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Current epidemiology of human plague in Madagascar

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ABSTRACT – From 1996 to 1998, 5 965 patients with suspected plague were identified in 38 districts of Madagascar (40% of the total population are exposed). Using standard bacteriology, 917 of them were confirmed or presumptive (C + P) cases. However, more than 2 000 plague cases could be estimated using F1 antigen assay. Two out of the 711 *Yersinia pestis* isolates tested were resistant to chloramphenicol and to ampicillin (both isolates found in the harbour of Mahajanga). Urban plague (Mahajanga harbour and Antananarivo city) accounted for 37.4% of the C + P cases. Bubonic plague represented 97.2% of the cases, and the lethality rate was still high (20%). In comparing the exposed population, plague was more prevalent in males (M:F sex ratio 1.3:1) and patients under 20 years (2.7% babies under two years). Buboes were mainly localised in the inguinal/femoral regions (55.8%). The epidemiological risk factors are discussed. © 2000 Éditions scientifiques et médicales Elsevier SAS

plague / *Yersinia pestis* / **epidemiology** / **lethality** / **resistance** / **Madagascar**

1. Introduction

Plague is one of the three quarantine diseases to be notified to the World Health Organisation (WHO). This zoonotic infection is caused by *Yersinia pestis* and transmitted among natural reservoirs by fleas. Humans are occasional hosts in the natural cycle of the disease. Bubonic plague, the classical form of the illness, is characterised by the swelling of local lymph nodes, which may occur following a bite by an infective flea. It may evolve into a highly infectious pneumonic form that can be spread from person to person through airborne droplets. The various aspects of plague infection have been reviewed recently [1].

More than 20 countries, mainly in Africa, report human plague cases to the WHO [2]. Madagascar and Tanzania would appear to be the most active plague foci. Indeed, the exact world situation of plague is not known since the epidemiological surveillance system and the laboratory capabilities vary from country to country.

Plague was originally introduced into Madagascar in 1898, during the last third pandemic. The history of its subsequent spread from the ports to the central highlands has been well described [3–6]. One century after its arrival, plague is still an acute public health problem that justifies an active national control program [7, 8]. Surveillance of the human cases and field research on the diagnosis and natural cycles have been intensified in recent years. This paper describes the trend and the current epidemiological situation of human plague in Madagascar from 1996 to 1998 from a file of 6 000 suspected and 1 000 confirmed cases.

2. Materials and methods

2.1. Patients and clinical samples

The Plague National Control Program recommends the treatment of all plague-suspected patients with streptomycin, and the collection of a clinical sample for retrospective biological confirmation. The following information is recorded for each patient: address, age, sex, clinical symptoms, date of onset, date of clinical diagnosis, epidemiological context (contact with plague patients or dead rodents).

From 1 January 1996 to 31 December 1998, 5 965 suspected cases were declared in five out of the six provinces of Madagascar: 98.8% of the patients were identified in the three provinces of Antananarivo, Fianarantsoa, and

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Table I. Bacteriological results of suspected plague patients declared in Madagascar from 1996 to 1998.

Year	*Bacteriological results – total (%)*				
	Confirmed[a]	Presumptive[b]	Negative	Not tested[c]	
1996	173	56	1068	332	1629 (27.3)
1997	384	75	2117	287	2863 (48)
1998	154	75	1016	228	1473 (24.7)
Total (%)	711 (11.9)	206 (3.4)	4201 (70.4)	847 (14.2)	5965 (100)

[a]*Y. pestis* positive; [b]microscopy positive; [c]no clinical sample available.

Mahajanga for a total of 38 districts. The population exposed to plague is estimated to be 5–6 million inhabitants (40% of the total population).

The notification forms and the clinical samples absorbed on a swab and transported in Cary Blair medium (bubo aspirate, sputum or postmortem liver or lung puncture) were sent to the Central Plague Laboratory (Institut Pasteur, Antananarivo). Biological results were available for only 85.8% (5 119) of the identified patients. Due to poor communications in Madagascar, the mean delay between the date of collection of the samples and the date of their arrival at the laboratory was 27.5 days.

