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Full Length Research Paper

Risk factors for Buruli ulcer in Côte d'Ivoire: Results of a case-control study, August 2001

Laurence Ahoua[1,2*], Aka N. Guetta[1], Euloge Ekaza[1], Samir Bouzid[1], Raymond N'Guessan[1] and Mireille Dosso[1]

[1]Bacteriological and virological laboratory, Institut Pasteur, 01 B. P. 490, Abidjan Côte d'Ivoire.
[2]Epicentre, 8 rue Saint Sabin 75011 Paris, France.

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A case-control study was carried out in 3 highly endemic regions of Côte d'Ivoire to study risk factors for Buruli ulcer. A case was defined as a Buruli ulcer occurring less than one year before the date of survey, resident in one of the regions investigated and there was no history of Buruli ulcer illness. Controls were selected from the general population by a two stage cluster sampling method. A total of 116 cases and 116 controls were included. For the cases, the male/female sex ratio was 0.84, the median age was 19.5 years and 40.5% were children ≤ 15 years. Biological results were obtained for 86 (74%) cases using skin exudate samples. Positive rates were 22.0, 22.1 and 27.9% respectively for smear examination, culture and PCR IS*2404*, respectively. After adjusting for possible confounders, no history of BCG vaccination (OR_a = 5.0, CI 1.7 - 14.3), presence of a case ≤15 years (OR_a = 8.3, CI 2.8 - 24.1), having a river/lake/dam near the housing (OR_a = 4.4, CI 1.6 - 12.2) and the type of place for fishing (p = 0.001) were associated with illness. Young children and women having daily water related activities were most at risk. Swab samples were not sensitive enough for Buruli ulcer diagnosis. There is an urgent need for a rapid field test to diagnosis Buruli Ulcer as PCR IS*2404* remains expensive for most of the endemic countries.

Key words: Ulcer, *Mycobacterium ulcerans*, Côte d'Ivoire, case-control study, risk factors.

INTRODUCTION

Buruli ulcer (BU) disease is the third most frequently reported mycobacterium infection in the world after tuberculosis and leprosis diseases (WHO, 2003). Endemics zones are located in rural areas in tropical and sub-tropical regions were BU occurs in cluster villages near swamps and slow flowing water sites. The reservoir of *Mycobacterium ulcerans* is environmental and several hypothesis on probable origin of the mycobacteria were described (Ross et al., 1997; Sizaire et al., 2006; WHO, 2003; van der Werf et al., 2005). In Côte d'Ivoire, BU is the second most frequent mycobacteriosis infection after tuberculosis (Kanga and Kacou, 2001) and number of cases is increasing each year. Between 1991 and 1994, 2246 cases of BU were diagnosed in the country. In1995, 5000 cases were reported in the south region of the country, near the tropical rain forest regions (No authors listed, 2003). In 1997, a national survey had reported 10 382 cases with an estimated prevalence of 0.32 per 1000 persons (Kanga and Kacou, 2001). The regions of Bouake and Daloa, in the Centre and West, are particularly affected. In 1998, the prevalence rate reached 22% in some of the villages (Kanga and Kacou, 2001). Because of its environmental reservoir, populations with outdoor activities are the most at risk in these endemic regions. Few studies have described risk factors for BU in African settings (Aiga et al., 2004; Gbery et al., 1996; Marston et al., 1995; Raghunathan et al., 2005). Results remained controversial and a great number of hypothesis are still under discussion, especially for the reservoir and the mode of transmission of the infection to humans

*Corresponding author. E-mail: epimail@epicentre.msf.org or lahoua@epicentre.msf.org. Phone: + 331-421-2849; Fax: + 331-40-21-28-03.

(Duker et al., 2006; Stienstra et al., 2001).

The prevalence rate and the number of newly reported endemic areas are constantly increasing each year, as reported by the National Program for Buruli ulcer (Kanga et al., 2006). The objective of this study was to identify individual and water-related risk factors for BU, in 2001, in three highly endemic regions of Côte d'Ivoire.

MATERIAL AND METHODS

A case-control study was conducted in August 2001 in the 3 following regions: the Bandama Valley region in the centre, the High-Sassandra region in the mid-west and the 18 Mountains region in the west.

