*Case Report*

# Sleeping sickness: The consequence of inappropriate diagnosis flow chart in case report

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**Sleeping sickness is a vector-borne parasitic disease with variable, non-specific and inconstant symptoms. Thus, clinical signs are insufficient for diagnosis. Definite diagnosis relies on evidence demonstration of trypanosome in body fluids through parasitological techniques. For reasons related to costs, workload and technical incapacities, these technics are not always performed and sleeping sickness examination is thus limited to serological and clinical investigations. We report a false alarm due to incorrect diagnosis about a sudden outbreak of sleeping sickness in a focus under control. This report shows that inadequate diagnosis methods can lead to downward and upward errors in the prevalence of a disease. There is a need to reinforce material and technical capacities of health centers in endemic areas.**

**Key words:** Sleeping sickness, diagnosis flow chart, case report.

## INTRODUCTION

Sleeping sickness or Human African trypanosomiasis (HAT) due to *T. b. gambiense* is a chronic disease with non-specifics, variables and inconstant symptoms (Dumas and Bisser, 1999; Burri and Brun, 2003). Only the enlargement of glands, specifically in the neck (Winterbottom's sign) observed in endemic area is the main sign of the infection. This sign appears at the early stage of the disease, but can remain unobserved. Thus, diagnosis of sleeping sickness relies on serological and parasitological tests (Cattand, 2001; Louis et al., 2001; Lejon and Büscher, 2002).

Diagnosis of the chronic form of sleeping sickness relies on the initial screening with the card agglutination test for trypanosomiasis, (CATT/*T. b. gambiense*) (Magnus et al., 1978) which is a cheap and quick test. Despite its good specificity, the positive predictive value (PPV) of the CATT on whole blood is limited because the test is used for mass screening of populations in which the prevalence of HAT rarely exceeds 5% (Truc et al., 2002; Jamonneau et al., 2000). The specificity of that serological test is further improved when performed on plasma or serum diluted to different concentrations (Van Meirvenne, 1999) but this is still insufficient. Thus, parasitological techniques, though having limited sensitivity (WHO, 1998; Ancelle et al., 1997), are absolutely essential for HAT diagnosis. Because of the common low parasitaemia (Chappuis et al., 2005), concentration technics such as micro hematocrit centrifugation (CTC) (Woo, 1970), or anion exchange chromatography (mAECT)

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**Abbreviations: CATT,** Card agglutination test for trypanosomiasis; **CSF,** cerebrospinal fluid; **CTC,** centrifugation en tube capillaire; **HAT,** human African trypanosomiasis; **MAECT,** mini anion exchange centrifugation technique; **NGO,** non-governmental organization; **PNLTHA,** programme National de lutte contre la trypanosomiase humaine Africaine; **PPV,** positive predictive value; **WHO,** world health organization.

(Lumsden et al., 1979) are recommended (WHO, 1998). These technics require appropriate materials, trained and qualified personnel.

A microscopic examination of aspirates from enlarged lymph nodes is also recommended as a low cost test with satisfactory results. For reasons related to costs, work-load and technical incapacities, these technics are not always performed in the field. The consequence is that downward and upward errors can be observed in the number of reported cases. We are reporting an example about a supposed outbreak of sleeping sickness in an endemic area under control in Chad.

## INVESTIGATION: CASE DETECTION

Moissala (8° 21' N, 17° 45' E) located in the southern region of Chad was known as a sleeping sickness focus under control, since no case was detected for more than 6 years and then, after a mass screening survey carried out in 2004 (National HAT control program (PNLTHA), unpublished). Surprisingly, by the end of year 2009, an outbreak of the disease was declared by a Non-Governmental Organization (NGO), locally implemented, with 182 patients passively diagnosed and treated since 2008.