2.2. Bacteriology

The bacteriology was the reference confirmatory test used, as recommended by WHO. The smears were stained (Gram or Wayson staining) and examined by microscopy. The bubo aspirates were extracted from the swab using 1 mL of sterile saline and cultured on YCIN media for 48 h at 28 °C. An aliquot was also injected into two mice. Suspected colonies of *Y. pestis* were confirmed using the specific phage test and commercialised biochemical microtests [9]. The remaining saline suspension was stored at -20 °C for F1 antigen test.

It was considered that plague was confirmed (C) in a patient when *Y. pestis* was isolated, was presumptive (P) when the culture was negative but the microscopy was positive; and was negative (N) when both tests were negative.

All the *Y. pestis* isolates were screened for their in vitro resistance to streptomycin, tetracycline, gentamycin, chloramphenicol, and sulfamethoxazole-trimethoprime, which are classically used for plague treatment, and to ampicillin, a large-spectrum drug against Gram-negative and Gram-positive bacteria frequently used to treat adenitis.

2.3. F1 antigen detection

To estimate the proportion of true plague patients overlooked by the reference bacteriological methods, 689 suspected samples collected from January to March 1997 were also tested for F1 antigen, using an immunocapture ELISA assay developed and kindly provided by the United States Naval Medical Research Institute (Bethesda, Maryland, USA). This assay was performed as previously described [10].

2.4. Statistical analysis

The data were managed using ACCESS 6.2 (Microsoft) and EPIINFO (CDC, Atlanta) software. The chi-square test with the Yates's correction when applicable, or the Fisher's exact test were used to compare percentages. The F test was used to compare the mean values. The census data of the population in 1996, was used to compare class age and sex of plague patients.

3. Results

3.1. Distribution of confirmed and presumptive plague patients

Using bacteriology as a confirmatory method *(table I)*, the total number of C + P patients was 917 (15.3% of the declared patients or 17.9% of the analysed patients). The characteristics of the suspected and C + P groups of patients are represented in *table II*. The sex ratio, median age and proportion of bubonic to pneumonic forms were not different between the two groups. About 80% of the cases were identified in the central highlands above 800 m altitude, and mainly in remote villages (68.9%). Urban plague was identified in two cities: the capital, Antananarivo (1 400 m altitude), and the coastal harbour of Mahajanga, where 11.5 and 19.6% respectively, of suspected cases were declared. Urban cases represented 37.4% of the C + P cases.

Figure 1 represents the trend of human plague in Madagascar from 1982 to 1998. After a quiet period from the '50s to the '70s, the disease steadily increased during the '80s, and remained stable during the '90s (the mean number of C + P cases was 230).

From 1996 to 1998, plague was confirmed in 30 districts of which 28 are located in the central highlands. The ten most prevalent districts in the country are shown in

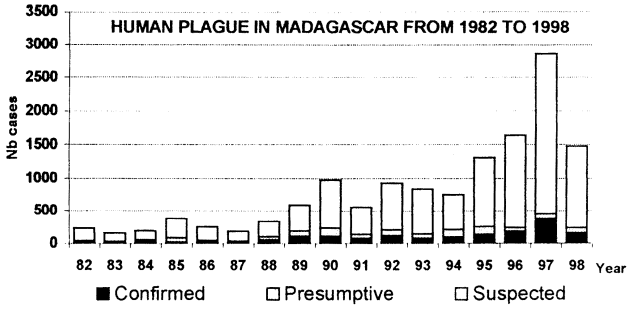

Figure 1. Confirmed and presumptive plague cases in Madagascar from 1980 to 1998, using bacteriology as reference method.

Table II. Characteristics of plague suspected and confirmed/presumptive (C + P) patients.

Characteristics	*Suspected patients*	*Confirmed/presumptive patients*
	Number = 5965	Number = 917
Male/Female (Sex Ratio)	3372 / 2593 (1.29: 1)	515 / 402 (1.28: 1)
Median age (25–75 percentile)	12 (6–20) years	15 (8–26) years
Bubonic/Pneumonic cases	5729 / 85 (151 unknown)	870 / 25 (22 unknown)
Highlands (%)	4794 (80.4)	652 (71.1)
Antananarivo city	*686 (11.5)*	*78 (8.5)*
Rural plague	*4108 (68.9)*	*574 (62.6)*
Mahajanga harbour (%)	1171 (19.6)	265 (28.9)
Number of districts concerned	38	30