Case definition

A case of BU was defined based on the World Health Organization clinical definition (Asiedu et al., 2003): presence of a node, papule, plaque or oedema developing into a painless ulcer with undermined edge that leads to an extensive scarring with deformity without appropriate therapy. A case of BU was selected for the study if the three following criteria were present; presence of a clinical BU occurring less than one year before the date of survey, village of residency included in one of the 3 endemic regions investigated, and no history of BU illness. Cases were selected in the in-patients and out-patients departments of BU treatment centres or at their homes. Controls were selected in the general population by a two stage cluster sampling method (Dodge and Romig, 1959; Leme-show and Stroh, 1988). A control was included in the study, if he/she had neither history nor current diagnosis of clinical BU infection. Cases and their controls were matched for sex, age and village of residency. An oral consent was obtained in presence of an eyewitness for each participants included in the study. Interviews, clinical examination and specimen collection were performed after obtaining oral consent in presence of an eye witness.

Laboratory examination

For the BU cases at the ulcerative stage of the disease, two laboratory tests were done to confirm clinical diagnosis. Two swabs of skin lesion were withdrawn for sampling. One sample was used to perform a direct smear microscopy examination of the skin exudate using the Zielh-Neelsen staining method. A positive result was defined as presence of acid-fast bacilli (AFB +) on the smear sample. The second sample was preserved in a cryotube containing 2 ml of cetylpyridium chloride (CPC) for culture and polymerase chain reaction (PCR). All samples were kept at +4°C for trans-port and than stored at –80°C until culture or PCR were performed. Culture of *M. ulcerans* was made using the standard method on solid media (Lowenstein–Jensen medium). The inoculated media was examined every week for a total incubation period of 9 weeks. The growth temperature was maintained between 30 and 33°C and lecture of the sample was done every 6 days on an overall period of 9 months. A positive result was defined by the detection of growth of *M. ulcerans* on the solid media. A (Single PCR) technique using the IS*2404* sequence of *M. ulcerans* was performed for DNA amplification. This sequence was chosen because of its high sensibility and specificity to *M. ulcerans* (Stinear et al., 1999; Guimaraes-Peres et al., 1999). A positive result was defined as presence of the IS*2404* sequence in the amplified products.

Statistical analysis

The risk factors were assessed for socio-demographic characteristics, BCG vaccine status and water related activities (type of water site near the house, daily activities, etc). All cases of BU were included for analysis regardless of the laboratory results (intention-to treat analysis). A conditional logistic regression analysis was performed to assess association between occurrence of illness and risk factors. For the univariate analysis, we calculated an odds ratio (OR) for matched series and its Cornfield 95% confidence intervals (CI) for each risk factor. The significant p-value of each OR was calculated using the McNemar χ^2 test. Only risk factors with a p-value ≤ 0.20 were selected for the multivariate analysis. A backward stepwise procedure using Hosmer and Lemeshow method was performed to examine important risk associations while adjusting for possible confounders (Hosmer and Lemeshow, 1989a). The paired variables such as sex, age and village of residency, were kept into the final model. The Hosmer-Lemeshow goodness of fit test was applied to evaluate the fit of the model (Bouyer et al., 1995, Hosmer and Lemeshow, 1989b).

Statistical analysis was made using Epi-Info version 6.04d (CDC, Atlanta, USA) and SPSS version 10.0 (SPSS Inc. Chicago, Illinois, USA) software.This study was approved by the Institutional Ethical Committee of Pasteur Institute and by the National Program against Mycobacterium ulcers (Programme National de lutte contre les Ulcères à Mycobactéries, PNUM) of Côte d'Ivoire.

RESULTS

Socio-demographic characteristics of the BU cases

Among the 116 cases of BU included in the study, 47 (40.5%) were from the Bandama valley region, 31 (26.7%) were from the 18 Mountains region and 38 (32.8%) from the High-Sassandra region (Figure 1).

