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1 **Extravascular dermal trypanosomes in suspected and confirmed cases of *gambiense* Human**
2 **African Trypanosomiasis.**

3

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28 **Short title**

29 Dermal trypanosomes in gHAT cases and suspects

30

31 **Keywords**

32 Skin, reservoir, Human African Trypanosomiasis, *Trypanosoma brucei gambiense*.

33

34 **Key points**

35 (37/40 words)

36 Live trypanosomes can remain undetected in the blood of individuals seropositive for sleeping

37 sickness. Here, we show that they could be infected with parasites in their extravascular

38 dermis, highlighting the skin as a potential reservoir for trypanosomes.

39

40 **Abstract**

41 (238/250 words)

42 Background: The diagnosis of *gambiense* Human African Trypanosomiasis (gHAT) typically
43 involves two steps: a serological screen, followed by the detection of living trypanosome
44 parasites in the blood or lymph node aspirate. Live parasites can, however, remain undetected
45 in some seropositive individuals, who we hypothesize are infected with *Trypanosoma brucei*
46 *gambiense* parasites in their extravascular dermis.

47 Methods: To test this hypothesis, we conducted a prospective observational cohort study in
48 the gHAT focus of Forecariah, Republic of Guinea. Of the 5,417 subjects serologically screened
49 for gHAT, 66 were enrolled into our study and underwent a dermatological examination. At
50 enrolment, 11 seronegative, 8 unconfirmed seropositive and 18 confirmed seropositive
51 individuals had blood samples and skin biopsies taken and examined for trypanosomes by
52 molecular and immuno-histological methods.

53 Results: In seropositive individuals, dermatological symptoms were significantly more
54 frequent, relative to seronegative controls. *T.b. gambiense* parasites were present in the
55 blood of all confirmed cases (n=18) but not in unconfirmed seropositive individuals (n=8).
56 However, *T. brucei* parasites were detected in the extravascular dermis of all unconfirmed
57 seropositive individuals and all confirmed cases. Skin biopsies of all treated cases and most
58 seropositive untreated individuals progressively became negative for trypanosomes 6 and 20
59 months later.

60 Conclusions: Our results highlight the skin as a potential reservoir for African trypanosomes,
61 with implications for our understanding of this disease's epidemiology in the context of its
62 planned elimination and underlining the skin as a novel target for gHAT diagnostics.

63 **Introduction**

64 The number of new cases of *gambiense* Human African Trypanosomiasis (gHAT or sleeping
65 sickness) has never been so low in the known epidemiological history of the disease, with only
66 ~1,500 new cases reported in 2017 [1, 2], and the World Health Organization (WHO) has
67 targeted gHAT elimination by 2030 [3]. This objective has been encouraged by the success of
68 active surveillance efforts that relies on a two-step diagnosis: an initial serological screen,
69 followed by microscope observation of blood, lymph or cerebrospinal fluid (CSF) to detect
70 extracellular trypanosomes and to confirm the serological diagnosis. However, some
71 seropositive individuals remain without a confirmed parasitological diagnosis for years and
72 have been recently described as being latent cases, raising the question as to whether
73 reservoirs of live parasites persist in these individuals [4].

74 *T. brucei s. l.* parasites are found in the extravascular compartment of various tissues of their
75 mammalian hosts, including the skin, albeit mostly under experimental conditions in animal
76 models rather than during the natural progression of the disease [5]. Recent studies have
77 revealed that substantial quantities of trypanosomes persist within the extravascular dermis
78 following experimental infection in mice with *T.b. gambiense* or *T. b. brucei*. These parasites
79 can be transmitted to the tsetse vector, even in the absence of detectable parasites in the
80 host's blood [6]. This study also reported a retrospective screening of archived skin biopsies
81 from a gHAT endemic region, which revealed the presence of some extravascular skin-
82 dwelling trypanosomes [6]. However, the species of these parasites was not identified and no
83 clinical records were available for the screened samples.

84 These observations raise the question as to whether *T.b. gambiense* might be found in the
85 skin of confirmed gHAT cases, as well as in unconfirmed seropositive individuals, in regions of
86 active disease transmission. To address this question, we performed a prospective

87 observational study in the Forecariah district in the Republic of Guinea, which is one of the
88 most active gHAT foci in Western Africa.

89 **Methods**

90 More details for material and methods are provided as Supplementary Data.

91 Ethical approval

92 All investigations were conducted in accordance with the Declaration of Helsinki and with the
93 approval of the National Ethical Committee of the Republic of Guinea (Study Diag-Cut-THA
94 032/CNERS/17 and amendment 038/CNERS/19).

95 Study enrolment, screening and case definitions

96 From May 2017 to February 2019, a total of 5,417 individuals were screened by the HAT
97 National Control Programme using the card agglutination test for trypanosomiasis, first on
98 whole-blood (CATTwb), then on plasma (CATTp) for validation, in 43 villages in the active gHAT
99 focus of the Forecariah District, Republic of Guinea. All subjects were classified as
100 seronegative, unconfirmed seropositive or confirmed seropositive according to the diagnostic
101 process presented in Table 1. All parasitologically confirmed cases were diagnosed and
102 treated by the HAT National Control Programme according to WHO recommendations and as
103 described previously [7]. All confirmed cases (CATTp $\geq 1/4$ with parasitological confirmation)
104 and all unconfirmed seropositive individuals (CATTp $\geq 1/4$ without parasitological
105 confirmation) were proposed for study enrolment. In total, 40 seronegative controls (39
106 CATTwb-negative and 1 CATTwb-positive CATTp $< 1/4$) were randomly selected from the 5,417
107 population, of which the first 29 individuals, enrolled in 2017, were only included in the
108 epidemiological and clinical analysis, and the last 11 individuals, enrolled in 2019, were
109 subjected to the entire protocol. Children under 16 years of age and pregnant women were

110 excluded from the study. Each participant was informed about the study's objectives and
111 provided written informed consent.

112 Field procedure and sampling

113 Participants underwent an epidemiological interview and a clinical examination, during which
114 dermatological symptoms including pruritus (skin itch) and dermatitis (skin inflammation),
115 were assessed at enrolment as well as at each subsequent follow-up at 6 and 20 months after
116 enrolment/treatment. Epidemiological and clinical parameters are detailed in Supplementary
117 Data. The absence of dermatitis lesions at the skin sampling site was verified and a 2mm
118 blood-free skin punch biopsy was sampled from the right back shoulder of all confirmed
119 seropositive cases, all unconfirmed seropositive individuals, and for the final 11 seronegative
120 controls. Touch preparations were obtained by gently rolling the biopsy on a clean glass slide
121 and Giemsa staining in the field. The positivity of a given slide was defined by the detection of
122 at least three trypanosomes. Biopsies were fixed for immuno-histochemistry and molecular
123 analyses. Plasma aliquots from blood samples were also obtained for serological trypanolysis
124 tests [8].

125 Immunohistochemical detection

126 Skin biopsy sections were stained with hematoxylin-eosin (HE) and Giemsa stains, and
127 immunolabelled with the *T. brucei*-specific anti-ISG65 antibody that targets the Invariant
128 Surface Glycoprotein 65 expressed at the surface of the mammalian host stages of *T. brucei*
129 *s.l.* parasites [9], and the *T. brucei*-specific anti-Hsp70 antibody that recognizes the
130 endoplasmic reticulum molecular chaperone heat-shock protein 70 homologue [10]. Slides
131 were blindly assessed by at least two readers. Slides from seronegative controls were mixed
132 with slides from seropositive cases in order to guarantee blind reading. The positivity of a
133 given skin-section slide was defined by the detection of at least three trypanosomes.