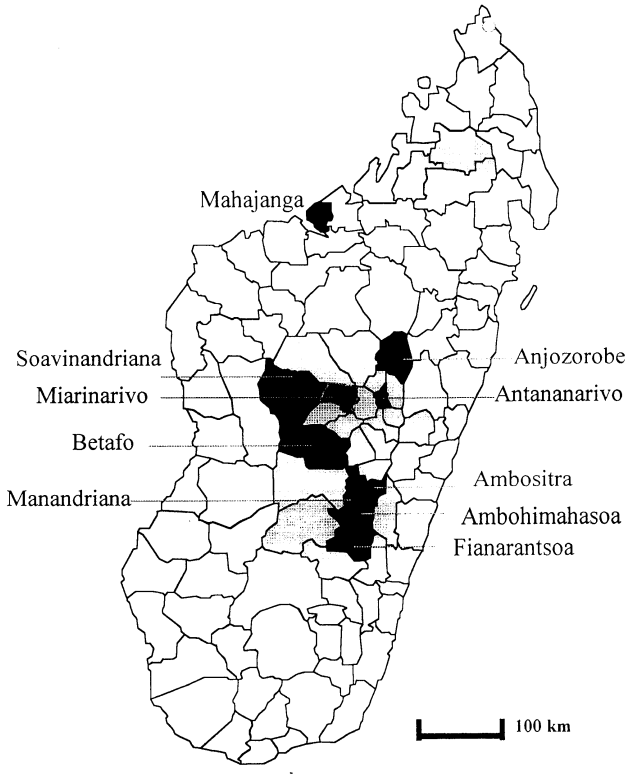

Figure 2. Geographical localisation of the ten most active plague districts in Madagascar from 1996 to 1998. The plague endemic districts are shown in grey and the ten most active districts in black.

figure 2: they accounted for 79.8% (732) of C + P cases during the period of study. The harbour of Mahajanga was by far the most active district (28.8%), followed by Ambositra (11%), central Antananarivo city (7%), and Betafo (6%) districts. Plague reemerged in October 98 in the district of Ikongo (southeast boundary of the endemic zone), after three decades of quiescence.

Actually, the number of plague cases was higher than that confirmed by bacteriology, as demonstrated by the detection of F1 antigen *(table III)*: 27.3% of the negative samples were F1 antigen positive. This data would suggest that 1 146 true patients were misdiagnosed among the 4 201 negative patients of table I. It should be noted that 35.5 and 34.2%, respectively, of the C + P cases were, surprisingly, F1 antigen negative. The diffusion of the F1 antigen out of the swab into the Cary Blair agar was demonstrated by extracting the agar.

3.2. Drug sensitivity of clinical *Y. pestis* isolates

All of the 711 isolates of *Y. pestis* were sensitive to the six antibiotics tested, with the exception of one strain resistant to ampicillin, and a second one to chloramphenicol. Both isolates were found in the Mahajanga harbour, where annual outbreaks of bubonic plague occur.

3.3. Season and periodicity

The monthly cumulative numbers of C + P cases are represented in *figure 3*. In the highlands, bubonic and pneumonic plague seasons extended from October to April, during the rainy tropical summer season. In Mahajanga harbour, where only bubonic plague was observed, the season extended from July to November during the dryer and cooler season on the west coast.

Table III. F1 antigen immunocapture assay on suspected clinical samples, according to the bacteriological results.

F1 Antigen ELISA	*Bacteriology*		
	Confirmed [a]	Presumptive [b]	Negative [c]
Positive (%)	129 (64.5)	23 (65.7)	124 (27.3)
Negative (%)	71 (35.5) [d]	12 (34.2) [d]	294 (64.7)
Total (%)	200 (100)	35 (100)	454 (100)

[a]*Y. pestis* culture positive; [b]*Y. pestis* culture negative, but microscopy positive; [c]culture and microscopy negative; [d]F1 antigen was finally found diffused in the Cary Blair agar, respectively, for 65/71 of the confirmed samples and 6/12 of the presumptive samples.

