Differences in pathogenicity and virulence of *Trypanosoma brucei gambiense* field isolates in experimentally infected Balb/C mice

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Abstract

Trypanosoma brucei gambiense (*T. b. gambiense*) is the major causative agent of human African trypanosomiasis (HAT). A great variety of clinical outcomes have been observed in West African foci, probably due to complex host-parasite interactions. In order to separate the roles of parasite genetic diversity and host variability, we have chosen to precisely characterize the pathogenicity and virulence of *T. b. gambiense* field isolates in a mouse model. Thirteen *T. b. gambiense* strains were studied in experimental infections, with 20 Balb/C infected mice per isolate. Mice were monitored for 30 days, in which mortality, parasitemia, anemia, and weight were recorded. Mortality rate, prepatent period, and maximum parasitemia were estimated, and a survival analysis was performed to compare strain pathogenicity. Mixed models were used to assess parasitemia dynamics, weight, and changes in Packed Cell Volume (PCV). Finally, a multivariate analysis was performed to infer relationships between all variables. A large phenotypic diversity was observed. Pathogenicity was highly variable, ranging from strains that kill their host within 9 days to a non-pathogenic strain (no deaths during the experiment). Virulence was also variable, with maximum parasitemia values ranging from 42 million to 1 billion trypanosomes/ml. Reduced PCV and weight occurred in the first two weeks of the infection, with the exception of two strains. Finally, the global analysis highlighted three groups of strains: a first group with highly pathogenic strains showing an early mortality associated with a short prepatent period; a second group of highly virulent strains with intermediate pathogenicity; and a third group of isolates characterized by low pathogenicity and virulence patterns. Such biological differences could be related to the observed clinical diversity in HAT. A better understanding of the biological pathways underlying the observed phenotypic diversity could thus help to clarify the complex nature of the host-parasite interactions that determine the resistance/susceptibility status to *T. brucei gambiense*.

Keywords: human African trypanosomiasis, *Trypanosoma brucei gambiense*, clinical diversity, phenotypic diversity, mouse model, pathogenicity, virulence, host-parasite interaction, multivariate analysis

Introduction

Trypanosoma brucei gambiense (*T. b. gambiense*) is the causative pathogenic agent of human African trypanosomiasis (HAT, or sleeping sickness) in West and Central Africa. It is responsible for 98% of all HAT cases reported in the last decade (Franco et al., 2014). The disease is classically described as a chronic infection characterized by an early hemolymphatic phase (phase 1), followed by a late meningo-encephalitic phase that leads to neurological disorders (phase 2) (Brun et al., 2010; WHO, 2013). Until recently, HAT was considered to be an invariably fatal disease, in the absence of any treatment (WHO, 1998). However, self-cured individuals have been reported, and the disease severity has been observed to range from asymptomatic carriers to acute forms (Garcia et al., 2000; Jamonneau et al., 2012; Truc et al., 1997). Other recent studies have reported subjects with latent infections who can maintain a long-lasting specific serological response, but in the absence of any detectable parasitemia (Garcia et al., 2000; Ilboudo et al., 2011).

This diversity in disease progression is likely due to host and parasite diversity, and to complex host-parasite interactions (Garcia et al., 2006). It is therefore important to clarify the respective contributions of the host and the parasite in their relationship, if the basis of this diversity is to be understood. Host factors that contribute to HAT clinical diversity have recently been revealed through identification of genetic polymorphisms associated with clinical outcomes, and by the differential response of cytokines in people presenting asymptomatic and aparasitemic latent infections versus parasitologically confirmed infections (Bucheton et al., 2011; Gineau et al., 2016; Ilboudo et al., 2012; Ilboudo et al., 2016). As a result, the concept of human trypanotolerance is now generally accepted (Berthier et al., 2016).

Concerning the *T. b. gambiense* parasite which is known to be mainly clonal and forms an homogenous genetic group (Weir et al., 2016), direct genotyping of field strains analyzed