Knowing that the number of patients diagnosed passively usually form a small proportion of the total cases declared in a focus (Legros et al., 2002), we concluded that there should be an epidemic and decided to put in place a control strategy including regular active case finding survey and vector control. With the help of the medical team of the NGO, we explored the area and built a map showing endemic villages (with declared patients) and villages at risk (Figure 1). Then, after information and education of local populations about the transmission of the disease and control strategy that will be put in place, a mass screening survey was carried out in all endemics villages declared by the NGO and loca-ted at the left side of the river. This survey was carried out by the end of the rainy season and endemic villages located at the right side of the river were still inaccessible.

The HAT diagnostic procedure in the field was performed according to the field algorithm established in the country since 2003 (Simarro et al., 2004; Louis et al., 2008) (Figure 2). Briefly, people were firstly screened using CATT test on whole blood, then direct examination of lymph node aspirate was performed on subjects positive with CATT on whole blood. CATT whole blood sero-positives were tested subsequently in CATT on serum at different dilutions and the end titre (highest dilution giving agglutination) was determined. Subjects negative with CATT on whole blood were released and did not undergo further microscopic examinations. Parasitological tests (CTC and mAECT) were only performed on subjects with CATT end titres ≥ 1:8. Patients (sleeping sickness cases) were subjects with trypanosomes seen by microscopy ("T+" cases) or subjects with CATT end titres ≥ 1:16 ("S+" cases).

As a patient is declared cured only when, within 2 years, no trypanosome is detected in body fluids and the cerebrospinal fluid (CSF) white blood cell count returned to normal (WHO, 1998), we also performed a follow-up of patients diagnosed within the last two years by the NGO. Their medical file was carefully inspected and they were asked to respond to a simple questionnaire (Table 1) concerning their medical care in the health center of the NGO.

## RESULTS

The active screening lasted for 12 days, from 8th to 19th December, 2009 and 25 villages were screened; 13,875

inhabitants (72.62% of estimated populations) were examined and 4 patients were detected, 2 in the first period and 2 in the second period (Table 2). All these cases were treated, respectively by pentamidine and Nifurtimox-Eflornithine Combinaison Therapy (NECT).Among the 182 patients diagnosed by the NGO from 2008 to 2009, only 74 (40.66%) were found in 17 villages. Their medical file showed that only CATT on whole blood was systematically performed to almost all patients. Anion exchange chromatography test and microscopic examination of lymph node aspirates was not performed but lumbar puncture and microscopic examination of cerebrospinal fluid (CSF) was considered as a diagnosis test (Figure 3). Likewise, the only pre-sence of cervical nodes was a key diagnosis test. After having analyzed medical files of these previous treated patients, we concluded that only 5 patients might have been really considered as sleeping sickness cases (Table 3).

## DISCUSSION

Analysis of medical files of patients diagnosed by the NGO showed that there has been an overestimate of cases reported. The number of cases reported during a given time is very important since it is the criterion that will determine epidemiological status (epidemic or endemic) of the disease in an area. In our current study, almost two hundred of sleeping sickness cases were reported in a period of one year in Moïssala by an NGO. This observation was similar to the situation prevailing in the beginning of 1990s in Democratic Republic of Congo and Angola (WHO, 1994). Yet, it is known that thanks to control measures, particularly active case finding surveys and treatment of all detected cases, the prevalence of sleeping sickness has considerably dropped in all country. The number of detected cases is inversely proportional to the number of examined persons (Kohagne Tongué and Louis, 2009; Simarro et al., 2011). A sudden outbreak of sleeping sickness detected by a passive surveillance is very surprising in an area where no case was diagnosed, four years before after a mass screening survey. The evolution of *T. b. gambiense* sleeping sickness form is silent and sly and passive cases are mainly in the second period of the disease (Legros et al., 2002).