134 PCR detection

135 DNAs were extracted from paraffin-embedded biopsies and blood samples with tissue-specific
136 commercial kits (Qiagen, Germany). For each sample, at least two PCRs were performed with
137 TBR primers targeting a DNA satellite repeated sequence (10,000 copies per cell) [11], and
138 TgsGP primers directed against the single copy *TgsGP* gene [12], for detecting *T. brucei s. l.*
139 and *T.b. gambiense* DNAs, respectively.

140 Data analyses

141 For epidemiological, clinical and diagnostic parameters, differences between seronegative
142 controls versus unconfirmed seropositive individuals and confirmed cases were assessed
143 using the following two-sided tests at 5% confidence: Fisher's exact tests for qualitative data
144 (Tables 2 and 3) and/or Mann-Whitney tests for quantitative data (age in Table 2). For the
145 follow-up analyses, differences between results at enrolment versus results at 6 months and
146 20 months after treatment/enrolment were assessed for each group using two-sided Fisher's
147 exact tests at 5% confidence (Table 4).

148 **Results**

149 Epidemiological and clinical results

150 Results of the initial screening of 5,417 individuals are shown in Table 1. Out of 5,377
151 seronegative subjects (CATTwb-negative or CATTp<1/4), 40 were enrolled as seronegative
152 controls, of whom 11 provided skin biopsies. A total of 40 seropositive individuals (CATTwb-
153 positive and CATTp ≥1/4) were identified during the survey, of which 12 tested negative upon
154 parasitological examination (0.22%) and 28 were confirmed as HAT cases (0.52%). Eight non-
155 confirmed seropositive individuals and 18 confirmed HAT cases had no exclusion criteria and
156 accepted to be enrolled in the study.

157 As shown in Table 2, the occurrence of dermatitis was significantly more frequent in confirmed
158 HAT cases (15/18, 83%, $P < 0.0001$) and non-confirmed seropositive individuals (5/8, 63%,
159 $P = 0.0166$) as compared to seronegative controls (7/40, 18%). Pruritus was the most frequent
160 dermatological sign in confirmed HAT patients (11/18, 61%), as compared to seronegative
161 controls (3/40, 8%). Among the various observed clinical manifestations of localized
162 dermatitis, we unambiguously identified typical cases of intertrigo (in 4/18 confirmed cases
163 versus 2/40 seronegative controls), pityriasis (in 3/18 versus 2/40), scabies (in 3/18 versus
164 1/40), dermatophytosis (in 3/18 versus 1/40), molluscum (in 3/18 versus 1/40), and ulceration
165 (in 3/18 versus 1/40). The main clinical manifestation in non-confirmed seropositive
166 individuals were eczema (3/8), intertrigo (2/8) and pityriasis (1/8). Apart from general pruritus
167 and intertrigo, all dermatological signs were observed in upper regions of the body, especially
168 on the thorax and arms.

169 Biological results

170 Plasma from all confirmed and unconfirmed seropositive cases, and from 11/40 seronegative
171 controls, was assessed using the trypanolysis test, which detects complement-mediated
172 immune responses activated by *T.b. gambiense*-specific antigens. All confirmed cases were
173 positive for the LiTat 1.3 antigen, and 89% (16/18) of these cases were positive for both the
174 LiTat 1.5 and 1.6 antigens (Table 3). Only 25% (2/8) of the unconfirmed seropositive individuals
175 were positive for all antigens, while the others remained negative for all three variants, as
176 seronegative controls.

177 A skin punch biopsy was sampled from all enrolled confirmed and unconfirmed seropositive
178 cases and from 11/40 seronegative controls. Dermal touch preparations were then generated
179 in the field and full-length trypanosomes were observed on slides from 81% (13/16) of the
180 confirmed cases and from 33% (2/6) of the unconfirmed seropositive individuals (Table 3 and

181 Supplementary Fig.1). One of the unconfirmed seropositive individuals who tested positive in
182 this dermal test, also tested positive in the trypanolysis test.

183 The skin biopsy samples were processed for immunohistochemistry analyses (IHC) in the lab.
184 Skin samples obtained from the seronegative controls (11/11) did not test positive for
185 trypanosomes (Table 3). By contrast, all unconfirmed seropositive individuals (8/8) and all
186 confirmed cases (18/18) were found to be positive at least following staining by a *T. brucei*-
187 specific anti-ISG65 antibody (Fig.1, Supplementary Fig.2 and Table 3). In addition, all samples
188 from non-confirmed seropositive individuals and confirmed cases were also found to be
189 positive following either unspecific Giemsa staining and/or unspecific HE staining and/or
190 labelling with a *T. brucei*-specific anti-Hsp70 antibody (Fig.1, Supplementary Fig.2 and Table
191 3). In positive skin sections, *T. brucei* parasites were evenly distributed in the reticular dermis,
192 and were occasionally associated with edema. No other parasites were detected in any of the
193 skin samples.

194 To confirm the identity of these skin-dwelling parasites, *T. brucei*-specific PCR (TBR-PCR)
195 assays were performed on total DNA extracted from fresh blood and from paraffin-embedded
196 skin samples. Both blood and skin DNA samples from the seronegative controls (11/11) were
197 found to be negative by the TBR-PCR assays. By contrast, 100% of blood (18/18) and 78% of
198 skin samples (14/18) from confirmed cases tested positively in the TBR-PCR assays. Parasite
199 DNA was only detected in the skin of unconfirmed seropositive individuals (6/8, 75%) but not
200 in their blood (0/8) (Table 3). *T.b. gambiense*-specific TgsGP-PCR assays were performed on
201 the same DNA samples and were positive for only 67% (12/18) of the blood samples of
202 confirmed cases (Table 3). We reasoned that the use of fresh skin biopsies would be more
203 appropriate than paraffin-embedded skin samples for TgsGP-PCR due to the low sensitivity of
204 this method targeting a single-copy gene. To test this hypothesis, we obtained fresh skin

205 samples from an outgroup of nine additional confirmed cases, who were identified in 2018 in
206 the same district by using the same study protocol (Supplementary Table 1). The fresh skin
207 samples from 89% (8/9) of these confirmed cases were found positive to TBR-PCR, and 33%
208 (3/9) were also found positive to TgsGP-PCR (Supplementary Table 1).

209 Follow-up results

210 The same panel of analyses were repeated at 6 and 20 months after study enrolment of the
211 unconfirmed seropositive individuals or after treatment of the confirmed cases (Table 4). In
212 total, 17/18 and 12/18 confirmed cases were followed-up at 6 months and 20 months after
213 treatment, respectively, with 12/18 confirmed cases followed-up 2 times, 5/18 followed-up
214 one time and 1 loss to follow-up (Table 4). Most of the clinical symptoms associated with the
215 stage-2 cases at enrolment, including dermatological signs, significantly decreased in
216 frequency during the first 6 months after treatment. Whereas all parasitological observations
217 and PCR results became negative within 6 months after treatment in all confirmed cases
218 (17/17), trypanosomes were still detected by histological methods in up to 38% of them (5/13
219 by IHC anti-ISG65). Twenty months after treatment, all CATTp and histological tests became
220 negative (12/12), with 2/3 confirmed cases remaining positive to the trypanolysis test (Table
221 4).