by polymorphic microsatellite markers could not identify any association between genetic diversity and either the clinical picture or the disease progression in humans (Jamonneau et al., 2002; Kabore et al., 2011). However, an important phenotypic diversity was observed in experimental infections of *T. b. gambiense* strains in a rodent model, revealing diverse mortality rates and indicating that parasitemia dynamics vary depending on the parasite strain (Beckers et al., 1981; Frézil et al., 1979; Giroud et al., 2016; Inoue et al., 1998; Turner et al., 1995). More recently, differences were observed at the proteome level in proteins expressed by a highly virulent strain and a low virulent strain, despite the fact that these strains could not be discriminated using microsatellite markers (Holzmuller et al., 2008). This study suggested that differences in genome expression are more likely to be involved than genetic diversity in determining the virulent and pathogenic traits of trypanosomes at the intraspecific level. However, while these studies have highlighted the existence of a phenotypic diversity related to parasitemia and mortality in rodent models, they do present various shortcomings: clinical features such as anemia and weight loss were not systematically recorded; different host species and a limited number of strains were used (Beckers et al., 1981; Inoue et al., 1998); experiments were not initially designed to study phenotypic diversity (Holzmuller et al., 2008; Inoue et al., 1998; Turner et al., 1995); and finally, not a single statistical analysis was performed that encompassed all variables.

Consequently, our global objective was to investigate the role of *T. b. gambiense* in determining the phenotypic diversity in infected hosts using appropriate experimental and statistical methods. More precisely, the aim of the present study was to characterize the phenotypic variability of 13 *T. b. gambiense* strains using experimental infections in the inbred Balb/C mouse model. For each stock, the pathogenicity (*i.e.* the ability to induce pathogenic effects: weight loss, anemia and mortality) and virulence (*i.e.* the capacity to multiply inside a host) were monitored in 20 mice during a 30-day post-infection period. We analyzed these traits

and their correlations using mixed linear models and multivariate analyses, and three groups of strains associated with different combinations of these variables were identified.

Material and Methods

Parasites

This study used 13 strains isolated from HAT patients diagnosed during medical surveys conducted by the National Control Program between 2000 and 2009 in the HAT mangrove foci of Guinea (Boffa, Forecariah and Dubreka) and the forest focus of Bonon in Côte d'Ivoire (Table 1). Trypanosome isolation in the field was performed by intraperitoneally inoculation in two Balb/C mice (produced in CIRDES from paternal strains purchased from Charles River laboratories, France) previously immunosuppressed with cyclophosphamide (300 mg/kg of Endoxan®, administered before infection and then every 5 days). After 3 to 6 passages in mice, isolated parasites were stored as stabilates in liquid nitrogen.

Experimental infections

For each strain, stabilates were thawed and then inoculated in immunosuppressed mice for parasite amplification. After amplification, harvested blood from mice containing trypanosomes was diluted into phosphate buffered saline glucose (PSG) to achieve a concentration of 10^5 parasites/ml in a total volume of 10 ml. For each strain, 20 immunosuppressed Balb/C mice (300 mg/kg of cyclophosphamide Endoxan®, administered on the day of inoculation and then every 5 days), six to eight weeks old, were intraperitoneally infected with 0.5 ml of a previously prepared solution containing $5*10^4$ trypanosomes per mouse. A control group of 20 immunosuppressed mice, monitored under the same experimental

conditions, was inoculated with the same protocol using PSG alone. Each batch of 20 mice was monitored for 30 days.

Evaluated parameters

Parasitemia was determined daily by direct microscopic examination (X400) of mouse tail blood (Herbert and Lumsden, 1976). A mouse was considered parasitologically negative when no trypanosomes were detected in 40 microscopic fields. Mortality was also recorded daily. Weight and PCV (Packed Cell Volume) were measured just before infection and three times per week. PCV, which is the percentage of red blood cells in a blood volume and is thus related to anemia, was measured using the micro-centrifugation method.

Statistical analyses

Univariate analysis

All statistical analyses were performed with R3.1.0 (R-Development-core-team, 2010). For each *T. b. gambiense* strain, the number of mice presenting a positive parasitemia during the 30-day follow-up was recorded, as well as their proportion. Then, the average prepatent period (*i.e.* the date at which a mouse was determined to be positive for parasitemia) and the average maximum parasitemia were estimated (in addition to their standard errors), taking into account only those mice in which at least one parasite was detected during the follow-up. The non-parametric Wilcoxon test was used to test for a significant difference in prepatent period and maximum parasitemia between strains. Survival analysis was performed with the R package Survival 2.37-7. Survival was modeled using the Kaplan-Meier estimate, and the non-parametric Peto & Peto modification of the Gehan-Wilcoxon test, which assigns higher weights

for early deaths, was used to test if survival curves differed significantly between *T. b. gambiense* strains (Harrington and Fleming, 1982). For this test, a moderately pathogenic strain, B4_I315, was chosen as a common reference for paired comparison between strains, in order to discriminate along a spectrum from strongly to weakly pathogenic strains. This strain was positioned at the coordinate 0 of the first axis of the Principal Component Analysis and next to the coordinate 0 of the second axis (see Multivariate Analysis).