The male/female sex ratio was 0.84 (53/63). Median age was 19.5 years (range: 0 to 71 years) and 47 (40.5%) were children under 15 years of age. The preponderant nationality was Ivorian (86/116, 74.1%). The main daily occupation was agricultural activity (59/116, 50.8%). Twenty nine (25%) cases did not have a profession.

Clinical and biological outcomes

On onset of symptoms, the most frequently reported clinical forms were oedema (54/116, 47%) and node associated to oedema (44/116, 38%). Of all cases, 102 (88%) had associated signs such as general signs (asthenia, fever or loss of weight) or visceral signs (diarrhoea, cough). A mean number of 1.5 lesions were diagnosed per patient. For 79 (71%) of the cases, the principal lesion was an ulcer with budged and irregular edges with no coating or granulation. The limbs were the most frequent location with a clear predominance of the lesions on the lower limbs (79/114, 69%) (Table 1). The other localisations reported were the abdomen/thorax (6 cases), the rear end/perinea (4 cases) and the head/

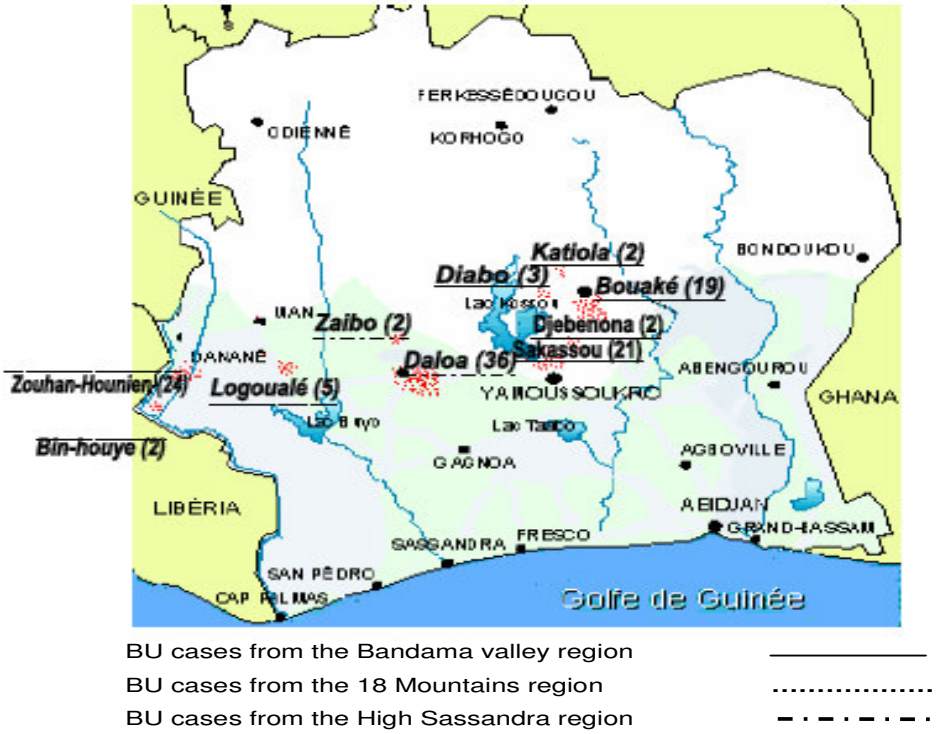

Figure 1. Geographical distribution of Buruli ulcer cases, Côte d'Ivoire, August 2001 (N = 116).

neck (2 cases). For biological analysis, smear examination was performed for 59 cases. Culture and PCR examination was performed for 86 cases. Positive rates were 22.0, 22.1 and 27.9%, respectively, for smear examination, culture and PCR IS*2404* (Table 1). In addition, 6 samples were negative for culture and positive for PCR.

Disease progression and treatment outcomes

The median length of illness was 4.2 months (range: 5 days to 13 months). The delay for diagnosis was 2.2 months (range: 1 day to 13 months) and the median time to medical treatment was 4 days (range: 0 to 4 months) (Table 2).