222 In total, 5/8 and 4/8 unconfirmed seropositive individuals were followed-up at 6 months and
223 20 months after enrolment, respectively, with 4/8 unconfirmed seropositive individuals
224 followed-up 2 times, 1/8 followed-up one time and 3/8 losses to follow-up (one death, one
225 pregnancy and one resignation) (Table 4). In 80% (4/5) of the unconfirmed seropositive
226 individuals who were monitored after enrolment, dermatological signs progressively
227 disappeared (Table 4). The four unconfirmed seropositive individuals who were negative to
228 the trypanolysis test at enrolment, became negative to CATTp, TBR-PCR on skin and IHC anti-

229 ISG65 at the same period. In contrast, the only trypanalysis-positive individual who could be
230 monitored at 6 months maintained a serological reactivity to CATTp. No parasite DNA was
231 detected by TBR-PCR in either blood or skin but the skin biopsy remained positive by IHC-
232 ISG65. Although this individual was lost to follow-up for the 20-month time-point, he was
233 diagnosed as a stage 1 case (CATTp 1/8, mAECT-BC +, CSF - and WBC 4) during an active
234 surveillance campaign that was led in November 2019 (i.e. after the end of this study) and was
235 treated accordingly.

236 **Discussion**

237 Here, we set out to investigate whether *T.b. gambiense* parasites might be found in the skin
238 of confirmed gHAT cases, as well as in unconfirmed seropositive individuals, in regions of
239 active disease transmission. Although this study is somewhat limited to a restricted population
240 and to the detection methods used, 100% of the confirmed cases and unconfirmed
241 seropositive subjects were found to carry extravascular trypanosomes in their skin.

242 Dermatological signs in gHAT

243 Our results indicate that dermatological symptoms might be an important aspect of gHAT's
244 clinical presentation. The few reports that exist on this topic in the literature describe a wide
245 array of skin pathologies associated with sleeping sickness, including pruritus, chancre, rashes
246 and localized edemas [13, 14]. However, detailed dermatological profiles of HAT cases have
247 mostly been derived from light-skinned travelers with imported HAT [14]. Whereas chancres
248 and rashes remain anecdotal, pruritus was the most commonly observed dermatological sign
249 in endemic cases (in up to 57% of stage-2 cases) [14]. Here, we observed a higher occurrence
250 of pruritus and dermatitis in unconfirmed seropositive individuals and in confirmed cases,
251 relative to seronegative controls (Table 2). The observed dermatitis profiles included some

252 conditions the etiologies of which might not be directly related to a trypanosome infection.
253 However, it could be hypothesized that the immune status of the infected host skin is
254 somehow altered by the presence of trypanosomes in a way that promotes the outcome of
255 dermatitis caused by other pathogens and/or increases skin sensitivity.

256 Trypanosome detection

257 The direct detection of trypanosomes in the human skin is not well documented in the
258 literature [13]. As there is no gold-standard approach for that purpose, we implemented seven
259 distinct molecular and immuno-histological methods in parallel, yet with their own specific
260 strengths and weaknesses. Here, dermal touch preparations were generated in the field in
261 sub-optimal ambient conditions (31°C at 75% humidity on average), which could explain the
262 unusual morphology of some trypanosomes that were probably altered by osmotic shock
263 while drying. Then, only a limited portion of each parasite is visible in the 2.5µm skin sections
264 because entire trypanosomes do not necessarily lie in the section plan. For the same reason,
265 the parasite nucleus, kinetoplast and flagellum are rarely all visible in the same given cell
266 section. However, the specificities of the anti-ISG65 and anti-Hsp70 antibodies enable to
267 unambiguously detect most *T. brucei* parasites within the extracellular dermal matrix, and this
268 is confirmed by TBR-PCR assays. Considering that *T.b. brucei* are non-infectious to humans and
269 killed within a couple of hours by human serum, the dermal parasites detected here, at least
270 in confirmed cases, are likely to be *T.b. gambiense* parasites, as confirmed by the positivity of
271 some direct TgsGP-PCR assays performed on fresh skin samples from an outgroup. However,
272 further genetic studies would be necessary to rule out the hypothesis of infections with a
273 peculiar *T.b. brucei* strain.

274 The detection of skin-dwelling parasites at enrolment in most of the 2mm skin punch biopsies
275 sampled from seropositive individuals indicates that skin-dwelling parasites might be present

276 over a considerable proportion of the skin surface. However, the precise dynamics of parasite
277 load and distribution in the extravascular dermal compartment over the course of an infection
278 remains unknown. According to historic (reviewed in [5]) and more recent [6] studies in
279 experimental animal models, skin-dwelling parasites could theoretically be detected in almost
280 the entire skin surface, yet with a variable distribution and at variable local densities.

281 A dermal reservoir of trypanosomes in non-confirmed seropositive individuals

282 One possible explanation for the persistence of disease foci in certain regions is the presence
283 of animal reservoirs [15]. Another possibility, as increasing evidence suggests, is that
284 traditionally used diagnostic approaches do not detect some *T.b. gambiense* infections among
285 seropositive cases [15]. Indeed, bloodstream parasite numbers in *T.b. gambiense* infections
286 can periodically fluctuate to less than 100 trypanosomes/ml, falling below the detection limit
287 of the most sensitive methods currently in use [16]. Another study estimated that 20-30% of
288 gHAT cases are missed in active case detection by standard parasitological techniques and are
289 left untreated [17]. These infected individuals might ultimately progress to clinical disease or
290 remain almost asymptomatic until undergoing a possible self-cure [15].

291 Here, routine molecular analyses confirmed the presence of *T. brucei* parasites in the skin of
292 unconfirmed seropositive individuals, including those testing negative to the LiTat 1.3
293 trypanolysis test known to be highly specific of *T.b. gambiense*. As previously observed in the
294 same transmission focus [7], trypanolysis-negative individuals rapidly became negative to
295 CATTp and this was associated with the disappearance of detectable dermal trypanosomes.
296 In such subjects, dermal infections could be transient and too short to allow *T.b. gambiense*
297 to invade the bloodstream and express the LiTat 1.3 antigen. Alternatively, these infections
298 could possibly be caused by other trypanosome species cross-reacting with the CATT. More
299 sensitive and extensive molecular analyses will be required to solve this question.

300 Only two unconfirmed seropositive individuals were positive to the LiTat 1.3 trypanolysis test
301 in this study. One died before the first follow-up and the other was lost to follow-up after six
302 months. Nevertheless, it is noteworthy that this last individual, who was still positive to
303 histological tests at six months, was eventually diagnosed as a stage 1 case during a medical
304 survey almost 2.5 years after enrolment. Systematic characterization and follow-up of dermal
305 trypanosomes in unconfirmed seropositive individuals testing positive to the LiTat 1.3
306 trypanolysis test would be required to better address the role of these individuals in the
307 transmission of *T.b. gambiense*.

308 Transmission and epidemiological contribution of dermal trypanosomes

309 Mathematical modelling recently predicted that, in the absence of any animal reservoirs,
310 these unconfirmed seropositive individuals could contribute to disease transmission by
311 maintaining an overlooked reservoir of skin-dwelling parasites [18]. The infected skin of
312 seropositive unconfirmed individuals could provide a population of parasites that are readily
313 accessible to the tsetse fly. Indeed, this mode of transmission has been demonstrated in
314 experimental animal models, in which skin-dwelling trypanosomes were efficiently
315 transmitted to the tsetse vector, even in the absence of detectable parasitemia [6, 19, 20].
316 However, the presence of the stumpy parasite forms that are assumed to be most adapted
317 for development in tsetse flies were not investigated here. This is an important question for
318 future studies to address, in order to estimate the actual infectivity potential of human skin-
319 dwelling parasites. Our reported observations should also be confirmed in a larger number of
320 unconfirmed seropositive individuals (including RDT-positive subjects), and the study scaled-
321 up to include other endemic transmission foci in Africa, in order to confidently determine the
322 actual prevalence of dermal trypanosomes.