Less than half of previous treated patients were found during our survey. That phenomenon is common in sleeping sickness foci. It has even been demonstrated that during a follow-up, 39% patients are found after six months, 44% after one year and 25% after two years (Grillet et al., 2004, report of the South Sudan Sleeping sickness program, unpublished). It is a consequence of the mobility of populations, enhanced by the absolute necessity of lumbar puncture that dissuades them from any report to medical team during follow-up.
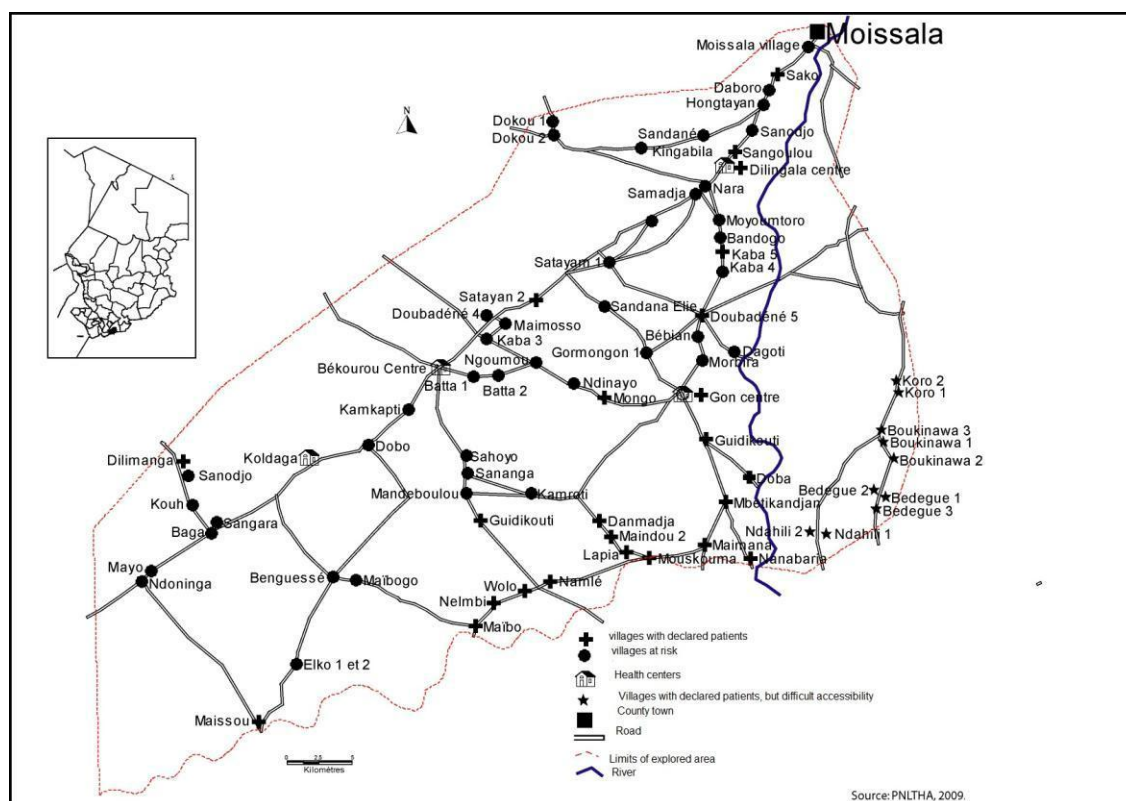
**Figure 1.** A map of the Moïssala focus showing endemics and villages at risk of HAT as observed by the NGO.

Nevertheless, the number of previous treated patients found demonstrates sufficiently the need to reinforce technical capacities for a reliable and sustainable control and surveillance of sleeping sickness. It seems obvious that medical team of this NGO was not well trained about diagnosis methods of HAT. A misunderstanding was observed concerning the presence of cervical node and the microscopic examination of lymph node aspirate in the diagnosis of sleeping sickness. Indeed, we noticed that subjects with enlargement of cervical nodes were automatically considered as sleeping sickness cases and treated. Different etiological factors play role in causation of lymphadenopathy (Agarwal et al., 2010), and lympadenopathy is very inconstant in the case of a HAT infection (Jannin et al., 1993). For that case, gland palpation cannot be used as a key diagnosis method of sleeping sickness. Likewise, positive CATT on whole blood can be induced by parasites others than trypanosomes, and high prevalence of CATT-positive individuals can be found even in areas where transmission has stopped (Jamonneau et al., 2010).