The first type of health care sought were the traditional practitioner (82/116, 71%), the health centres (26/116, 22%) and auto-medication (8/116, 7%). Among all cases, 83% (94/113) had no complications, 16% (18/113) had an associated bacterial infection and 1% (1/113) had signs of malnutrition. At the time of the survey, 50% (57/113) had an extension of the lesion, 37% (41/113) had a stabilized lesion and 13% (15/113) the type of disease progression were significantly different if the patient previously used traditional medicine or not (χ^2 test, p = 0.20 and p = 0.69, respectively).

Risk factors for Buruli ulcer

A total number of 116 cases and 116 matched controls were included in the analysis. There was no difference between cases and controls in socio-demographic characteristics: age, sex and village of residency (Table 3). In the univariate analysis, individual factors associated with illness were having no history of BCG vaccination (OR = 2.4, CI 1.4 - 4.3), being of another nationality than Ivorian (OR = 5.0, CI 1.1 - 22.8), being a child not attending school (OR = 3.3, CI 1.2 - 10.0), having a BU ase 15 years of age or less in the circle of relatives (ORc = 5.6, CI 1.6 - 19.3) and having a direct contact with this case (OR = 4.0, CI 1.1 - 14.2). In addition, residency in another region on onset of symptoms was also associated with a higher risk of illness (OR = 5.5, CI 1.2 - 24.8).

In this case, the regions cited were known to be in the univariate analysis were wearing pants or boots, the number of BU cases in endemic for BU disease: High-Sassandra, the 18 mountains, Middle-Cavally, Low-Cavally and the South lagoon regions. Individual factors not associated with illness the circle of relatives, were cured (Table 2). Neither the type of complication nor the type of relation shared with the BU cases and being a family case (Table 4). Two water related risk factors were found to be significantly associated with illness: a river, a lake

Table 1. Clinical and biological characteristics of the BU cases, Côte d'Ivoire, August 2001 (N = 116).

Parameter	Value
Total number of cases	116
Clinical form at start* n (%):	
Œdema	54 (47)
Node and oedema	44 (38)
Node and/or papule	14 (12)
Other forms	4 (3)
Associated signs[ζ] n (%):	102 (88)
General signs (asthenia, fever or loss of weight)	99 (85)
Visceral signs (diarrhoea, cough)	39 (34)
Mean number/person [CI]	1.5 [1.3 –1. 7]
Principal lesion[ξ] n (%) :	
Ulcer	79 (71)
Node/papula	13 (12)
Œdema	7 (6)
Other	12 (11)
Adjoining secondary lesions n (%)	28 (24)
Distant secondary lesions n (%)	7 (6)
Locations of the lesions[‡] n (%):	
Inferior limbs	79 (69)
Superior limbs	28 (25)
Other	7 (6)
Positive *M. ulcerans* rate n (%):	
Zielh-Neelsen (59 cases analysed)	13 (22.0)
Culture (86 cases analysed)	19 (22.1)
PCR IS*2404* (86 cases analysed)	24 (27.9)

*Only the most frequent clinical forms are described, the other forms may be associated. [ζ]A case can have more than one associated sign. [ξ]5 principal lesions not reported. [‡]2 locations not reported. A case can have more than one location.

or a dam located near the housing compared to a pump, a well or a swamp (OR = 2.6, CI 1.4 - 4.8) and the type of water source used for cooking or drinking (Likelihood Ratio χ^2 test, p = 0.03). Water related risk factors not associated with illness were the distance from the housing to the water point, the type of water source used for washing and laundry, swimming, type of place for fishing, type of farming and using rain water for farming (Table 5). Because of missing data, 57 cases and 57 controls were included in the multivariate analysis. When adjusting for possible confounders, no history of BCG vaccination (OR_a = 5.0, CI 1.7 - 14.3), presence of a BU cases 15 years of age or less (OR_a = 8.3, CI 2.8 - 24.1), having a river, a lake or a dam near the housing (OR_a = 4.4, CI 1.6 - 12.2) and the type of place for fishing (Likelihood Ratio χ^2 test, p = 0.001) were significantly associated with illness (Table 6). The final multivariate model was tested for goodness of fit, using the Hosmer-Lemeshow goodness of fit χ^2 test. There was no statistical difference between the observed and predicted values (χ^2 =14.03; 8 ddl; p = 0.08) and 81% of the values were correctly classified by the model.