323 Our results raise questions about the strategies used to diagnose this disease, which currently
324 focus on detecting parasites in the blood and lymph. If the human skin is indeed a reservoir
325 for trypanosomes, it could represent a novel target for diagnostics, and it could: (i) allow more
326 carriers to be treated; (ii) help to determine a more accurate estimate of the true prevalence
327 of the disease; and (iii) help to identify as yet undetected reservoirs in both human and animal
328 populations. The development of less invasive and field-adapted diagnostic methods to detect
329 extravascular dermal trypanosomes, such as the serological detection of skin-related
330 biomarkers or the identification of specific bio-physical profiles by skin scanning, would
331 benefit to these goals. The current WHO recommendation, based on risk-benefit analyses, is
332 to not treat unconfirmed seropositive individuals without knowing if they have an active
333 infection [1]. Importantly, we observed that the routinely administered trypanocide
334 treatments (Pentamidine for stage-1 and NECT for stage-2 cases) efficiently targeted both
335 bloodstream and dermal trypanosomes in all the patients followed-up over 20 months. With
336 the promise of new cheaper, less toxic and easier to administer drugs on the horizon, the
337 policy of treating unconfirmed seropositive individuals could possibly be reconsidered.
338 Indeed, the new drug Acoziborole, that requires a single oral administration, could hopefully
339 be the next revolutionary treatment against gHAT. As gHAT approaches its elimination targets,
340 we propose from our findings that the current algorithms, used to identify and manage
341 disease cases, could be adapted to include the detection of skin-dwelling parasites, which
342 likely represent a previously unaccounted for anatomical reservoir.

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357 **Author contributions**

358 MarC, AMS and NRKS conducted the clinical study in the field and commented on the
359 manuscript. HI, IS, CT, CC, ACo, ACr, OC, ECA and JMB performed sample analyses and
360 commented on the manuscript. MamC and VJ held logistical aspects, analyzed part of the data
361 and commented on the manuscript. AML, BB and BR designed the study, organized logistical
362 aspects, analyzed the data and wrote the manuscript as co-last authors.

363 **Competing interest**

364 All authors declare no financial relationships with any organizations that might have an
365 interest in the submitted work in the previous three years, no other relationships nor activities
366 that could appear to have influenced the submitted work, and no other relationships or
367 activities that could appear to have influenced the submitted work.

368 **Data and material availability**

369 Upon request, the original protocol and associated forms, as well as an anonymized dataset,
370 could be obtained from the corresponding author rotureau@pasteur.fr.

371 **Transparency statement**

372 The lead author affirms that the manuscript is an honest, accurate and transparent account
373 of the study being reported, that no important aspect of the study has been omitted, and that
374 any discrepancies from the study as originally planned have been explained.

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427

428 **Figures**

429 **Fig. 1. Extravascular trypanosomes in the dermal matrix of human skin biopsies.**

430 For each enrolled study subject, paraffin-embedded skin biopsy sections were stained either
431 with (A) a specific anti-ISG65 antibody (brown) or (B) with Giemsa (purple) and screened at a
432 100x magnification. Representative trypanosome sections from confirmed stage-1 (subject
433 1044) and stage-2 cases (subjects 1035, 1036, 1037, 1039 and 1042), as well as from
434 unconfirmed seropositive individuals (subjects 1046, 1065 and 1066) are shown. The scale

435 bars represent 10µm. More images of extravascular *T. brucei* parasites in human skin biopsies
436 are available in Supplementary Fig2.

437 **Tables**

438 **Table 1. Diagnostic process, number of subjects and results.**

439 CATTwb / CATTp: card agglutination test for trypanosomiasis on whole blood / plasma; mAECT
440 BC / LN aspirate: mini anion-exchange column technique on buffy coat / lymph node aspirate;
441 WBC: white blood cells; CSF: cerebrospinal fluid; ND: not determined. *Highest plasma
442 dilution with a positive result.

443

Groups	Diagnostic process				No. subjects		
	1- Serological screening	2- Serological validation	3- Parasitological confirmation	4- Staging	Screened	Enrolled	Followed-up
	CATTwb / RDT	CATTp*	mAECT BC / LN aspirate observation	Parasites in CSF No. WBC in CSF			
Seronegative	- +	ND < 1/4	ND	ND	5 377	40	0
Seropositive	+	\geq 1/4	-	ND	12	8	5
Stage 1				no	8	4	4
Stage 2	+	\geq 1/4	+	yes	18	14	13
ND				ND	2	0	0
All					28	18	17
Total					5 417	66	22

445 **Table 2. Epidemiological and clinical characteristics of case subjects.**

446 For each group and each parameter, total values correspond to the numbers of subjects for
447 which a value was available (n/total). p values were obtained by comparing one by one the
448 parameters of each group of seropositive subjects (unconfirmed and all confirmed) to those
449 of seronegative controls using two-sided Fisher's exact tests or * two-sided Mann-Whitney
450 tests at 5% confidence. LN: lymph nodes.

451

Parameters	Groups (n=66)						
	Seronegative (n=40)	Seropositive (n=8)		Confirmed (n=18)			
		n/total (%) or mean (SD)	n/total (%) or mean (SD)	p values	Stage 1 (n=4)	Stage 2 (n=14)	All (n=18)
	n/total (%) or mean (SD)	n/total (%) or mean (SD)	p values	n/total (%) or mean (SD)	n/total (%) or mean (SD)	n/total (%) or mean (SD)	p values
Epidemiological							
Age (n=66)	37.9 (14)	36.6 (18)	0.7647*	31.0 (17)	35.6 (15)	34.6 (15)	0.3502*
Male sex (n=66)	22/40 (55%)	3/8 (38%)	0.4538	2/4 (50%)	5/14 (36%)	7/18 (39%)	0.3950
HAT case(s) in the family since 2010 (n=65)	11/40 (28%)	2/7 (29%)	>0.9999	2/4 (50%)	5/14 (36%)	7/18 (39%)	0.5404
Occupational risk (n=66)	17/40 (43%)	4/8 (50%)	0.7155	2/4 (50%)	5/14 (36%)	7/18 (39%)	>0.9999
Clinical							
Swollen LN (n=65)	5/39 (13%)	6/8 (75%)	0.0010	4/4 (100%)	13/14 (93%)	17/18 (94%)	< 0.0001
Any dermatological symptoms (n=66)	8/40 (20%)	5/8 (63%)	0.0252	4/4 (100%)	13/14 (93%)	17/18 (94%)	< 0.0001
Dermatitis (n=66)	7/40 (18%)	5/8 (63%)	0.0166	4/4 (100%)	11/14 (79%)	15/18 (83%)	< 0.0001
Pruritus (n=66)	3/40 (8%)	2/8 (25%)	0.1887	0/4 (0%)	11/14 (79%)	11/18 (61%)	< 0.0001
Asthenia (n=65)	17/39 (44%)	4/8 (50%)	>0.9999	4/4 (100%)	14/14 (100%)	18/18 (100%)	< 0.0001
Fever (n=63)	6/38 (16%)	1/7 (14%)	>0.9999	2/4 (50%)	9/14 (64%)	11/18 (61%)	0.0013
Weight loss (n=61)	6/39 (15%)	3/8 (38%)	0.1672	2/4 (50%)	6/10 (60%)	8/14 (57%)	0.0046
Eating disorders (n=66)	4/40 (10%)	1/8 (13%)	>0.9999	0/4 (0%)	7/14 (50%)	7/18 (39%)	0.0250
Headache (n=65)	23/39 (59%)	6/8 (75%)	0.6918	3/4 (75%)	13/14 (93%)	16/18 (89%)	0.0322
Circadian rhythm disruptions (n=66)	3/40 (8%)	1/8 (13%)	0.5303	0/4 (0%)	5/14 (36%)	5/18 (28%)	0.0925
Sexual dysfunctions (n=65)	4/39 (10%)	1/8 (13%)	>0.9999	0/4 (0%)	5/14 (36%)	5/18 (28%)	0.1236
Behaviour changes (n=63)	4/39 (10%)	0/7 (0%)	>0.9999	0/4 (0%)	3/13 (23%)	3/17 (18%)	0.6624

453 **Table 3. Serological, molecular and histological analysis results from blood and skin samples.**

454 For each group and each parameter, total values correspond to the numbers of subjects for
455 which a value was available (n/total). p values were obtained by comparing one by one the
456 parameters of each group of seropositive subjects (unconfirmed and all confirmed) to those
457 of seronegative controls using two-sided Fisher's exact tests at 5% confidence. VAT: variable
458 antigen type; PCR: polymerase chain reaction; TgsGP: *Trypanosoma brucei gambiense* surface
459 glycoprotein; HE: haematoxylin-eosin; IHC: immuno-histochemistry; Hsp70: heat shock
460 protein 70; ISG65: invariant surface glycoprotein 65; ND: not determined.