It is assumed that in addition to subjects positive after parasitological tests, individuals with a CATT end-dilution titer of 1:16 or 1:32 can also be considered as sleeping sickness cases (Simarro et al., 1999). However, the PPV of a CATT titer ≥ 1/16, like any other diagnostic test, depends on the prevalence of the disease in the tested

population. It is moderate in low endemicity areas and higher when the prevalence of disease raises (Chappuis et al., 2004). According to the sleeping sickness diagnosis flow chart, CATT titration test must be performed for all positive CATT whole blood-subjects but with a negative lymph node aspirate. We also noticed during our investigation that treatment was performed by the medical team of the NGO without examination of CSF that determines the stage of the disease. In the absence of sufficiently specific clinical signs and blood tests indicating the evolution from first- to second-stage HAT, staging of patients still relies on examination of CSF obtained by lumbar puncture (Chappuis et al., 2005). It is a vital step in the diagnosis process. More than 19% of the previous treated patients investigated were false sleeping sickness cases. This means the outbreak of the disease (182 cases between 2008 and 2009) declared in Moïssala was only a consequence of inappropriate diagnosis tests, as confirmed by our mass screening survey. Consequences of this unfitness and incompetence are: (i) At individual level, subjects have been wrongly considered as affected by a disease with no effective treatment (Nok, 2003), a disease of which are available only a handful of drugs plagued by various problems, ranging from oral inabsorption, acute toxicities, short durations of action, and low efficacies to the emergence of trypanosomal resistance (Wang, 1995;
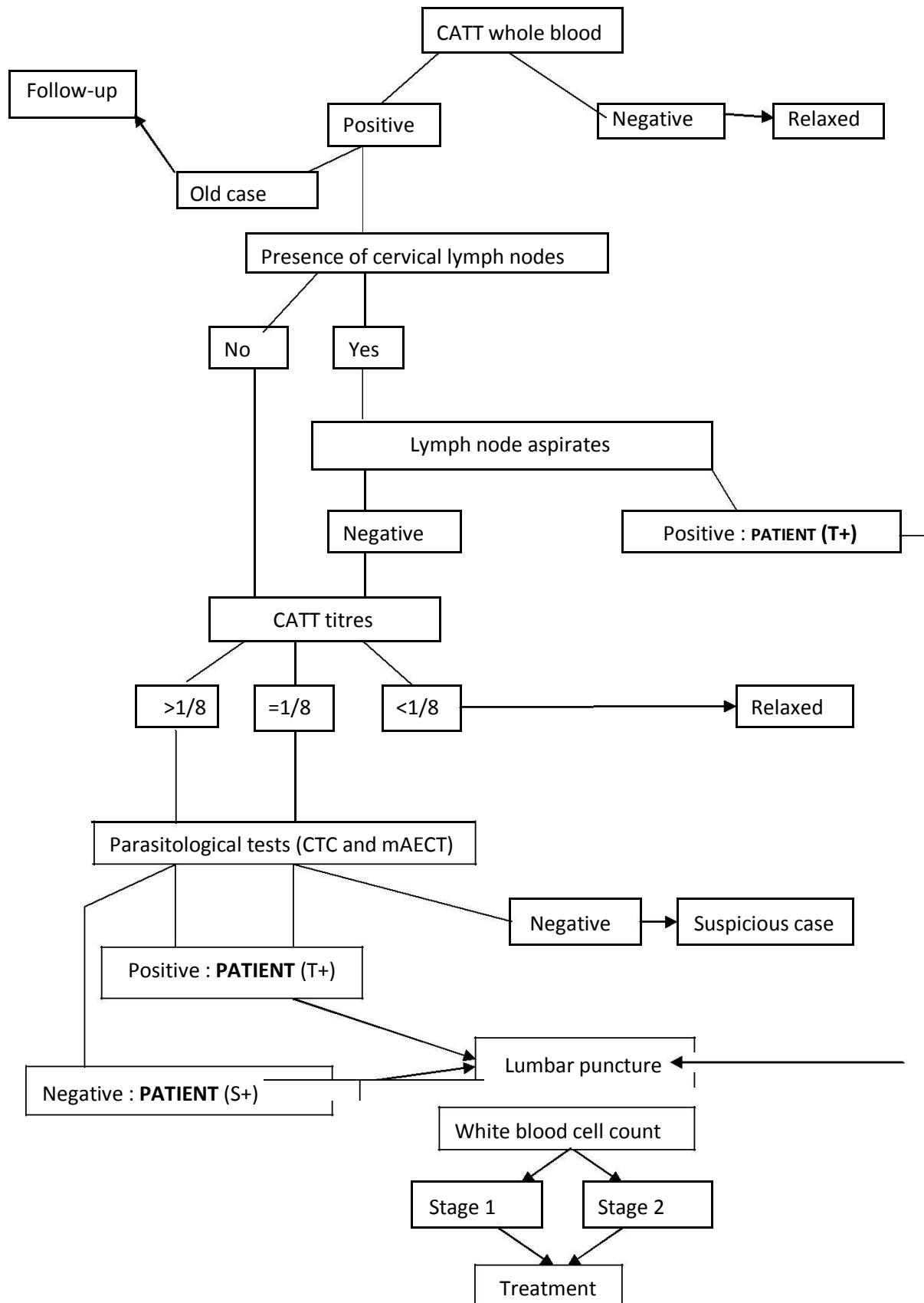
**Figure 2.** Field algorithm for sleeping sickness diagnosis.