For longitudinal analyses of PCV, weight and parasitemia, mixed models were performed that take into account intra-correlation measures within mice that were modeled as random variables, using the *nlme* R package (Pinheiro et al., 2014). Due to mortality during the monitoring process, analyses on PCV and weight were performed on truncated data at 13 DPI (days post infection) for PCV, and 15 DPI for weight.

Several models were tested (comprising quadratic effect of time, interaction between random effects and slope), and the model that seemed to best fit the data was used. Briefly, for fit comparison, models were run using the Maximum Likelihood algorithm (Pinheiro and Bates, 2000). Random effects were compared using the likelihood ratio test, and fixed effects were chosen according to the individual *t*-test and single model ANOVA (F-test). Diagnostic plots were edited to check model assumptions, namely that the within-animal errors were independent and identically normally distributed, and independent of the random and fixed effects, and that random effects were normally distributed and independent of the mouse effect. Once the model comparisons were made, parameters were estimated using the Restricted Maximum Likelihood algorithm (Pinheiro and Bates, 2000).

The final model for PCV was:

$$PCV_{ijk} = \mu + S_i \times t_j + a_k + \varepsilon_{ijk} \quad (1)$$

where PCV_{ijk} is the PCV of a mouse k infected by the strain i on t_j, a_k represents the animal (k = 1, …, 240), S (i = 1, …, 13) is the *T. b. gambiense* strain, and t is the time after infection, represented numerically (from 0 to 13 DPI). The within-animal errors ε_{ijk} followed a Gaussian distribution N (0, $\sigma^2\delta^2_{Si}$), allowing the modeling of heteroscedasticity among strains (δ^2_{Si}). Random effects were also normally distributed, with $a_k \sim N$ (0, σ^2).

The same model was used for weight, where W_{ijk} is the weight of a mouse k infected by the strain i on t_j:

$$W_{ijk} = \mu + S_i \times t_j + a_k + \varepsilon_{ijk} \qquad (2)$$

After graphical observations, parasitemia was modeled according to a simple logistic model computed in the *nlme* R package (Pinheiro et al., 2014), which follows the standard equation:

$$y(x) = \frac{\phi_1}{1+\exp\left[\left(\phi_2 - x\right)/\phi_3\right]} \qquad (3)$$

so that Φ_1 is the asymptotic value of y as x approaches infinity, Φ_2 is the value of x for which $y = \Phi_1/2$ (which represents the inflection point of the curve and is here after referred to as the medium parameter), and Φ_3 is a scale parameter. When applied to parasitemia, this equation became:

$$PA_{ijk} = \frac{\phi_1 + S_{1i} + a_k}{1+\exp\left[\left(\phi_2 + S_{2i} - x\right)/\phi_3\right]} \qquad (4)$$

where PA_{ijk} is the parasitemia of a mouse k infected with by the *T. b. gambiense* strain S_i on t_j, S_{1i} and S_{2i} are the strain fixed effects, and a_k represents the animal random effect. The within-animal errors ε_{ijk} followed a Gaussian distribution $N(0, \sigma^2\delta^2_{Si})$, which allowed the modeling of heteroscedasticity among strains, with $a_k \sim N$ (0, σ^2).

Multivariate analysis

Seven synthetic variables were estimated per strain from the above models: the mortality modeled by the Kaplan-Meier estimate with higher weight to account for early death (DMw, Table 2), the slope in PCV (PCVsl=S_j equation 1, Table 2), the slope in weight (Wsl=S_j equation 2, Table 2), the average prepatent period (PP, Table 3), the parasitemia asymptote (PAasym=S_{1i}, equation 4, Table 3), the parasitemia medium parameter (PAmed=S_{2i}, equation 4, Table 3), and the average maximum parasitemia (PAmax, Table 3). The Spearman rank correlation test was performed between these seven variables. Principal component analysis (PCA) was implemented on these variables using the Ade4 R package (Chessel, 2004), and a hierarchical clustering was performed using the Ward distance.

Results

The control group did not display any mortality. PCV and weight remained stable during the experiment. One of the 20 Balb/C mice died promptly after inoculation with the *T. b. gambiense* strain T33_1_7, and was removed from the analyses. All mice inoculated with the 17_3_SANG, 17_3_SUC, B4_C191, B4_F303 and Yenb17_4 strains exhibited positive parasitemia during the follow-up. Some mice (from 1 to 5 depending on the strain) from the eight remaining strains did not exhibitany detectable parasites (Table 2). These mice were removed from subsequent analyses, as a precaution in case they had not been infected.