461

Parameters	Groups (n=37)					
	Seronegative (n=11)	Seropositive (n=8)	Confirmed (n=18)			p values
			Stage 1 (n=4)	Stage 2 (n=14)	All (n=18)	
	n/total (%)	n/total (%)	n/total (%)	n/total (%)	n/total (%)	p values
Trypanolysis						
LiTat 1.3 positive (n=36)	0/10 (0%)	2/8 (25%)	4/4 (100%)	14/14 (100%)	18/18 (100%)	<0.0001
LiTat 1.5 positive (n=36)	0/10 (0%)	2/8 (25%)	4/4 (100%)	12/14 (86%)	16/18 (89%)	<0.0001
LiTat 1.6 positive (n=36)	0/10 (0%)	2/8 (25%)	4/4 (100%)	12/14 (86%)	16/18 (89%)	<0.0001
Positive for all VATs (n=36)	0/10 (0%)	2/8 (25%)	4/4 (100%)	12/14 (86%)	16/18 (89%)	<0.0001
Negative for all VATs (n=36)	10/10 (100%)	6/8 (75%)	0/4 (0%)	0/14 (0%)	0/18 (0%)	<0.0001
PCR on blood						
TBR positive (n=37)	0/11 (0%)	0/8 (0%)	4/4 (100%)	14/14 (100%)	18/18 (100%)	<0.0001
TgsGP positive (n=37)	0/11 (0%)	0/8 (0%)	2/4 (50%)	10/14 (71%)	12/18 (67%)	0.0004
Negative for all PCRs on blood (n=37)	11/11 (100%)	8/8 (100%)	0/4 (0%)	0/14 (0%)	0/18 (0%)	<0.0001
PCR on skin						
TBR positive (n=37)	0/11 (0%)	6/8 (75%)	1/4 (25%)	13/14 (93%)	14/18 (78%)	<0.0001
TgsGP positive (n=37)	0/11 (0%)	0/8 (0%)	0/4 (0%)	0/14 (0%)	0/18 (0%)	>0.9999
Negative for all PCRs on skin (n=37)	11/11 (100%)	2/8 (25%)	3/4 (75%)	1/14 (7%)	4/18 (22%)	<0.0001
Histology						
Dermal touchpreps (n=22, 3 reads)	ND	2/6 (33%)	1/3 (33%)	12/13 (92%)	13/16 (81%)	
HE section (n=36, 1 read)	0/11 (0%)	6/8 (75%)	4/4 (100%)	8/13 (62%)	12/17 (71%)	0.0003
Giemsa section (n=37, 2 reads)	0/11 (0%)	4/8 (50%)	0/4 (0%)	14/14 (100%)	14/18 (78%)	<0.0001
IHC Hsp70 (n=31, 1 read)	0/11 (0%)	1/4 (25%)	1/4 (25%)	11/12 (92%)	12/16 (75%)	0.0002
IHC ISG65 (n=37, 3 reads)	0/11 (0%)	8/8 (100%)	4/4 (100%)	14/14 (100%)	18/18 (100%)	<0.0001
Negative for all reads (n=37)	11/11 (100%)	0/8 (0%)	0/4 (0%)	0/17 (0%)	0/18 (0%)	<0.0001

463 **Table 4. Clinical, serological, molecular and histological follow-up analyses at 6 and 20**
464 **months after enrolment.**

465 Total values correspond to the numbers of subjects for which a value was available (n/total).
466 For each group of subjects, p values were obtained by comparing one by one the parameters
467 recorded at 6 months and 20 months after treatment/enrolment to those obtained at
468 enrolment, using two- sided Fisher's exact tests at 5% confidence. LN: lymph nodes; CATT:
469 card agglutination test for trypanosomiasis; VAT: variable antigen type; PCR: TBR polymerase
470 chain reaction; Hsp70: heat shock protein 70; ISG65: invariant surface glycoprotein 65; ND:
471 not determined.