**First side**

**Second side**

**Figure 3.** Medical file of a patient diagnosed by the NGO showing the use of lumbar puncture as a diagnosis test after the negativity of CATT on whole blood.

**Table 1.** Simplified questionnaire for patients found during follow-up.

| Question | Proposed answer | | | |
|---|---|---|---|---|
| Where were you pricked before treatment? | Finger | Elbow | Neck | Back |
| | | | | |
| Have you been palpated at the neck? | Yes | | No | |
| | | | | |
| Have you been hospitalized? | Yes | | No | |
| | | | | |
| How long did you treatment last? | 7 days | 10 days | 14 days | Others |
| | | | | |
| Where were you pricked during treatment? | Buttock | Elbow | Both | Elsewhere |

Checchi et al., 2008); (ii) at the country level, National statistics of that disease have been wrongly increased; (iii) at the international level, costly control measures provided by regional and international institutions have been wrongly expended.

**CONCLUSION**

A good achievement of laboratory tests is important for the diagnosis of all disease, and more, for the diagnosis of sleeping sickness where there are no pathognomonic symptoms. Parasitological techniques, though having limited sensitivity, are absolutely essential. Microscopic examination of lymph node aspirate is low cost and can be easily performed in health centers if personnel are well trained. There is an urgent need to regularly organize training course about diagnosis methods of sleeping sickness. This course might concern all people involved in the fight against the disease, either living in endemic areas or not, for accurate information that will be helpful for control and surveillance.