DISCUSSION

In this study, cases were more frequently young women and children less than 15 years of age. Culture was the main adult activity and children were more frequently not attending school and going along with their parents to work in the field. Clinical aspects were characteristic consisting in 1 to 2 ulcers located in the lower limbs for more than 70% of the cases. Most of the patients accessed professional health care at a late stage of the disease, preferring to be initially treated by a traditional practitioner. However, the disease progress and frequency of complications did not significantly differ whether the BU case was previously treated by a traditional practi-

Table 2. Evolution and treatment outcomes of the BU cases, Côte d'Ivoire, August 2001 (N = 116).

Parameter	Value
Total number of cases	116
Median length of illness (range)	4.2 months (5 days to 13 months)
Median diagnosis delay (range)	2.2 months (1 days to 13 months)
Median time to treatment (range)	4 days (0 to 4 months)
First type of health care sought n (%):	
traditional practitioner	82 (71)
the health centre	26 (22)
auto-medication	8 (7)
Treatment received n (%)*:	
medicinal plants	89 (77)
antibiotic	17 (15)
surgery	6 (5)
None	3 (3)
Complications n (%)ζ:	
none	94 (83)
sur-infection	18 (16)
Undernourished	1(1)
Disease progression n (%)ζ:	
extension	57 (50)
stabilisation	41 (37)
Cured	15 (13)

*One treatment not reported. ζ 3 cases had the type of complication and the type of disease progression not reported.

Table 3. Socio-demographic characteristics of cases and controls, (n = 232) Côte d'Ivoire, August 2001.

Socio-demographic characteristics	Bandama valley (Bouake region)			18 Mountains (Man region)			High-Sassandra (Daloa region)		
	Cases	Controls	p	Cases	Controls	p	Cases	Controls	p
n	47	47	-	31	31	-	38	38	-
Sex ratio (Male/Female)	0.95	0.96	1.0	0.72	0.72	1.0	0.81	0.80	1.0
Age (years): median (range)	20 (4-70)	20 (4-70)	0.8	22 (2-71)	23 (1-76)	0.8	15 (0-57)	15 (0-58)	0.8
Ivorian nationality n (%)	40 (85)	43 (92)	0.3	30 (97)	31 (100)	0.3	30 (79)	34 (89)	0.2
Usual activity									
Farming	22 (47)	17 (37)	0.6	17 (55)	12 (39)	0.3	20 (53)	14 (37)	0.5
Child attending school	6 (13)	10 (21)		6 (19)	10 (32)		7 (18)	12 (32)	
Child not attending school	8(17)	10 (21)		5 (16)	3 (10)		9 (24)	10 (26)	
Other	11 (23)	10 (21)		3 (10)	6 (19)		2 (5)	2 (5)	

tioner or not. The positive rates for smear examination and culture were similar (22%). Whereas the PCR IS*2404*, had a 28% positive rate and was more sensitive in detecting positive samples than culture. In our study, the risk factors associated with illness were having no history of BCG vaccination, having a BU case 15 years of age or less in the circle of relatives, living near a river, a lake or a dam and the type of place for fishing. However, there are some limits to our study. The coding system used for collecting data for the variables related to the type of water source, was based on an added coding (that is, 1, 2, 4, 8, 16, etc…) where the combination of two

Table 4. Individual risks factors associated with Buruli ulcer matched for age, sex and village of residency, univariate analysis. Côte d'Ivoire, August 2001 (N = 232).