Parameters	Confirmed																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
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	Enrollment	6 months	20 months	Enrollment	6 months	20 months	Enrollment	6 months	20 months	Enrollment	6 months	20 months	Enrollment	6 months	20 months																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																															
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Clinics																	Asthenia	3/5 (60%)	1/5 (20%)	0.5238	4/4 (100%)	0.4 (0%)	0.0286	4/4 (100%)	0.4 (0%)	0.0286	2/3 (67%)	0.4286	13/13 (100%)	3/13 (23%)	0.0001	3/9 (33%)	0.0011	Swollen LN	4/5 (80%)	1/4 (25%)	0.2063	4/4 (100%)	2/3 (67%)	0.4286	4/4 (100%)	2/3 (67%)	0.4286	2/3 (67%)	0.4286	12/13 (92%)	2/11 (18%)	0.0005	3/9 (33%)	0.0066	Any dermatological symptoms	4/5 (80%)	1/5 (20%)	0.2063	4/4 (100%)	1/4 (25%)	0.2063	4/4 (100%)	1/4 (25%)	0.1429	0.3 (0%)	0.0286	12/13 (92%)	5/13 (38%)	0.0112	3/9 (33%)	0.0066	Fever	1/5 (20%)	1/5 (20%)	>0.9999	2/4 (50%)	0.4 (0%)	0.4286	2/4 (50%)	0.4 (0%)	0.4286	0.3 (0%)	0.4286	9/13 (69%)	0.13 (0%)	0.0005	1/9 (11%)	0.0115	Headache	5/5 (100%)	2/5 (40%)	0.1667	3/4 (75%)	0.4444	3/4 (75%)	0.4444	3/4 (75%)	0.1429	0.3 (0%)	0.1429	12/13 (92%)	2/13 (15%)	0.0002	4/9 (44%)	0.0231	Pruritus	1/5 (20%)	0/5 (0%)	>0.9999	0.4 (0%)	0.4 (0%)	>0.9999	0.4 (0%)	0.4 (0%)	>0.9999	0.3 (0%)	>0.9999	10/13 (77%)	3/13 (23%)	0.0169	2/9 (22%)	0.0274	Weight loss	2/5 (40%)	2/5 (40%)	>0.9999	2/4 (50%)	1/4 (25%)	>0.9999	2/4 (50%)	1/4 (25%)	>0.9999	0.3 (0%)	0.4286	5/9 (56%)	0.13 (0%)	0.0048	0.9 (0%)	0.0294	Dermatitis	4/5 (80%)	1/5 (20%)	0.2063	4/4 (100%)	0.4 (0%)	0.2063	4/4 (100%)	0.4 (0%)	0.1429	0.3 (0%)	0.0286	10/13 (77%)	5/13 (38%)	0.1107	3/9 (33%)	0.0789	Eating disorders	1/5 (20%)	1/5 (20%)	>0.9999	0.4 (0%)	0.4 (0%)	>0.9999	0.4 (0%)	0.4 (0%)	>0.9999	0.3 (0%)	>0.9999	6/13 (46%)	0.13 (0%)	0.0149	1/9 (11%)	0.1649	Sexual dysfunctions	1/5 (20%)	3/5 (60%)	0.5238	0.4 (0%)	0.4 (0%)	>0.9999	0.4 (0%)	0.4 (0%)	>0.9999	0.3 (0%)	>0.9999	5/13 (38%)	5/13 (38%)	>0.9999	1/9 (11%)	0.3330	Circadian rhythm disruptions	1/5 (20%)	1/5 (20%)	>0.9999	0.4 (0%)	0.4 (0%)	>0.9999	0.4 (0%)	0.4 (0%)	>0.9999	0.3 (0%)	>0.9999	4/13 (31%)	1/13 (8%)	0.3217	1/9 (11%)	0.3602	Behaviour changes	0/4 (0%)	1/5 (20%)	>0.9999	0.4 (0%)	0.4 (0%)	>0.9999	0.4 (0%)	0.4 (0%)	>0.9999	0.3 (0%)	>0.9999	2/12 (17%)	0.13 (0%)	0.2200	0.9 (0%)	0.4857	Diagnosis																	CATTwb	5/5 (100%)	4/5 (80%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	2/3 (67%)	0.4286	13/13 (100%)	10/13 (77%)	0.2200	4/9 (44%)	0.0048	CATTp	5/5 (100%)	1/5 (20%)	0.0476	4/4 (100%)	0.4 (0%)	0.0286	4/4 (100%)	0.4 (0%)	0.0286	1/3 (33%)	0.1429	13/13 (100%)	3/13 (23%)	0.0001	0.9 (0%)	<0.0001	Parasitology	0/5 (0%)	0/5 (0%)	>0.9999	4/4 (100%)	0.4 (0%)	0.0286	4/4 (100%)	0.4 (0%)	0.0286	0.3 (0%)	0.0286	13/13 (100%)	0.13 (0%)	<0.0001	0.9 (0%)	<0.0001	Trypanolysis																	LiTat 1.3 positive	1/5 (20%)	1/5 (20%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	3/3 (100%)	>0.9999	13/13 (100%)	10/11 (91%)	0.2200	5/9 (56%)	0.0172	LiTat 1.5 positive	1/5 (20%)	1/5 (20%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	2/3 (67%)	0.4286	11/13 (85%)	8/11 (73%)	0.6299	5/9 (56%)	0.1778	LiTat 1.6 positive	1/5 (20%)	1/5 (20%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	0.3 (0%)	0.0286	11/13 (85%)	6/11 (54%)	0.1819	0.9 (0%)	0.0002	Positive for all VATs	1/5 (20%)	1/5 (20%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	0.3 (0%)	0.0286	11/13 (85%)	6/11 (54%)	0.1819	0.9 (0%)	0.0002	Negative for all VATs	4/5 (80%)	4/5 (80%)	>0.9999	0.4 (0%)	0.4 (0%)	>0.9999	0.4 (0%)	0.4 (0%)	>0.9999	0.3 (0%)	>0.9999	0/13 (0%)	1/11 (9%)	0.4583	3/9 (33%)	0.0545	TBR PCR																	Blood	0/5 (0%)	0/5 (0%)	>0.9999	4/4 (100%)	0.4 (0%)	0.0286	4/4 (100%)	0.4 (0%)	0.0286	0.3 (0%)	0.0286	13/13 (100%)	0.13 (0%)	<0.0001	0.9 (0%)	<0.0001	Skin	3/5 (60%)	0/5 (0%)	0.1667	1/4 (25%)	0.4 (0%)	>0.9999	1/4 (25%)	0.4 (0%)	>0.9999	0.3 (0%)	>0.9999	12/13 (92%)	0.13 (0%)	<0.0001	0.9 (0%)	<0.0001	Histology																	Dermal touchpreps	1/3 (33%)	0/2 (0%)	>0.9999	1/3 (33%)	1/4 (25%)	>0.9999	1/3 (33%)	1/4 (25%)	>0.9999	ND	ND	11/12 (92%)	2/13 (15%)	0.0002	ND	ND	IHC Hsp 70	1/4 (25%)	ND	>0.9999	1/4 (25%)	ND	>0.9999	1/4 (25%)	ND	>0.9999	0.3 (0%)	>0.9999	11/12 (92%)	ND	ND	0.9 (0%)	<0.0001	IHC ISG65	5/5 (100%)	1/5 (20%)	0.0476	4/4 (100%)	0.4 (0%)	0.0286	4/4 (100%)	0.4 (0%)	0.0286	0.3 (0%)	0.0286	13/13 (100%)	5/13 (38%)	0.0016	0.9 (0%)	<0.0001	Negative for all reads	0/5 (0%)	2/5 (40%)	0.4444	4/4 (100%)	0.0079	0.0079	0.4 (0%)	2/4 (50%)	0.4286	2/3 (67%)	0.1429	0/13 (0%)	1/13 (8%)	>0.9999	9/9 (100%)	<0.0001
Asthenia	3/5 (60%)	1/5 (20%)	0.5238	4/4 (100%)	0.4 (0%)	0.0286	4/4 (100%)	0.4 (0%)	0.0286	2/3 (67%)	0.4286	13/13 (100%)	3/13 (23%)	0.0001	3/9 (33%)	0.0011																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
Swollen LN	4/5 (80%)	1/4 (25%)	0.2063	4/4 (100%)	2/3 (67%)	0.4286	4/4 (100%)	2/3 (67%)	0.4286	2/3 (67%)	0.4286	12/13 (92%)	2/11 (18%)	0.0005	3/9 (33%)	0.0066																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
Any dermatological symptoms	4/5 (80%)	1/5 (20%)	0.2063	4/4 (100%)	1/4 (25%)	0.2063	4/4 (100%)	1/4 (25%)	0.1429	0.3 (0%)	0.0286	12/13 (92%)	5/13 (38%)	0.0112	3/9 (33%)	0.0066																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
Fever	1/5 (20%)	1/5 (20%)	>0.9999	2/4 (50%)	0.4 (0%)	0.4286	2/4 (50%)	0.4 (0%)	0.4286	0.3 (0%)	0.4286	9/13 (69%)	0.13 (0%)	0.0005	1/9 (11%)	0.0115																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
Headache	5/5 (100%)	2/5 (40%)	0.1667	3/4 (75%)	0.4444	3/4 (75%)	0.4444	3/4 (75%)	0.1429	0.3 (0%)	0.1429	12/13 (92%)	2/13 (15%)	0.0002	4/9 (44%)	0.0231																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