Mortality analysis

Eight strains displayed a 100% mortality rate before the end of the experiment (17_3_SANG, 17_3_SUC, B4_I315, CB5_1, S14_5_1, S7_2_2, T33_1_7 and Yenb17_4).

Strain 68_6 was the only strain during the 30-day follow-up that did not display any mortality. Intermediate profiles with partial mortality (from 7 to 16 mice) were observed in the four remaining strains (Table 2, Figure 1). The global survival analysis showed significantly different mortality rates between mice infected by the 13 strains (*P*-value $<10^{-5}$). A paired comparison of the strains was performed with the moderately pathogenic strain B4_I315 chosen as a reference, indicating that four strains (B4_C191, B4_F303, Ko117_2 and 68_6) induced a significantly later mortality, whereas three strains (17_3_SUC, S14_5_1 and Yenb17_4) elicited an earlier mortality (Table 2). The median survival times of the five remaining strains (17_3_SANG, CB5_1, MBONR1, S7_2_2 and T33_1_7) were not significantly different from B4_I315.

PCV and weight

Significant reduction in PCV and weight were observed in all strains, except for 68_6 and Ko117_2 (indicating that the slopes of PCV and weight for these strains were not significantly different from 0). The statistical analysis of the PCV slope revealed that four strains (17_3_SANG, MBONR1, S14_5_1 and S7_2_2) were not significantly different from B4_I315, whereas four strains (17_3_SUC, 68_6, Ko117_2 and Yenb17_4) displayed a significantly more gradual decline in PCV than B4_I315, and four strains (B4_C191, B4_F303, CB5_1 and T33_1_7) had a marked reduction in PCV. Concerning the decrease in weight, two strains (17_3_SANG and S7_2_2) were not significantly different from B4_I315, four strains (68_6, B4_C191, B4_F303 and Ko117_2) displayed a more gradual decline in weight than B4_I315, and six strains (17_3_SUC, CB5_1, MBONR1, S14_5_1, T33_1_7 and Yenb17_4) exhibited a more robust weight loss (Table 2).

Analyses of parasitemia

The average prepatent period was estimated between 3.53 and 15.4 days (Table 3). The comparative analysis using B4_I315 as a reference revealed that six strains (S14_5_1, T33_1_7, 17_3_SUC, Yenb17_4, B4_F303 and B4_C191) had a significantly earlier onset of parasites in the blood, whereas two strains (68_6 and Ko117_2) had a significantly later onset (Table 3). The average maximum parasitemia ranged from 42.47 to 1,000 million trypanosomes/ml of blood, with an estimated value of 736 (s.d. 315) for B4_I315. Six strains showed similar maximum levels of parasitemia (B4_C191, B4_F303, Ko177_2, S7_2_2, T33_1_7 and Yenb17_4), five strains displayed significantly lower maximum parasitemia values than B4_I315 (17_3_SUC, 68_6, CB5_1, MBONR1 and S14_5_1), and strain 17_3_SANG showed a higher maximum parasitemia value (Table 3). Parasitemia development was modeled by a logistic regression (Figure 2). Two parameters corresponding to the inflection point of the logistic curve, the estimated theoretical asymptotic value of the parasitemia (PAasym) and the medium parameter (PAmed), showed significant differences between strains (Table 3).

Multivariate analysis

The correlation estimates and the PCA were performed to obtain a global view of strain pathogenicity (estimated by the survival analysis, and by monitoring anemia and weight) and virulence (related to parasitemia). Early death correlated significantly with a sharp drop in weight and an early rise in parasitemia, but not as high as the recorded maximum parasitemia or the estimated asymptotic value (Table 4, Fig 3.A). Moreover, early death determined the first PCA axis, which separated strains according to their pathogenicity (Fig 3.B, left: low pathogenic strains; right: highly pathogenic strains). The second PCA axis was drawn using the

estimated maximum parasitemia (moving upwards along the axis represents higher recorded parasitemia values). Weight loss was significantly associated with an early death and, to a lesser extent, an early rise in parasitemia (Table 4). No significant correlation was found between the drop in PCV and other variables at the strain level. The clustering highlighted three groups of strains that correspond to the three PCA colors (Fig. 3B). Neither groups nor synthetic variables associated with the country of origin (non-significant *p*-values using a Wilcoxon test, with the country as an explanatory variable).