**Table 2.** Total number of examined persons and cases detected per villages.

| VILLAGES | PEOPLE | | | CATT DILUTION TEST | | | PARASITOLOGICAL TEST | | | | | | DETECTED CASES | | | P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Estimated | Examined | % | <1/8 | =1/8 | >1/8 | PG - | PG + | CTC - | CTC + | mAECT - | mAECT + | T+ | S+ | Total | |
| Dilingala | 1 926 | 1 717 | 89,15 | 10 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Sangoulou | 486 | 353 | 72,63 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Kaba 4 | 630 | 647 | >100 | 20 | 2 | 1 (µ filaria) | 8 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Kaba 5, Bandogo | 573 | 493 | 86,03 | 16 | 1 | 0 | 4 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Doubadéné 5 | 1 387 | 958 | 69,07 | 22 | 0 | 0 | 4 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | |
| Gon centre | 1 890 | 1 380 | 73,02 | 34 | 2 | 0 | 7 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | |
| Maissou | 575 | 377 | 65,56 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Elko 1, Elko 2 | 460 | 223 | 48,48 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Maibo | 663 | 489 | 73,76 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Namlé, Wolo | 591 | 351 | 59,39 | 3 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Danmadja, Nelmbi | 380 | 351 | 92,37 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Maidou 2 | 90 | 73 | 81,11 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Mouskouma, Lapia | 188 | 122 | 64,89 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Maimana | 526 | 246 | 46,77 | 2 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | |
| Nana Baria | 86 | 42 | 48,84 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Mbetikandjan | 517 | 336 | 64,99 | 8 | 0 | 1 | 3 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 2 | |
| Doba | 124 | 80 | 64,52 | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Guitikouti | 750 | 574 | 76,53 | 15 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Mongon | 923 | 476 | 51,57 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Ndinayo | 1 020 | 722 | 78,78 | 13 | 0 | 0 | 3 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | |
| Total | 13 785 | 10 010 | 72,62 | 160 | 7 | 3 | 38 | 2 | 6 | 1 | 3 | 1 | 3 | 1 | 4 | |

LP: lumbar puncture, T+: cases with trypanosomes (after parasitological tests), PG: lymph node aspirate examination, S+: cases without trypanosom centrifugation technic, P1: first stage of the disease, mAECT: anion exchange chromatography technic , P2: second stage of the disease, NECT: Nifurti treatment received, Penta: pentamidine.

**Table 3.** Diagnosis tests used by the NGO to detect previous treated cases found.

| Village | CATT whole blood | | [1]Thick or thin blood film | | CATT titration or dilution | | | Presence of cervical lymph nodes | | Examination of lymph nodes aspirate | | CTC | mAECT | [2]Lumbar puncture as a diagnosis |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | - | + | - | + | <1/8 | =1/8 | >1/8 | - | + | - | + | + | + | + |
| Dilingala | 1 | 2 | NP | NP | NP | NP | NP | 1 | 2 | NP | NP | NP | NP | NP |
| Kaba 5 | 0 | 6 | 0 | 4 | NP | NP | NP | 4 | 2 | NP | NP | NP | NP | NP |
| Doubadéné 5 | 0 | 1 | NP | NP | NP | NP | NP | 0 | 1 | NP | NP | NP | NP | NP |
| Gon centre | 0 | 4 | 2 | 2 | NP | NP | NP | 2 | 2 | NP | NP | NP | NP | NP |

**Table 3.** Contd.

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Maïssou | 0 | 1 | 0 | 1 | NP | NP | NP | 0 | 0 | NP | NP | NP | NP | NP |
| Maïbo | 1 | 12 | 7 | 6 | NP | NP | NP | 6 | 5 | NP | NP | NP | NP | 2 |
| Namlé et Wolo | 0 | 1 | 0 | 1 | NP | NP | NP | NP | NP | NP | NP | 1 | NP | NP |
| Danmadja et Nelmbi | 0 | 2 | 0 | 2 | NP | NP | NP | NP | NP | NP | NP | NP | NP | NP |
| Maidou 2 | 0 | 5 | 1 | 4 | 1 | 1 | NP | NP | NP | NP | NP | NP | NP | NP |
| Mouskouma et Lapia | 4 | 12 | 4 | 8 | 2 | 1 | 1 | NP | NP | NP | NP | NP | NP | 4 |
| Maïmana | 0 | 6 | 3 | 3 | 1 | 1 | NP | NP | 2 | NP | NP | NP | NP | NP |
| Nana Baria | 1 | 2 | 0 | 1 | 1 | 1 | 1 | NP | NP | NP | NP | NP | NP | 1 |
| Mbetikadjan | 0 | 9 | 3 | 6 | NP | NP | 1 | 6 | 2 | NP | NP | NP | NP | NP |
| Guitikouti | 1 | 3 | 1 | 2 | 3 | 1 | 1 | NP | NP | NP | NP | NP | NP | 1 |

[1]not used in the algorithm, [2]Lumbar puncture was performed either as the only diagnosis test, or after the negative of CATT whole blood test, NP: not per

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