariate models. Second, we attempted blood and other biological sampling only once, but it was impossible in some cases because of children's poor health status and a high occurrence of dehydration. We excluded 28% of observations in the global model because of missing data for microbiological variables. However, a comparison of the total study population with the subset of 291 children without any missing data showed that they shared similar characteristics (data not shown), which strongly suggests the absence of selection bias. Third, the inherent bias common to all case-control studies (mainly recall bias, retrospective design, and temporality, because risk factors were assessed after the development of noma) is certainly present in our study, especially in the assessment of nutritional habits and environmental life factors. However, noma is a rare disease and the case-control approach used was the most appropriate design to study such a disorder.

We are confident that these findings could be extrapolated to a larger population of children in Niger and surrounding countries. In terms of sociodemographic and clinical characteristics, our study population was representative of children with noma in sub-Saharan Africa. Cases were managed according to WHO recommendations, thus making our findings generalisable to the overall at-risk population.

This study does not provide evidence for the existence of one bacterial pathogen as a cause for the disease, including previous suspects, but the results do strongly suggest that an altered oral microbiota increases the risk of disease, independent of sociodemographic and environmental factors. Our findings show that the eradication of noma disease needs concerted efforts to alleviate poverty, promote improved nutrition of both pregnant women and infants, and help to teach parents to recognise early signs of the disease. The 2012 report on the progress of the UN Millennium Development Goals emphasises that the target of reducing extreme poverty has been reached 5 years ahead of the 2015 deadline in Niger.[39] Despite this achievement, an urgent need remains to associate health campaigns aimed at improving oral care with existing successful strategies against malnutrition in sub-Saharan countries where noma is especially prevalent.[1,9,11]

Contributors

DB-M, AG-A, SH, and DP had responsibility for the overall study design, management, conduct, analysis, and writing of the paper. DB-M, AG-A, SH, and DP were involved in the interpretation, synthesis, and writing of the paper. DB-M, BP-C, DM, AG, J-EB, and DP were involved in the field visits and site study monitoring. PF, AH, and JS were responsible for the development of microbiological techniques. DBM, AM, PF, AH, JS, AG-A, and DP contributed to the data analysis and interpretation, and writing. The Geneva Study Group on Noma conceived and organised the study and provided a forum for discussion throughout the analysis. All authors reviewed and approved the final version.

Conflicts of interest

We declare that we have no conflicts of interest.

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