Am. J. Trop. Med. Hyg., 70(4), 2004, pp. 390–394

TRYPANOSOMA BRUCEI GAMBIENSE TRYPANOSOMIASIS IN TEREGO COUNTY, NORTHERN UGANDA, 1996: A LOT QUALITY ASSURANCE SAMPLING SURVEY

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Abstract. We estimated the pre-intervention prevalence of *Trypanosoma brucei gambiense* (*Tbg*) trypanosomiasis using the lot quality assurance sampling (LQAS) methods in 14 parishes of Terego County in northern Uganda. A total of 826 participants were included in the survey sample in 1996. The prevalence of laboratory confirmed *Tbg* trypanosomiasis adjusted for parish population sizes was 2.2% (95% confidence interval = 1.1–3.2). This estimate was consistent with the 1.1% period prevalence calculated on the basis of cases identified through passive and active screening in 1996–1999. Ranking of parishes in four categories according to LQAS analysis of the 1996 survey predicted the prevalences observed during the first round of active screening in the population in 1997–1998 ($P < 0.0001$, by chi-square test). Overall prevalence and ranking of parishes obtained with LQAS were validated by the results of the population screening, suggesting that these survey methods may be useful in the pre-intervention phase of sleeping sickness control programs.

INTRODUCTION

Trypanosoma brucei gambiense (*Tbg*) trypanosomiasis (sleeping sickness) is a chronic parasitic disease that has a long initial asymptomatic phase and that is 100% lethal in the absence of treatment. Humans acquire infection through the bite of an infected *Glossina* (tsetse fly), usually in selected human-vector contact sites.[1] In the foci of the disease, villages where active transmission occurs have higher prevalences. Interventions are based upon a combination of 1) active screening for the treatment of all cases and 2) vector control. Such programs are resource-intensive because they require mobile teams for mass screening, a laboratory diagnosis capacity, and treatment facilities capable of caring for a high number of patients.

When sleeping sickness re-emerges in an historic focus, estimation of the overall prevalence will allow overall planning of resources, while identification of high prevalence villages will determine which sites may host active transmission. Classic survey methods allow estimating the overall prevalence, but they cannot be used to identify high prevalence villages because the sample size required, one stratum per village, would be too large. Lot quality assurance sampling (LQAS) is an alternative sampling method originally designed to test batches of industrial products and efficiently identify those where the acceptable failure rate is exceeded.[2] While traditional survey methods aim at estimating a quantified parameter, LQAS is based upon the qualitative testing of a hypothesis that is accepted or rejected. Thus, LQAS provides less information than traditional methods. However, it requires much smaller sample sizes and was found to be useful in a number of public health settings, including immunization coverage surveys.[3] LQAS has never been used for sleeping sickness prevalence surveys. However, the ability to compare strata within a larger sample makes it a method that is adapted to the heterogeneous distribution of *Tbg* trypanosomiasis in populations.

Since 1980, *Tbg* trypanosomiasis has re-emerged in northern Uganda as in other sub-Saharan African historic foci.[4–8] The National Sleeping Sickness Control Program (NSSCP) has conducted control activities in the West Nile region (Arua and Moyo district) in collaboration with Médecins Sans Frontières since 1986 using diagnosis and treatment protocols defined in an agreement with the World Health Organization. From 1987 to 1991, 80–90% of the population of the western part of the Moyo district was screened through passive and active methods and 4,822 patients were diagnosed and treated in the treatment center of Moyo Hospital between 1987 and 1992.[5] A second center, exclusively oriented toward sleeping sickness, opened a passive screening program in September 1991 in Adjumani in the eastern part of the Moyo district. This was followed between 1992 and 1996 by an active mass screening campaign that resulted in the treatment of 5,423 patients (as of December 1999, Médecins Sans Frontières unpublished data). In September 1995, while sleeping sickness was under control in the Moyo district, cases of this disease were reported in Terego County in the neighboring district of Arua . Thus, a sleeping sickness diagnosis and treatment center was opened in the village of Omugo in the Arua district. As of April 1996, 447 Terego county residents with *Tbg* trypanosomiasis were self-referred for diagnosis and treatment at the center, suggesting active current local transmission. To better direct the planned active screening efforts, we conducted a cross-sectional survey in April–May 1996. The objectives of the survey were to 1) obtain a pre-intervention estimate of the prevalence of *Tbg* trypanosomiasis in Terego County and 2) identify high prevalence parishes that required an intervention before the others. We thus used the LQAS methods to achieve these two objectives while maintaining a manageable sample size.