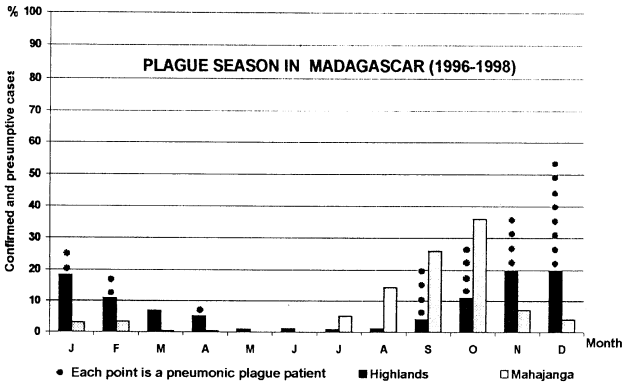

Figure 3. Bubonic and pneumonic plague seasons in the highlands and in Mahajanga harbour.

Although plague occurred annually in the same quarters in Antananarivo and Mahajanga, no clear periodicity could be defined in the rural villages.

3.4. Sex and age

In both the suspected and the C + P groups of patients, the frequency of the disease was higher in males than females ($P < 10^{-4}$) *(table II)*.

The distribution of C + P cases according to sex and age is shown in *table IV*. In comparing the whole population exposed to plague, the difference in age and sex concerned specifically children and young adults < 20 years old. When considering only age, the disease was significantly more prevalent in the age class 10–19 years ($P = 0.017$) and less prevalent in the > 50 years class age ($P < 0.01$).

3.4.1. Plague in babies

Of the 25 C + P babies aged ≤ two years (12 females and 13 males), 19 were *Y. pestis*-confirmed and 6 were presumptive for plague. Ten of these babies were declared in Mahajanga harbour. Of the four infants under six months, two were aged only one month (both culture-positive). The lethality rate in this group was 20%.

3.5. Clinical symptoms

Temperatures above 39 °C were observed in 72% of the C + P patients. Bubonic plague was by far the most common clinical form of the disease (97.2%). Pneumonic plague was observed only in the highlands and accounted for 2.8% of the cases (no difference between sex).

Buboes were usually unilateral, but exceptionally could be bilateral or multiple, and their size had no relation to severity of the disease. They were typically found in inguinal/femoral (55.8%), axilliary (20.3%) and

Table IV. Confirmed and presumptive (C + P) plague patients according to age and sex.

Age	*Confirmed or presumptive patients*		
	Total (%)	Female (%)	Male (%) [a]
0–09 years	264 (30.2)	102 (38.6)	162 (61.4)[b]
10–19 years [d]	268 (30.7)	109 (40.6)	159 (59.4)[c]
20–29 years	167 (19.1)	80 (47.9)	87 (52)
30–39 years	84 (9.6)	39 (46.4)	45 (53.6)
40–49 years	49 (5.6)	25 (51)	24 (49)
> 50 years [e]	42 (4.8)	24 (57)	18 (43)
Total (%)	874 [f] (100)	379 (43.4)	495 (56.6)

[a]global sex ratio male:female 1.3:1 ($P < 10^{-4}$); [b]plague more prevalent according to sex and age ($P = 4.\ 10^{-4}$); [c]plague more prevalent according to sex and age ($P = 2.10^{-3}$); [d]plague more prevalent in this class age ($P = 0.017$); [e]plague less prevalent in this class age ($P < 0.01$); [f]age unknown for 43 patients of the 917 C + P patients.

Table V. Localisation of adenitis in bubonic confirmed/presumptive (C + P) plague patients, according to age.

Age	*Bubo Localisation*					
	Inguinal/femoral	Axilliary	Cervical/ maxilliary	Others	Unknown	Total
0–9 years	124	61	57	5	13	260
0–2	*11*	*7*	*4*	*1*	*0*	*26*
3–5	*50*	*22*	*18*	*2*	*4*	*96*
6–9	*32*	*32*	*32*	*2*	*9*	*138*
10–19 years	144	49	34	8	25	260
20–29 years	108	27	12	3	12	162
30–39 years	46	19	5	2	9	81
40–49 years	27	9	4	0	5	45
> 50 years	23	7	2	0	5	37
Total (%)	472 (55.8)	172 (20.3)	114 13.5)	18 2.1)	69 (8.2)	845[a] (100)

[a] age unknown for 25 out of 870 bubonic plague patients.