Variable	Cases (n = 116)	Controls (n = 116)	OR	95%CI
History of BCG vaccination				
Yes	62 (53)	88 (76)	Ref	
No	54 (47)	28 (24)	2.4	1.4-4.3
Nationality				
Ivorian	100 (86)	108 (93)	Ref	
Other	16 (14)	8 (7)	5.0	1.1-22.8
Wearing boots				
Always	4 (3)	9 (8)	Ref	
Sometimes or never	112 (97)	107 (92)	2.2	0.7-7.3
Wearing pants				
Always	14 (12)	12 (10)	Ref	
Sometimes or never	102 (88)	104 (90)	0.8	0.3 – 2.2
Attending school				
Yes	19 (16)	32 (28)	Ref	
No	97 (84)	84 (72)	3.3	1.2-10.0
Residency in another region on onset of symptoms				
No	105 (91)	114 (98)	Ref	
Yes	11 (9)	2 (2)	5.5	1.2-24.8
BU case in the circle of relatives :				
Number of BU cases in the circle of relatives				
No case	51 (44)	46 (40)	Ref	
>=1 case	65 (56)	70 (60)	0.7	0.4-1.5
Age of the BU case in the circle of relatives				
>15 years	9 (16)	34 (49)	Ref	
≤15 years	49 (84)	36 (54)	5.6	1.6-19.3
Type of contact with the BU case				
Indirect	47 (72)	59 (85)	Ref	
Direct	18 (28)	10 (16)	4.0	1.1-14.2
Type of relation with the BU case				
No contact	50 (44)	46 (40)	Ref	
House/meal	38 (33)	29 (25)	1.1	0.5-2.4
Yard	5 (4)	16 (14)	0.3	0.1-0.9
Village/other	22 (19)	23 (21)	0.7	0.3-1.7
Family case				
No	20 (31)	30 (44)	Ref	
Yes	45 (70)	39 (56)	1.8	0.8-4.0

answers or more was possible. Consequently we could not accurately discriminate between 2 answers.

The association between the type of water source and illness was improperly estimated with a probable under-estimation of the odd ratios. Matching for sex, age and place of residency, had the advantage of controlling for certain potential confounders that could have influenced the association between the independent risk factors and the disease. Nevertheless, when some risk factors are closely related to the matched variables, a confounding bias may appear. In our study, the location of residency was closely linked to the population's life style and their daily activities. Consequently, location of residency was a confounder for the water related risk factors leading to a probable underestimation of the odd ratios of these particular expositions. Finally, we did not assess the risks factors for the subgroup of PCR positive skin exudate BU cases (n = 24). Because of the too few outcome events

Table 5. Water related risks factors associated with Buruli ulcer matched for age, sex and village of residency, univariate analysis. Côte d'Ivoire, August 2001 (N = 232).

Variable	Cases (n=116)	Controls (n=116)	OR	95%CI
Type of water point near the housing				
Pump/well/swamp	41 (37)	65 (56)	Ref	
River/Lake/dam	71 (63)	51 (44)	2.6	1.4-4.8
Distance to the water point				
>500m	31 (28)	27 (23)	Ref	
<=500m	81 (72)	89 (77)	0.7	0.3-1.5
Water source for cooking/drinking*				
Tap water/pump	35 (30)	20 (17)	Ref	
Well	33 (28)	36 (31)	0.3	0.1 -0.8
Swamp	35 (30)	50 (43)	0.2	0.1-0.6
River/lake/dam	13 (12)	10 (9)	0.5	0.1-1.5
Water source for washing				
Tap water/pump	38 (33)	31 (27)	Ref	
Well	37 (32)	31 (27)	1.2	0.4-3.0
Swamp	24 (21)	41 (35)	0.3	0.1-0.9
River/lake/dam	17 (14)	13 (11)	0.9	0.3-2.7
Water source for laundry				
Pump/well	66 (57)	52 (45)	Ref	
Swamp	24 (21)	34 (29)	0.4	0.2-0.9
River	20 (17)	21 (18)	0.6	0.2-1.6
Other	6 (5)	9 (8)	0.5	0.2-1.6
Place for drying laundry				
Other	33 (29)	42 (38)	Ref	
Clothes line	82 (71)	69 (62)	1.5	0.8-2.9
Swimming				
No	79 (68)	78 (67)	Ref	
Yes	37 (32)	38 (33)	0.9	0.5-1.8
Place for fishing				
No fishing	73 (63)	66 (57)	Ref	
Swamp	13 (11)	7 (6)	1.2	0.4-3.5
River/lake/dam	30 (26)	43 (37)	0.5	0.2-1.1
Type of farming				
No farming	7 (6)	9 (8)	Ref	
Farming without watering	12 (10)	10 (9)	1.9	0.4-9.4
Farming with watering	36 (31)	25 (21)	2.1	0.5-8.1
Farming with irrigation	61 (53)	72 (62)	0.7	0.2-3.1
Using rain water for farming				
No	37 (34)	46 (43)	Ref	
Yes	72 (66)	61 (57)	1.6	0.8-3.5

*Likelihood Ratio χ^2 test, p = 0.03.

compared to the too many independent variables to be analysed. The risk estimates would have been unreliable with a possibility of over fitting the model (Concato et al., 1993).