Discussion

Phenotypic diversity

This study was established to characterize the phenotypic diversity of 13 *T. b. gambiense* strains, by recording variables linked to their pathogenicity and virulence in infected Balb/C mice. We chose to use field strains collected recently from HAT in patients in West Africa, with a low number of passages in mice. Presence of mixed infections cannot be ruled out but these strains were intentionally not cloned because cloning in rodents may induce biases by selecting clones and mother strains (Penin et al., 1996; Postan et al., 1987). This is the first study to make the association, in a large number of strains, between weight, PCV, mortality, and parasitemia. Our experimental design was based on an inbred mouse model with 20 replicates per strain, in order to overcome the problems associated with host diversity. Virulence can be defined as the capacity of organisms to multiply inside a host, while pathogenicity is the ability to induce mortality and tissue lesions (Andrade et al., 1985; Devera et al., 2003; Holzmuller et al., 2008). Our analysis demonstrates that the studied strains were divided into 3 groups regarding mortality: a group with highly pathogenic strains that induced early death; another group with moderately pathogenic strains (with death occurring during the

time course of the experiment); and a final group, represented by one weakly pathogenic strain that did not provoke any death during the experiment. Early death was associated with a short prepatent period, a quick rise in parasitemia (assessed by the variable PAmed), and early weight loss. The medium pathogenicity variable was linked to a high level of parasitemia, whereas the low pathogenic strain showed a later rise and a lower level of parasitemia. Rapid weight loss was a better indicator of pathogenicity in the infection model with Balb/C mice than anemia, as highlighted by the significant correlation between weight loss and early death. Our data demonstrate two different sides to this virulence: the early rise in parasitemia, resulting in a short prepatent period; and the level of parasitemia that was reached during the infection. Furthermore, highly pathogenic strains did not allow sufficiently prolonged survival in mice so that they could exhibit high parasitemia, although they did present a short prepatent period.

Diversity in pathogenicity and virulence was previously described in laboratory rodents infected with *T. b. gambiense* stocks (Beckers et al., 1981; Frézil et al., 1979; Giroud et al., 2016; Holzmuller et al., 2008; Inoue et al., 1998; Turner et al., 1995) but with some short comings, as presented earlier. In comparison to our results, Frézil et al. (1979) observed a higher pathogenicity that was associated during passages with a shorter incubation period (Frézil et al., 1979). Holzmuller et al. (2008) also described a high variability in virulence and pathogenicity in *T. b. gambiense* strains (Holzmuller et al., 2008), and highlighted two extreme strains: a low pathogenic strain that displays a high average parasitemia, and a highly pathogenic strain with a low average parasitemia. However, they did not observe the same trend between pathogenicity and the prepatent period as reported here. Considering the results in Balb/C mice from Inoue et al. (1998), three groups of strains appear to emerge that correspond to the three groups described in our study: a group with two strains showing high pathogenicity and a short prepatent period; another group with two strains displaying medium pathogenicity and high maximum parasitemia; and a final group of four strains showing low pathogenicity

and smaller maximum parasitemia values.. Finally, Turner et al. (1995) estimated that the parasite population growth rate was a determining factor of its pathogenicity (Turner et al., 1995) (here, the authors employ the term 'virulence' to describe the pathogenic symptoms), which supports the relationship that we have highlighted between early mortality and a short prepatent period.

Molecular bases of phenotypic diversity

Importantly, our study demonstrates that host death is not elicited by the parasite concentration in the blood itself, since early death was not associated with a high absolute parasitemia value. Infected mice being inbreed, we thus hypothesize that differences at the proteomics level are responsible for pathogenicity, as well as the phenotypic diversity of different strains (Holzmuller et al., 2008). African trypanosomes are strictly extracellular and have developed efficient immune escape mechanisms to evade/manipulate the host immune response in order to complete their life cycle/transmission (Stijlemans et al., 2016). For this, they sporadically vary their main exposed membrane surface glycoprotein (termed variable surface glycoprotein or VSG) to elude antibody recognition (Pays et al., 2006). In addition, they produce a large number of biologically active molecules that are involved in causing trypanosomiasis (Igbokwe et al., 1994); these molecules can either be secreted/excreted, or they can be released during parasite death (Geiger et al., 2010). Bezie et al. (2014) and Stijlemans et al. (2016) recently reviewed some of the virulence and pathogenic factors of the parasite, such as VSG, trypanosome enzymes, B-cell mitogens, Kinesin, and T lymphocyte triggering factors (Bezie et al., 2014). Our results leads us to suspect that variety in produced VSG or other released/excreted/secreted molecules (regardless of their quantity) could induce the observed clinical diversity in mice. It is worth noting, however, that the mice were immunosuppressed in