Table VI. Lethality rate on plague suspected and plague confirmed/presumptive patients, according to the clinical form of the disease.

Clinical form	*Suspected patients*			*C + P patients*		
	Survivors (%)	Deaths (%)	Total (%)	Survivors (%)	Deaths (%)	Total (%)
Bubonic	5054 (94)	320 (6)	5374 (100)	707 (81.3)	163 (18.7)	870 (100)
Pneumonic	64 (78)	18 (22)	82 (100)	18 (72)	7 (28)	25 (100)
Unknown [a]	48 (43.6	62 (56.4)	110 (100)	8 (36.4)	14 (63.6)	22 (100)
Total (%)	5166 (92.8)	400 (7.1)	5566 (100)	733[b] (79.9)	184[c] (20)	917 (100)

[a]clinical form not reported (not clinically examined after death, or presumably septicaemic plague without visible symptoms) b: 323 females and 410 males c: 79 females and 105 males.

cervical/maxillary (13.5%) regions, and their localisation did not differ with sex, but did with age of the patients *(table V)*. The groin buboes were relatively less frequent, and the cervical/submaxillary buboes more frequent in the group of children under ten years than in other age classes ($P < 10^{-8}$).

3.6. Lethality

The lethality rate was 6.7% (400/5 965) of the suspected patients, and 20% (184/917) of the C + P patients *(table VI)*. It was higher in 1998 (25.3%, $P = 0.01$) than in 1996 (20.5%) or 1997 (17.2%), and did not differ according to the clinical form, the sex or the age of the patients. Patients with unknown clinical form had a higher lethality rate (63.6 versus 19%, $P < 10^{-5}$). This group may be composed of septicaemic cases or deaths that were notified but never examined by health personnel.

The confirmation rate was higher in mortal cases than in survivors (46 versus 13%, $P < 10^{-7}$).

4. Discussion

Plague was responsible in Madagascar for thousands of victims during the first decades of the 20th century [3-5]. No more cases were reported in the coastal area after 1927, until 1991, when a severe outbreak of bubonic plague occurred in the Mahajanga harbour, today the most active district of Madagascar [11]. Plague is a threat for 5–6 million inhabitants, including thoses in the capital Antananarivo [8]. The conditions that encouraged its reemergence at the end of the '70s, are multiple and complex. The global reasons are probably the dramatic reduction of the country's resources devoted to public hygiene, and the low socioeconomic conditions of the majority of the population.

The main objective of the Plague National Control Program in Madagascar is to reduce mortality and morbidity due to plague, based on clinical diagnosis and free treatment of all suspected cases, chemoprophylaxis of the contact population, and the control of fleas by spreading insecticide. Clinical over-diagnosis of patients being a routine situation, the retrospective biological confirmation of the disease is therefore highly justified. In most of the endemic countries, plague occurs in inaccessible areas where qualified personnel, material for collection of samples or laboratory means are scarce. In comparison to other countries, the situation in Madagascar is rather good since clinical samples could be obtained for 86% of the suspected patients. However, the excessively long delay to convey samples reduces the chance of isolating the plague bacilli. The global confirmation rate (confirmed and presumptive patients) was only 18% of the individuals tested, and varied from centre to centre according to the accuracy of clinical diagnosis and the operational difficulties. Using the F1 antigen capture assay, 27.3% of the culture-negative patients were actually true plague cases, suggesting that the number of confirmed cases should be theoretically increased by 1 146 cases, or even more, considering that samples were missing for 847 suspected patients. The difficulty of ensuring a reliable laboratory diagnosis of plague from field-collected samples is a concern in most of the plague endemic countries. Thus, it is difficult to appreciate the real magnitude of plague in the world.

Considering the plague season, notable differences exist between the highlands and the coastal city of Mahajanga. The seasonal prevalence and the periodicity of the disease are linked to many factors such as climatic conditions (atmospheric humidity), the variation of the total number of rats and of the proportion of immune-to-susceptible rats, as well as the variation in the average number of fleas per rat (flea index). There is a close correlation between the plague and the flea seasons, and the transmission of the infection from rat to man is conditional upon the number of infected fleas and living rats available at a given time. The influence of local factors should also be kept in mind, such as the movement of grain after harvest, leading to the migration of the rats with their fleas, the damage caused on rat shelters by heavy rains or the accumulation of filth.