METHODS

Diagnosis of *Tbg* trypanosomiasis is made through serologic screening followed by parasitologic diagnostic procedures. Serologic testing is conducted using the card agglutination trypanosomiasis test (CATT),[9] a serologic test that can be performed with increasing dilutions of serum to increase its specificity. Parasitologic diagnostic procedures are based upon microscopic examination of lymph nodes aspirates, blood, or cerebrospinal fluid for evidence of *Tbg*.

Case definitions. Three levels of case definition with increasing specificity were used. First, a positive CATT result

was defined as a visible agglutination on the card test using a whole blood specimen. This case definition was most sensitive but least specific. Second, a serologically suspected case was defined as a positive CATT result using a serum at a 1:4 dilution. This case definition had an intermediate level of sensitivity and specificity. Third, a confirmed case of *Tbg* trypanosomiasis was defined as parasitologic evidence of *Tbg* infection in the blood, lymph nodes, or cerebrospinal fluid. This case definition was less sensitive but most specific.

Sampling methods. The survey population was defined as the population of the 24 parishes of Terego County. This sample was stratified by parish. In each of the 24 parishes, households were randomly selected from a list obtained from the local authorities and key informants. Within each selected household, one individual was selected at random.

Laboratory testing. All participants were screened in the field using a CATT on whole blood. Patients with a positive CATT result were referred to the diagnosis and treatment center for a CATT on a serum specimen diluted 1:4 and diagnostic investigations according to the algorithm used in the NSSCP.[5] Briefly, serologically suspected cases were investigated for parasitologic evidence of *Tbg* infection. Patients presenting with cervical lymphadenopathies underwent a glandular puncture for microscopic analysis. When there was no evidence of *Tbg* infection in any lymph node, blood samples were examined using the Woo concentration technique,[10] followed by the quantitative buffy coat technique if the Woo test result was negative.[11] Finally, a lumbar puncture for microscopic analysis of cerebrospinal fluid was done for all serologically suspected cases. These suspected cases for which no infection could be detected during the initial visit were actively followed-up by specialized community health workers (sleeping sickness assistants) and brought back to the Omugo Center for four visits during the next 12 months, as per the national protocol.

Statistical analysis. *Analysis of the survey.* The LQAS method allowed testing the hypothesis that the prevalence in a given parish was below a certain threshold.[2] If the observed number of cases among the n subjects selected in a parish was less or equal a critical number (d), the prevalence could be considered to be lower than a high threshold (T1) in this parish with a defined alpha error. If n exceeded d, the prevalence could not be considered to be lower than the high threshold (T1) in this parish with the defined alpha error. Power calculations then suggested that in these parishes, there was probability equal to 1 - the beta error that the prevalence exceeded a lower threshold (T2).

Parishes were ranked in categories according to the LQAS analysis results for the three case definitions used. Parishes that exceeded the critical number d for the three case definitions were ranked in the ++++ category, parishes that exceeded the critical number for two were ranked in the +++ category, parishes that exceeded the critical number for one were ranked in the ++ category, and the remainder that never exceeded the critical number d were ranked in the + category.

Following this parish-specific LQAS statistical analysis, all parishes were pooled to measure the overall prevalence in Terego County, as in an ordinary stratified random sample.[3] Prevalence estimates were adjusted for population sizes since an equal number of participants was selected from each parish that in fact had different population sizes.