our experimental infection model, and infection in mice model does not reflect what occurs during a natural infection in Human host. Nevertheless, the observed phenotypic differences between isolates demonstrate that these parasite strains differ in terms of their biological mechanisms at play during the infection course. The characterization of these biological pathways appears to be an attainable goal in our collection of field-isolated strains. Key determinants could then be followed up in human subjects that display contrasting resistance/susceptibility statuses, in order to assess their role in the course of a natural infection.

The results reported here and in the literature prompt the question: is the observed phenotypic diversity between parasite strains due to genetic diversity or epigenetic factors? In Holzmuller et al. (2008), phenotypic and proteomic differences were not associated with genetic diversity at microsatellites markers, suggesting that epigenetic factors contribute to phenotypic diversity. Moreover, trypanosomes are known to exhibit great plasticity in genome expression, which allows them to grow within insects or mammals (Beschin et al., 2014; Eyford et al., 2011) and within various tissues (Capewell et al., 2016; Trindade et al., 2016) and to switch their variant gylcoproteins surfaces (Horn, 2014). The genomic sequence analysis of 85 *T. brucei* isolates recently highlighted the very low genetic diversity of *T. brucei gambiense* group 1, in comparison with other *T. brucei* groups, and its monophyly, strongly suggesting that gene expression differences maybe more important in *T. brucei gambiense*. Five strains used in our current analysis (S7_2_2_, S14_5_1, B4_F303, CB5_1, Yenb17_4) were sequenced (Weir et al., 2016) and there seems to be no association between the phenotypic group highlighted here and the phylogenic tree drawn by Weir et al. (2016). Finally, combining the genomic, transcriptomic, and proteomic analyses at an intraspecific level should clarify the molecular bases of phenotypic diversity in *T. b. gambiense* strains. After the detection of phenotypic diversity in these 13 strains, the next necessary step would be to correlate this diversity to the genotype, transcriptome, and proteome.

There are also interesting questions related to the existence of this phenotypic variability in the *T. b. gambiense*, whereas it is a homogenous and recent genetic group (Weir et al., 2016). This could be partly explained by the HAT screening and treatment strategy that has been used so far to control the disease. Currently, only microscopically confirmed patients receive treatment. Passive detection is also known to essentially detect patients exhibiting severe symptoms. It can thus be speculated that the algorithms used so far have put a selective pressure on the parasite, favoring *T. b. gambiense* strains that are both weakly pathogenic and/or virulent. In light of the elimination goal, the existence of such a category of parasite strains may require the development of new algorithms and more sensitive diagnostic tools. Importantly, the effort for isolating strains from the patients in the field must be accentuated, as well as the improvement of the isolation techniques. This is essential to work on strains whose features reflect as much as possible the biological reality.

Conclusions

We have demonstrated an important phenotypic variability in pathogenicity and virulence patterns in a collection of 13 *T. b. gambiense* field isolates. The mechanisms and causes underlying this biological variability remain poorly understood. Nevertheless, further investigation will help identify the key parasite molecules involved in this process and their role in determining the outcome of infection in humans.

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Figure legends

Fig. 1: Survival curves of BalbC mice infected by 13 different strains of *Trypanosoma brucei gambiense*.

The different strains are represented by different colors and line types. DPI: Days Post Infection.

Fig 2: Parasitemia evolution for the 13 strains (in parasites/ml of blood).

Each panel represents the parasitemia evolution for all infected mice for one strain. For each *T. brucei gambiense* strain, data at the individual level are represented by different colors. DPI: Days Post Infection.

Fig 3: PCA using seven synthetic variables.

A. Correlation circle of the variables on the first factorial plan. DMw death modeled by the Kaplan-Meier estimate (the variable increases when early death occurs); PCV slope and Weight slope: slope in PCV and weight evolution (a negative slope means a fall in PCV or weight respectively); PP (average prepatent period); PAmed and PAasym (medium parameter and asymptotic value of parasitemia estimated by the logistic regression); PA max (average maximum parasitemia).
B. Projection of the 13 different *T. brucei gambiense* strains on the first factorial plan. Colors are related to the three clusters highlighted by the clustering.