In Madagascar, rats are the main epizootic responsible, and the recent involvement of the shrew *Suncus murinus* in Mahajanga harbour is still under investigation. The universal flea *Xenopsylla cheopis* is the indoor vector of plague in urban, as well as in rural zones, while the endemic flea *Synopsyllus fonquerniei* is the outdoor and rural vector in Madagascar. The abundance of the human flea and lice found in the population of the highlands raises the controversial question of their potential role in transmission of the disease [12, 13]. Although some positive results have been obtained in the laboratory with *Pulex irritans,* and transmission by these insects has been proven in Manchuria, the human flea is not taken into practical account as a whole. As a matter of fact, far more

outbreaks of bubonic plague would have been observed in Madagascar. The emergence of three new *Y. pestis* ribotypes in the Ambohimahasoa and Ambositra districts raises the question of the possible involvement of endemics in mammals and fleas in the selection of these new variants [14].

The use of antibiotics after the '50s has consistently contributed to the reduction in the frequency of pneumonic plague. Thus, conversely to what was observed at the beginning of the century, the worldwide most frequent clinical form of plague is now bubonic plague. In Madagascar, pneumonic plague is restricted to the highlands and is closely linked to the late diagnosis of bubonic plague cases. Its spread is favoured by local traditions (traditional healing, funeral ceremonies), and the poor social conditions of the population (impairment of general resistance of individuals, ignorance of hygiene measures, confinement in small and badly ventilated houses) [15].

We found plague more prevalent in males than females, in adolescents and young adults than in people over 50 years old. Most investigators agree that the differences in the age, sex, or ethnic groups of bubonic plague are only due to difference in exposure to infection, but not to intrinsic factors [16]. However, a random sample seroepidemiological survey in the population of the highlands, using anti-F1 antibody marker, suggested a higher susceptibility of males to develop the disease for a similar exposure [17]. Incidence of bubonic plague is usually low among young children up to five years old, probably because of less contact with infective fleas, rather than due to maternal protective antibodies [16]. In Madagascar, babies are subjected to the same risk factors as other members of the family (sleeping on the floor in a single room), explaining the relative high incidence of bubonic plague among babies ≤ two years [13].

Buboes are generally more frequent in the inguinal/femoral (55 to 70%) than axilliary (15 to 21%) or cervical/maxillary (5 to 20%) region [16]. However, in Madagascar, when considering children < ten years old, a relatively lower frequency of groin buboes, and a higher frequency of cervical/maxillary buboes were noticed.. The bubo localisation did not affect the prognosis of the disease in our study.

Despite the existence of efficient and cheap treatment, the world lethality rate still varies from 5 to 20%, depending on the country being considered. It is often related to misdiagnosis or late diagnosis of the first cases, combined with low access to medical care. In Madagascar, the high lethality rate is explained, at least partially, by the frequent occurrence of plague in remote villages. Efforts must be made to educate villagers to the plague symptoms and preventive measures, and to train health workers in case management in order to prevent the emergence of drug resistance [18, 19]. In 1995, a multiple high-level resistance to all antibiotics recommended for plague treatment and one streptomycin-resistant isolate have been detected in clinical samples. Both forms of resistance were carried by a self-transferable plasmid [20, 21]. Since the date of their discovery, a careful surveillance of all the *Y. pestis* isolates from human, rats and fleas was performed and no other isolates of these two types could be found locally or in other parts of Madagascar. It is probable that the site of the genetic transfer was not in the natural environment, but perhaps in the patient. However there is great concern about finding other forms of resistance and the risk of their spreading in the urban zones. During the last three years, monoresistant *Y. pestis* isolates were found in the Mahajanga harbour (one ampiciliin and one chloramphenicol clinical isolate) and in the capital Antananarivo (one tetracycline-resistant rat isolate and two ampicillin-resistant flea isolates). The genetic basis supporting the type of resistance is under investigation.

A reliable surveillance system and a good plague control program is obviously lacking in many countries. The current implementation of the WHO Integrated Disease Surveillance and the cooperation plan agreed upon by all the African countries are expected to improve the situation.

Acknowledgments

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