Validation of the survey results. To validate the ranking of parishes obtained with the LQAS analysis, prevalences calculated during the initial active screening conducted between 1997 and 1998 were compared with the ranking of parishes in the four LQAS categories. The years 1997–1998 represented the first active screening round following the survey because no active screening could be done immediately after the survey in 1996 due to civil unrest in the county.

To validate the overall county prevalence estimate, the prevalence estimate obtained through the survey was compared with the period prevalence calculated on the basis of the total number of cases identified as a result of combined active and passive screening after the survey from 1996 to 1999. In all calculations, 1991 national census figures were projected to 1996 using a 3% crude annual growth rate as reported in the 1991 national census.

Sample size. Use of the LQAS tables allowed an arbitrary choice of n and d.[2] These indicated that if 1) $n = 59$ individuals were selected in each parish and 2) the number of identified cases was less than or equal to $d = 2$, the prevalence in the parish could be hypothesized not to exceed the T1 10% threshold with an alpha risk of 5%. Power calculations for such a sample size indicated that in parishes were the number of cases exceeded $d = 2$, the probability that the prevalence exceeded the T2 threshold 2% was 80%.[2] These choices of n and d were adapted to the decisions expected to be made on the basis of the results of the survey.

The expected overall sample size for Terego County was 1,416 (i.e., 24 strata of 59 individuals). Given the 1996 Terego County population size (115,203) and assuming a prevalence of 5%, this sample size was sufficient to obtain a 1.1% precision at a 95% confidence level.

Human subjects. Participants, although selected at random for the survey, underwent the routine diagnosis and treatment procedure recommended by the NSSCP. Thus, the survey was considered as initial evaluation and not as research. Patients identified with confirmed *Tbg* trypanosomiasis during the survey were offered treatment. Serologic suspects were also offered counseling and explained the need for follow-up diagnostic evaluations four times during one year.[5] The survey protocol was reviewed and approved by the Ugandan Ministry of Health and by the Arua District Medical Office.

RESULTS

Survey results. *Description of the survey sample.* Only 14 of the 24 parishes of Terego County could be surveyed because the fieldwork was interrupted in June 1996 due to civil unrest. In these 14 parishes (combined estimated 1996 population size = 63,502), 826 persons were included in the survey

TABLE 1
Age and sex distribution of the survey sample in Terego County in northern Uganda, 1996

Age (years)	Female		Male		Total
<5	35	52.2%	32	47.8%	67
5–14	67	43.8%	86	56.2%	153
15–29	105	41.3%	149	58.7%	254
30–59	126	43.9%	161	56.1%	287
≥60	23	35.4%	42	64.6%	65
Total	356	43.1%	470	56.9%	826

TABLE 2

Number of positive CATT results, serologically suspected cases, and cases of *Tbg* trypanosomiasis in the 14 parishes surveyed in Terego County in northern Uganda, 1996*

Parish	Number of cases among the 59 participants in each parish			Category
	Positive CATT	Serologically suspected	Tbg trypanosomiasis	
Ogunu	7†	5‡	4§	++++
Yiddu	7†	4‡	1	+++
Bura	7†	3‡	2	+++
Erea	6†	4‡	1	+++
Azapi	5†	2	0	++
Otrevu	4†	2	2	++
Angazi	4†	1	0	++
Onzoro	3†	1	1	++
Aripia	2	2	2	+
Owayi	2	2	1	+
Paranga	2	0	0	+
Anufira	1	1	1	+
Obi	1	1	1	+
Orivu	0	0	0	+
Total	51	28	16	–

* CATT = Card Agglutination Trypanosomiasis Test; *Tbg* = *Trypanosoma brucei gambiense.*

† Parish for which the number of positive CATT results exceeded the critical number (80% probability of a prevalence exceeding 2%).