Pruritus	1/5 (20%)	0/5 (0%)	>0.9999	0.4 (0%)	0.4 (0%)	>0.9999	0.4 (0%)	0.4 (0%)	>0.9999	0.3 (0%)	>0.9999	10/13 (77%)	3/13 (23%)	0.0169	2/9 (22%)	0.0274																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
Weight loss	2/5 (40%)	2/5 (40%)	>0.9999	2/4 (50%)	1/4 (25%)	>0.9999	2/4 (50%)	1/4 (25%)	>0.9999	0.3 (0%)	0.4286	5/9 (56%)	0.13 (0%)	0.0048	0.9 (0%)	0.0294																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
Dermatitis	4/5 (80%)	1/5 (20%)	0.2063	4/4 (100%)	0.4 (0%)	0.2063	4/4 (100%)	0.4 (0%)	0.1429	0.3 (0%)	0.0286	10/13 (77%)	5/13 (38%)	0.1107	3/9 (33%)	0.0789																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
Eating disorders	1/5 (20%)	1/5 (20%)	>0.9999	0.4 (0%)	0.4 (0%)	>0.9999	0.4 (0%)	0.4 (0%)	>0.9999	0.3 (0%)	>0.9999	6/13 (46%)	0.13 (0%)	0.0149	1/9 (11%)	0.1649																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
Sexual dysfunctions	1/5 (20%)	3/5 (60%)	0.5238	0.4 (0%)	0.4 (0%)	>0.9999	0.4 (0%)	0.4 (0%)	>0.9999	0.3 (0%)	>0.9999	5/13 (38%)	5/13 (38%)	>0.9999	1/9 (11%)	0.3330																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
Circadian rhythm disruptions	1/5 (20%)	1/5 (20%)	>0.9999	0.4 (0%)	0.4 (0%)	>0.9999	0.4 (0%)	0.4 (0%)	>0.9999	0.3 (0%)	>0.9999	4/13 (31%)	1/13 (8%)	0.3217	1/9 (11%)	0.3602																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
Behaviour changes	0/4 (0%)	1/5 (20%)	>0.9999	0.4 (0%)	0.4 (0%)	>0.9999	0.4 (0%)	0.4 (0%)	>0.9999	0.3 (0%)	>0.9999	2/12 (17%)	0.13 (0%)	0.2200	0.9 (0%)	0.4857																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
Diagnosis																	CATTwb	5/5 (100%)	4/5 (80%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	2/3 (67%)	0.4286	13/13 (100%)	10/13 (77%)	0.2200	4/9 (44%)	0.0048	CATTp	5/5 (100%)	1/5 (20%)	0.0476	4/4 (100%)	0.4 (0%)	0.0286	4/4 (100%)	0.4 (0%)	0.0286	1/3 (33%)	0.1429	13/13 (100%)	3/13 (23%)	0.0001	0.9 (0%)	<0.0001	Parasitology	0/5 (0%)	0/5 (0%)	>0.9999	4/4 (100%)	0.4 (0%)	0.0286	4/4 (100%)	0.4 (0%)	0.0286	0.3 (0%)	0.0286	13/13 (100%)	0.13 (0%)	<0.0001	0.9 (0%)	<0.0001	Trypanolysis																	LiTat 1.3 positive	1/5 (20%)	1/5 (20%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	3/3 (100%)	>0.9999	13/13 (100%)	10/11 (91%)	0.2200	5/9 (56%)	0.0172	LiTat 1.5 positive	1/5 (20%)	1/5 (20%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	2/3 (67%)	0.4286	11/13 (85%)	8/11 (73%)	0.6299	5/9 (56%)	0.1778	LiTat 1.6 positive	1/5 (20%)	1/5 (20%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	0.3 (0%)	0.0286	11/13 (85%)	6/11 (54%)	0.1819	0.9 (0%)	0.0002	Positive for all VATs	1/5 (20%)	1/5 (20%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	0.3 (0%)	0.0286	11/13 (85%)	6/11 (54%)	0.1819	0.9 (0%)	0.0002	Negative for all VATs	4/5 (80%)	4/5 (80%)	>0.9999	0.4 (0%)	0.4 (0%)	>0.9999	0.4 (0%)	0.4 (0%)	>0.9999	0.3 (0%)	>0.9999	0/13 (0%)	1/11 (9%)	0.4583	3/9 (33%)	0.0545	TBR PCR																	Blood	0/5 (0%)	0/5 (0%)	>0.9999	4/4 (100%)	0.4 (0%)	0.0286	4/4 (100%)	0.4 (0%)	0.0286	0.3 (0%)	0.0286	13/13 (100%)	0.13 (0%)	<0.0001	0.9 (0%)	<0.0001	Skin	3/5 (60%)	0/5 (0%)	0.1667	1/4 (25%)	0.4 (0%)	>0.9999	1/4 (25%)	0.4 (0%)	>0.9999	0.3 (0%)	>0.9999	12/13 (92%)	0.13 (0%)	<0.0001	0.9 (0%)	<0.0001	Histology																	Dermal touchpreps	1/3 (33%)	0/2 (0%)	>0.9999	1/3 (33%)	1/4 (25%)	>0.9999	1/3 (33%)	1/4 (25%)	>0.9999	ND	ND	11/12 (92%)	2/13 (15%)	0.0002	ND	ND	IHC Hsp 70	1/4 (25%)	ND	>0.9999	1/4 (25%)	ND	>0.9999	1/4 (25%)	ND	>0.9999	0.3 (0%)	>0.9999	11/12 (92%)	ND	ND	0.9 (0%)	<0.0001	IHC ISG65	5/5 (100%)	1/5 (20%)	0.0476	4/4 (100%)	0.4 (0%)	0.0286	4/4 (100%)	0.4 (0%)	0.0286	0.3 (0%)	0.0286	13/13 (100%)	5/13 (38%)	0.0016	0.9 (0%)	<0.0001	Negative for all reads	0/5 (0%)	2/5 (40%)	0.4444	4/4 (100%)	0.0079	0.0079	0.4 (0%)	2/4 (50%)	0.4286	2/3 (67%)	0.1429	0/13 (0%)	1/13 (8%)	>0.9999	9/9 (100%)	<0.0001																																																																																																																																																																																																																													
CATTwb	5/5 (100%)	4/5 (80%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	2/3 (67%)	0.4286	13/13 (100%)	10/13 (77%)	0.2200	4/9 (44%)	0.0048																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
CATTp	5/5 (100%)	1/5 (20%)	0.0476	4/4 (100%)	0.4 (0%)	0.0286	4/4 (100%)	0.4 (0%)	0.0286	1/3 (33%)	0.1429	13/13 (100%)	3/13 (23%)	0.0001	0.9 (0%)	<0.0001																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
Parasitology	0/5 (0%)	0/5 (0%)	>0.9999	4/4 (100%)	0.4 (0%)	0.0286	4/4 (100%)	0.4 (0%)	0.0286	0.3 (0%)	0.0286	13/13 (100%)	0.13 (0%)	<0.0001	0.9 (0%)	<0.0001																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
Trypanolysis																	LiTat 1.3 positive	1/5 (20%)	1/5 (20%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	3/3 (100%)	>0.9999	13/13 (100%)	10/11 (91%)	0.2200	5/9 (56%)	0.0172	LiTat 1.5 positive	1/5 (20%)	1/5 (20%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	2/3 (67%)	0.4286	11/13 (85%)	8/11 (73%)	0.6299	5/9 (56%)	0.1778	LiTat 1.6 positive	1/5 (20%)	1/5 (20%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	0.3 (0%)	0.0286	11/13 (85%)	6/11 (54%)	0.1819	0.9 (0%)	0.0002	Positive for all VATs	1/5 (20%)	1/5 (20%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	0.3 (0%)	0.0286	11/13 (85%)	6/11 (54%)	0.1819	0.9 (0%)	0.0002	Negative for all VATs	4/5 (80%)	4/5 (80%)	>0.9999	0.4 (0%)	0.4 (0%)	>0.9999	0.4 (0%)	0.4 (0%)	>0.9999	0.3 (0%)	>0.9999	0/13 (0%)	1/11 (9%)	0.4583	3/9 (33%)	0.0545	TBR PCR																	Blood	0/5 (0%)	0/5 (0%)	>0.9999	4/4 (100%)	0.4 (0%)	0.0286	4/4 (100%)	0.4 (0%)	0.0286	0.3 (0%)	0.0286	13/13 (100%)	0.13 (0%)	<0.0001	0.9 (0%)	<0.0001	Skin	3/5 (60%)	0/5 (0%)	0.1667	1/4 (25%)	0.4 (0%)	>0.9999	1/4 (25%)	0.4 (0%)	>0.9999	0.3 (0%)	>0.9999	12/13 (92%)	0.13 (0%)	<0.0001	