In endemic regions of West Africa, the majority of BU cases live in rural zones near natural waterways where access to tap water is uncommon (Amofah et al., 1993; Berliat, 1999). Socio-demographic findings in our study

Table 6. Risks factors of Buruli ulcer matched for sex, age and village of residency. Multivariate analysis, final model. Côte d'Ivoire, August 2001 (N = 114).

Variable	OR_a	95%CI
History of BCG vaccination		
Yes	Ref	
No	5.0	1.7-14.3
Age of the BU case in the circle of relatives :		
>15 years	Ref	Ref
≤15 years	8.3	2.8-24.1
Type of water point near the housing :		
Swamp/pump/well	Ref	-
River/lake/dam	4.4	1.6-12.2
Place for fishing		
No fishing	Ref	-
Swamp	2.8	0.2-34.2
River/lake/dam	0.1	0.03-0.4
Age (1- year increments)	1.0	0.97-1.03
Sex		
Male	Ref	-
Female	0.7	0.3-1.9
Region of residency		
Bouake	Ref	
Man	1.5	0.3-8.1
Daloa	1.6	0.6-4.9

OR_a = Adjusted Odds ratio; 95%CI = 95% Confidence Interval.

are similar to the ones described in other studies in Côte d'Ivoire (Marston et al., 1995; Berliat, 1999; Kanga and Kacou, 2001). Similarly, clinical aspects are typical consisting, in 60 to 69% of the cases, in an ulcer of the lower limbs (Berliat, 1999; Darie et al., 1993; Kanga and Kacou, 2001; Songne et al., 2001). The treatment cost of surgery is too expensive and hospitalisation is time consuming for the people living in poor-resource settings. Thus, traditional medicine is frequently employed in African rural regions (Guedenom et al., 1995; Médecins, 1997; Barthelme et al., 2001; Aka N'Guetta, 2001; No authors listed, 2002).

In endemic regions, a positive smear examination and the presence of *M. ulcerans* growth in culture, confirms the clinical diagnosis of BU. Quality of the laboratory results vary greatly according to the type of biological sample and the method used for their analysis. Studies in Côte d'Ivoire have shown that positive smear rates for the AFB+ range from 13 to 16% in chronic lesions and increased over 35% if the skin exudate samples when withdrawn from more recent lesions not treated by local medication (Darie et al, 1993; Ehuie, 2000; N'Guessan et al., 2001; Ekaza, 2004). In our study, the positive smear rate was 22%. More than two third of the cases had applied traditional medicine on their lesions which may have reduced the chances of obtaining a superior rate. These findings suggest that skin exudate microscopy is not a good laboratory examination for the diagnosis of BU because of the weak sensibility of the method. *M. ulcerans* culture is time consuming, varying from 6 weeks to 6 months and results are various (Palomino and Portaels, 1998). The positive culture rate found in our study (22%) is similar to the ones described elsewhere, were positive culture rates of clinical suspected lesions ranged from 9 to 34% (Guimaraes-Peres et al., 1999).

The PCR for diagnosing *M. ulcerans* infection is frequently used in industrialized countries, giving UB positive rates raging from 19 to 31% in smear samples and more than 80% in skin biopsies (Guimaraes-Peres et al., 1999; Lagarrigue et al., 2000; Aka N'Guetta, 2001). This method has also allowed to identify different geographical sub-groups of *M. ulcerans* (Chemlal et al., 2001a,b), and in 1997, PCR IS*2404* had identified for the first time in environmental water samples (Ross et al., 1997; Stinear et al., 2000). In our study, the PCR IS*2404* had a positive rate of 28% and was more sensitive in detecting *M. ulcerans* than culture. Even if the PCR method is a good tool for laboratory diagnosis (Ekaza et al., 2004), it still remains expensive for most of the limited resource countries. Heavy equipment with adequate