‡ Parish for which the number of serologically suspected cases exceeded the critical number (80% probability of a prevalence exceeding 2%).

§ Parish for which the number of *Tbg* trypanosomiasis cases exceeded the critical number (80% probability of a prevalence exceeding 2%).

sample. Among participants, the proportion of males was 57% (Table 1) and the median age was 26 years (range = 0–87).

Parish ranking using LQAS analysis. The number of parishes for which there was an 80% probability that the prevalence exceeded 2% increased with the sensitivity of the case definition (Table 2). When the confirmed *Tbg* trypanosomiasis case definition (least sensitive case definition) was used, only one parish (Ogunu) was considered to have an 80% probability of exceeding the 2% threshold. This parish was ranked in the + + + + category. When the serologically suspected case definition was considered (definition with intermediate sensitivity), an additional three parishes (Yiddu, Bura, and Erea) were considered to have an 80% probability of exceeding the 2% threshold. These three parishes were ranked in the + + + category. When a positive CATT result (most sensitive case definition) was considered as the outcome of interest, an additional four parishes (Azapi, Otrevu, Amgazi, and Onzoro) were considered to have an 80% probability of exceeding the 2% threshold. These four parishes were ranked in the + + category. Six remaining parishes that were considered to have a 95% probability of being under the 10% threshold for the most sensitive case definition were ranked in the + category. Overall, ranking allowed a mapping of the area studied to determine which parishes should be actively screened by a mobile team before the others (Figure 1).

Estimated prevalence in the whole county. Of the 826 individuals in the sample, 51 (6.5%, 95% confidence interval [CI] = 4.7–8.3) had a positive CATT result, 28 (3.6%, 95% CI = 2.3–5.0) were serologically suspected cases, and 16 (2.2%, 95% CI = 1.1–3.2) were confirmed *Tbg* trypanosomiasis case patients. Of these, 14 (87.5%) had cerebrospinal fluid abnormalities suggesting late stage infection. Based upon the prevalence estimate of the survey and the projected 1996 population size, the number of confirmed *Tbg* trypanosomiasis cases in the 14 surveyed parishes was estimated between 699 and 2,032 cases.

Validation of the survey results. *Validation of parish ranking.* The prevalence of *Tbg* trypanosomiasis calculated on the basis of the 1997–1998 first round of active screening in the 14 parishes decreased with each of the four prevalence categories that had been defined on the basis of the LQAS survey (0.9%, 0.3%, 0.2%, and 0.1% for the ++++, +++, ++, and + groups, respectively; $\chi^2 = 92.7$, degrees of freedom = 3, $P < 0.0001$) (Table 3).

Validation of the estimated prevalence in the whole county. In the 14 parishes where the survey was conducted, 683 (prevalence = 1.1%) new cases of *Tbg* trypanosomiasis were diagnosed through passive (n = 463, 67.8%) and active (n = 220, 32.2%) screening between July 1996 and December 1999. This prevalence was at the lower 95% confidence limit of the prevalence estimate provided by the survey.

DISCUSSION

This cross-sectional survey provided a pre-intervention estimate of the prevalence of *Tbg* trypanosomiasis in the 14