0.9 (0%)	<0.0001	Histology																	Dermal touchpreps	1/3 (33%)	0/2 (0%)	>0.9999	1/3 (33%)	1/4 (25%)	>0.9999	1/3 (33%)	1/4 (25%)	>0.9999	ND	ND	11/12 (92%)	2/13 (15%)	0.0002	ND	ND	IHC Hsp 70	1/4 (25%)	ND	>0.9999	1/4 (25%)	ND	>0.9999	1/4 (25%)	ND	>0.9999	0.3 (0%)	>0.9999	11/12 (92%)	ND	ND	0.9 (0%)	<0.0001	IHC ISG65	5/5 (100%)	1/5 (20%)	0.0476	4/4 (100%)	0.4 (0%)	0.0286	4/4 (100%)	0.4 (0%)	0.0286	0.3 (0%)	0.0286	13/13 (100%)	5/13 (38%)	0.0016	0.9 (0%)	<0.0001	Negative for all reads	0/5 (0%)	2/5 (40%)	0.4444	4/4 (100%)	0.0079	0.0079	0.4 (0%)	2/4 (50%)	0.4286	2/3 (67%)	0.1429	0/13 (0%)	1/13 (8%)	>0.9999	9/9 (100%)	<0.0001																																																																																																																																																																																																																																																																																																	
LiTat 1.3 positive	1/5 (20%)	1/5 (20%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	3/3 (100%)	>0.9999	13/13 (100%)	10/11 (91%)	0.2200	5/9 (56%)	0.0172																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
LiTat 1.5 positive	1/5 (20%)	1/5 (20%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	2/3 (67%)	0.4286	11/13 (85%)	8/11 (73%)	0.6299	5/9 (56%)	0.1778																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
LiTat 1.6 positive	1/5 (20%)	1/5 (20%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	0.3 (0%)	0.0286	11/13 (85%)	6/11 (54%)	0.1819	0.9 (0%)	0.0002																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
Positive for all VATs	1/5 (20%)	1/5 (20%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	4/4 (100%)	4/4 (100%)	>0.9999	0.3 (0%)	0.0286	11/13 (85%)	6/11 (54%)	0.1819	0.9 (0%)	0.0002																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
Negative for all VATs	4/5 (80%)	4/5 (80%)	>0.9999	0.4 (0%)	0.4 (0%)	>0.9999	0.4 (0%)	0.4 (0%)	>0.9999	0.3 (0%)	>0.9999	0/13 (0%)	1/11 (9%)	0.4583	3/9 (33%)	0.0545																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
TBR PCR																	Blood	0/5 (0%)	0/5 (0%)	>0.9999	4/4 (100%)	0.4 (0%)	0.0286	4/4 (100%)	0.4 (0%)	0.0286	0.3 (0%)	0.0286	13/13 (100%)	0.13 (0%)	<0.0001	0.9 (0%)	<0.0001	Skin	3/5 (60%)	0/5 (0%)	0.1667	1/4 (25%)	0.4 (0%)	>0.9999	1/4 (25%)	0.4 (0%)	>0.9999	0.3 (0%)	>0.9999	12/13 (92%)	0.13 (0%)	<0.0001	0.9 (0%)	<0.0001	Histology																	Dermal touchpreps	1/3 (33%)	0/2 (0%)	>0.9999	1/3 (33%)	1/4 (25%)	>0.9999	1/3 (33%)	1/4 (25%)	>0.9999	ND	ND	11/12 (92%)	2/13 (15%)	0.0002	ND	ND	IHC Hsp 70	1/4 (25%)	ND	>0.9999	1/4 (25%)	ND	>0.9999	1/4 (25%)	ND	>0.9999	0.3 (0%)	>0.9999	11/12 (92%)	ND	ND	0.9 (0%)	<0.0001	IHC ISG65	5/5 (100%)	1/5 (20%)	0.0476	4/4 (100%)	0.4 (0%)	0.0286	4/4 (100%)	0.4 (0%)	0.0286	0.3 (0%)	0.0286	13/13 (100%)	5/13 (38%)	0.0016	0.9 (0%)	<0.0001	Negative for all reads	0/5 (0%)	2/5 (40%)	0.4444	4/4 (100%)	0.0079	0.0079	0.4 (0%)	2/4 (50%)	0.4286	2/3 (67%)	0.1429	0/13 (0%)	1/13 (8%)	>0.9999	9/9 (100%)	<0.0001																																																																																																																																																																																																																																																																																																																																																																																																							
Blood	0/5 (0%)	0/5 (0%)	>0.9999	4/4 (100%)	0.4 (0%)	0.0286	4/4 (100%)	0.4 (0%)	0.0286	0.3 (0%)	0.0286	13/13 (100%)	0.13 (0%)	<0.0001	0.9 (0%)	<0.0001																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
Skin	3/5 (60%)	0/5 (0%)	0.1667	1/4 (25%)	0.4 (0%)	>0.9999	1/4 (25%)	0.4 (0%)	>0.9999	0.3 (0%)	>0.9999	12/13 (92%)	0.13 (0%)	<0.0001	0.9 (0%)	<0.0001																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
Histology																	Dermal touchpreps	1/3 (33%)	0/2 (0%)	>0.9999	1/3 (33%)	1/4 (25%)	>0.9999	1/3 (33%)	1/4 (25%)	>0.9999	ND	ND	11/12 (92%)	2/13 (15%)	0.0002	ND	ND	IHC Hsp 70	1/4 (25%)	ND	>0.9999	1/4 (25%)	ND	>0.9999	1/4 (25%)	ND	>0.9999	0.3 (0%)	>0.9999	11/12 (92%)	ND	ND	0.9 (0%)	<0.0001	IHC ISG65	5/5 (100%)	1/5 (20%)	0.0476	4/4 (100%)	0.4 (0%)	0.0286	4/4 (100%)	0.4 (0%)	0.0286	0.3 (0%)	0.0286	13/13 (100%)	5/13 (38%)	0.0016	0.9 (0%)	<0.0001	Negative for all reads	0/5 (0%)	2/5 (40%)	0.4444	4/4 (100%)	0.0079	0.0079	0.4 (0%)	2/4 (50%)	0.4286	2/3 (67%)	0.1429	0/13 (0%)	1/13 (8%)	>0.9999	9/9 (100%)	<0.0001																																																																																																																																																																																																																																																																																																																																																																																																																																																										
Dermal touchpreps	1/3 (33%)	0/2 (0%)	>0.9999	1/3 (33%)	1/4 (25%)	>0.9999	1/3 (33%)	1/4 (25%)	>0.9999	ND	ND	11/12 (92%)	2/13 (15%)	0.0002	ND	ND																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
IHC Hsp 70	1/4 (25%)	ND	>0.9999	1/4 (25%)	ND	>0.9999	1/4 (25%)	ND	>0.9999	0.3 (0%)	>0.9999	11/12 (92%)	ND	ND	0.9 (0%)	<0.0001																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
IHC ISG65	5/5 (100%)	1/5 (20%)	0.0476	4/4 (100%)	0.4 (0%)	0.0286	4/4 (100%)	0.4 (0%)	0.0286	0.3 (0%)	0.0286	13/13 (100%)	5/13 (38%)	0.0016	0.9 (0%)	<0.0001																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
Negative for all reads	0/5 (0%)	2/5 (40%)	0.4444	4/4 (100%)	0.0079	0.0079	0.4 (0%)	2/4 (50%)	0.4286	2/3 (67%)	0.1429	0/13 (0%)	1/13 (8%)	>0.9999	9/9 (100%)	<0.0001																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														