facilities, qualified and trained personnel are practical issues that are mandatory for such settings. A rapid diagnostic tool, easy to use in the field, would be urgently needed to help diagnose and treat in its earlier stage the mycobacterial infection. Findings on individual risk factors for illness are still under debate. In Uganda in 1967, the presence of a positive Tuberculin test or an history of BCG vaccination was significantly associated with a decrease in BU incidence (No authors listed, 1969). In the early 90's, this association was not con-firmed even though more than a third of the cases had a history of BCG vaccination (Amofah et al., 1993). Our study confirms the association between BCG vaccination and illness, where the risk of illness is five times higher if there is no history of BCG vaccination. BCG immunization has shown to play a cross-reactive protective role against UB disease (Coutanceau et al., 2006). The presence of a mycobacterial antigen 85 (Ag85A), found in *M. ulcerans* and in the tuberculosis vaccine *Mycobacterium bovis* BCG, can significantly reduce the bacterial load in the footpads of *M. ulcerans* infected mice (Tanghe et al., 2001). Family cases are infrequent and no significant association has yet been found between BU and belonging to the same family (Smith, 1970). Our results confirm these findings. However, a BU case in the circle of relatives of 15 years or less was at increased risk of illness. A direct contamination, therefore, still remains infrequent (Berliat, 1999; Marsollier et al., 2002). In 1995, wearing pants, a shirt or boots while farming was shown to be protective against illness (Marston et al., 1995; Raghunathan et al., 2005). In our study, we did not find a significant protection by wearing protective clothes.

Several hypotheses on the mode of transmission have been given (Wansbrough-Jones and Phillips, 2006). A previous skin trauma on site of the lesion does not seem necessary for the inoculation of the germ (Meyers et al., 1974). Potential vector, such as aquatic bugs from the Belostomadiae, Naucoris and Diplonychus types, could play a role in the transmission of the germ and represent a passive reservoir of *M. ulcerans*. The penetration of the germ would be favoured by a cuteanous breach by bite or sting of these insects (Portaels et al., 1999; Songne et al., 2001; Marsollier et al. 2002; Marsollier et al., 2003). Individual immune susceptibility, such as Th1 type immune response, may also play a protective role against onset of symptoms among exposed persons to *M. ulcerans* (Stienstra et al., 2001; Gooding et al., 2002). We found children to have more frequently Buruli ulcer infection than adults, probably because of the less mature immune system and their craze to play in swampy areas (Gooding et al., 2002).

Results on the association between illness and factors related to daily activities, such as farming, fishing and swimming have given various results elsewhere (Aiga et al., 2004; Marston et al., 1995). The only water related risk factors we found to be associated to BU, was the type of place for fishing. These findings demonstrate that water related risk factors and daily activities are most probably indirectly related to illness. It is more of an environmental change (e.g. artificial lake or dam construction), or a recent modification in the ecosystem (longer rainy seasons, increased humidity), that enabled the creation of a favourable environment for the growth of *M. ulcerans*. Daily activities in rural areas are mostly performed outdoors and time of exposition to the germ is longer which increases the risk of infection, regardless of the mode of contamination. Further epidemiological and environmental research is warranted not only to identify potential vectors of *M. ulcerans*, but also to better understand their role in the transmission of BU disease to humans. Correlating the geographical distribution of the BU cases and the environmental location of these vectors' shelters could help better understanding its mode of transmission. Appearing in the late 80's in Côte d'Ivoire, BU disease is spreading rapidly in new endemic areas and represents a real Public Health threat to the rural population touching preferentially young children and women. A simple field test for diagnosing *M. ulcerans* in Buruli lesions is still lacking.

The access to treatment still remains unaffordable to most of the patients. The social and economical impacts are matchless, with a great risk of dropping out of school or a desocialization for those suffering of amputations or limb deformities. Health education should aim the women and children. It should promote individual protective measures to fight against individual risk factors, more specifically for those having daily water related activities.

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