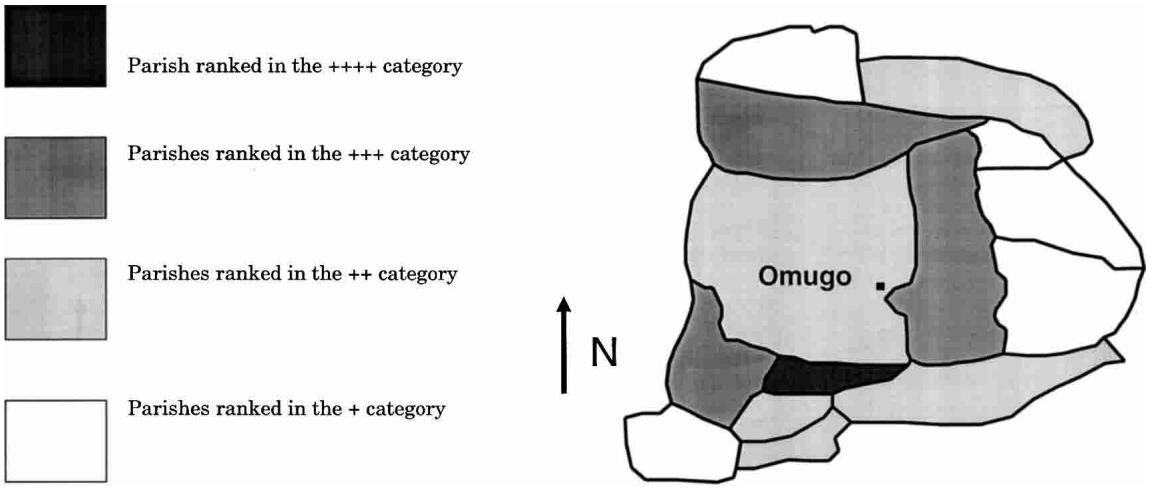

FIGURE 1. Ranking of parishes in categories on the basis of lot quality assurance sampling analysis in Terego County in northern Uganda, 1996.

TABLE 3

Comparison between the categories obtained through the LQAS analysis of the 1996 survey and the prevalence obtained during the first round of active screening (1997–1998) in Terego County in northern Uganda*

Parish	Category according to the 1996 LQAS survey	Prevalence based upon the first round of active screening in Terego County, 1997–1998		
		Tbg cases	Total	Prevalence (%)
Ogunu		42	4,605	0.9
Total		42	4,605	0.9†
Yiddu		15	4,359	0.3
Bura		11	4,138	0.3
Erea		11	2,093	0.5
Total		37	10,590	0.3†
Azapi		3	4,519	0.1
Otrevu		8	2,872	0.3
Angazi		NA	NA	NA
Onzoro		12	3,792	0.3
Total		23	11,183	0.2†
Aripia		5	3,112	0.2
Owayi		5	4,309	0.1
Paranga		2	3,474	0.1
Anufira		8	2,683	0.3
Obi		5	3,583	0.1
Orivu		0	4,194	0
Total		25	21,355	0.1†
Total		127	47,733	0.1

* LQAS = lot quality assurance sampling; *Tbg* = *Trypanosoma brucei gambiense;* NA = not available.

† General chi-square for the comparison of *Tbg* trypanosomiasis prevalence among the four categories = 92.7, degrees of freedom = 3, $P < 0.0001$.

parishes that could be visited in Terego County. Ranking of the parishes through LQAS testing allowed defining priorities for the planning of active case finding campaigns, and the overall estimate was valuable to plan the long-term activities of the diagnosis and treatment center in Omugo.

If this survey had not been conducted, two options would have been available to estimate the prevalence of *Tbg* trypanosomiasis in Terego County. First, period prevalence estimates based upon cases self-referred at the center could have been used. The period prevalence estimates based upon self-referred patients would have required months of activities at the center. In addition, it may have underestimated the prevalence in parishes located far from the center because residents with little access to the center may have been less likely to present themselves for diagnosis and treatment. Alternatively, prevalence estimates could have been based upon the first round of active screening campaigns in all parishes. This second method would have required more time and more financial resources, but would have provided a more reliable estimate. The LQAS survey did not require a first round of active screening. It provided a ranking of the parishes according to their expected prevalence levels. This ranking allowed directing efforts for the first round of active screening and was later validated by the results of the first round of active screening. Finally, it provided an unbiased overall estimate of the prevalence in the county that was validated by the total number of patients ultimately diagnosed through passive and active screening between July 1996 and December 1999.

Surveys using the classic cluster sample methods are useful in providing overall estimates of diseases prevalence.[12] However, these surveys cannot measure prevalence estimates for each visited cluster and they are not adapted to the heterogeneous distribution of *Tbg* trypanosomiasis in endemic areas. Cross-sectional surveys using the LQAS method address some of the limitations of traditional methods. First, LQAS provides an opportunity to test the hypothesis that the prevalence of disease in certain communities exceeds a certain threshold with a high sensitivity but a low specificity.[13] In this survey, use of LQAS dichotomous testing for three case definitions of increasing specificity allowed ranking parishes according to the specificity of the case definition. Second, LQAS with single random sampling in every village is less sensitive than cluster sampling to the heterogeneous distribution of *Tbg* trypanosomiasis in the population. Finally, use of stratified single random sampling allowed us to generate estimates for the parishes that had been visited despite the curtailing of the study at mid-course. For this study, a traditional stratified survey could also have estimated the prevalence in each visited strata. However, a traditional stratified survey would have been limited by the issue of sample size. Had we analyzed our data as a traditional stratified survey to compare prevalences across parishes, our limited sample size would have lead to large confidence intervals that would have not allowed us to form any conclusions. As an example, the prevalence of a positive CATT result in Ogunu (7 of 59, 11.8%, 95% CI = 4.9–23) would not have been significantly different from the prevalence of a positive CATT result in Obi (1 of 59, 1.7%, 95% CI = 0.04–9.1).

This survey had several limitations. Despite our attempt to sample randomly, the representation of the sample was not what had been aimed for. First, the proportion of males among participants suggested that they were over-represented. This over-representation cannot be confirmed since the proportion of males in the 1996 population was unknown and could have been affected by population movements associated with civil unrest in the district. Second, the heterogeneous distribution of *Tbg* trypanosomiasis within villages may have lead to the selection of biased samples within parishes. While this factor could have affected the LQAS ranking of the parishes, it is unlikely that it would have biased the overall prevalence since underestimates and overestimates would be expected to cancel each other out at the county level. Third, sampling of a single individual per household over-represented small households and under-represented large households. However, there is no data to suggest that the prevalence of *Tbg* trypanosomiasis varies according to the household size. Finally, the reduction in overall sample size secondary to the early termination of the survey increased the width of our confidence interval. However, our estimate was still compatible with the period prevalence obtained with the validation method.

Cross-sectional surveys using LQAS are based upon single random sampling in communities. Single random sampling requires a sampling frame, time, attention, and expertise. In the specific field of *Tbg* trypanosomiasis, it is also necessary to have an infrastructure to diagnose and treat survey participants before a survey is initiated. In addition, results of LQAS surveys are sometimes difficult to communicate to public health audiences that are not familiar with its principles.[14] Finally, an LQAS survey will never replace mass screening campaigns that remain the gold standard for estimating village-specific prevalences of *Tbg* infection, in part because prevalence levels below 10% make it difficult to estimate prevalence with a satisfying degree of precision during sur-

veys. For all these reasons, surveys using LQAS to assess the prevalence of *Tbg* trypanosomiasis cannot be currently recommended as a standard method for initial evaluation. However, in some circumstances, expertise may be present, laboratory capacity may be available, public health audiences may understand advanced LQAS analysis, and mass screening campaigns may be difficult to organize rapidly. In such settings, cross-sectional surveys using LQAS may be a useful tool to estimate prevalence and to identify small communities (e.g., parishes, villages) that should be screened during the first round of active screening campaigns. Availability of such data would allow that scarce resources available to sleeping sickness control programs be used in a more cost-effective way.

Received February 19, 2003. Accepted for publication August 14, 2003.

Acknowledgments: We are grateful to Dr. Mark White for suggestions on the survey methods and to Dr. Anne Moore for suggestions on the manuscript.

Financial support: This study was supported by Médecins Sans Frontières and by a grant from the European Development Fund EU/EDF/DG8.

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