1 **_Ornithodoros savingyi_ – the tick vector of _Candidatus_ Borrelia kalaharica in**

2 **Nigeria**

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8

9 Running title: *Ca.* Borrelia kalaharica in *Ornithodoros savingyi* ticks

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25

26

27 Abstract

28 Endemic tick-borne relapsing fever (TBRF) has not been documented in Nigeria, yet
29 clinically compatible cases have been described and soft tick species are endemic in
30 surrounding countries. Consequently, our aim was to investigate if TBRF associated
31 *Borrelia* were present in Nigeria. To address this, we examined 49 soft tick pools to
32 identify the tick species and screen for *Borrelia*. The tick species was revealed by 16S
33 rRNA amplification and Sanger sequencing to be *Ornithodoros savignyi,* an
34 aggressive multi-host rapidly feeding species with significant veterinary impact. We
35 detected *Borrelia* in three of 49 pooled samples (6%). Molecular analysis of amplified
36 16S rRNA, flagellin and intragenic spacer fragments disclosed that this *Borrelia* was
37 synonymous with the recently described *Candidatus* Borrelia kalaharica described in
38 a tourist returning to Germany from South Africa. Given the widespread endemic
39 range of this tick vector, TBRF should be considered as part of the differential
40 diagnosis in patients with fever returning from arid areas of Africa and further afield.

41 **Introduction**

42 Application of molecular approaches has resulted in a knowledge explosion regarding
43 relapsing fever borreliosis. These organisms are notoriously challenging to isolate and
44 clinical cases present without characteristic diagnostic hallmarks that would alert a
45 clinician to consider relapsing fever as part of the differential diagnosis. Many cases
46 are mis-diagnosed as other conditions, more commonly encountered, such as malaria
47 (1). Once considered, it has been found that tick-borne relapsing fever (TBRF) can
48 have an alarmingly high prevalence and result in significant impact upon the health of
49 populations, particularly within developing countries such as seen in Senegal (2).
50
51 Our knowledge of both species diversity and understanding of ecology and
52 epidemiology of TBRF and their vectors is rapidly expanding (3). Typically, relapsing
53 fever spirochetes are transmitted by soft *Ornithodoros* tick species, though *B.*
54 *recurrentis*, a louse-borne relapsing fever (LBRF) and the newly recognised *B.*
55 *miyamotoi* are transmitted by clothing lice and hard *Ixodes* tick species respectively
56 and thus form notable exceptions. Epidemiological knowledge of which species are
57 prevalent in which countries is similarly evolving. Amongst this emerging knowledge,
58 new and poorly characterised species are being described, largely through
59 investigation of arthropod vectors and reservoir/accidental vertebrate species.
60 Examples include descriptions of borreliosis in bats and penguins (4-6). Whether
61 these species will have relevance for human health remains to be resolved and might
62 follow the pattern seen for *B. miyamotoi* where the spirochete was initially described
63 in 1994, but human infections were not recognised until 2011 (7). Conversely, human
64 infection can serve as a sentinel for detection of novel species. Indeed, this has
65 recently been the case for detection of a novel TBRF species endemic to Iran (8, 9),

66 and more recently with a report of a new TBRF, *Candidatus* Borrelia kalaharica in a
67 tourist returning from a holiday in Southern Africa (10). This patient from Germany
68 had clinical signs compatible with relapsing fever and raised clinical awareness in this
69 region, probably through recent introductions of LBRF amongst the influx of African
70 refugees into Germany (11, 12). What had not been determined was the tick vector
71 and consequently our understanding of the potential epidemiological spread of this
72 newly described Candidatus species.
73
74 Clinical descriptions of potential TBRF have emerged from Nigeria (local
75 newspapers/personal communication), however diagnostic methods in this resource
76 poor setting were not able to substantiate these claims. Extensive studies of
77 *Ornithodoros* tick species have not included Nigeria (13), thus leaving a knowledge
78 gap regarding presence (if any) of *Ornithodoros* ticks in Nigeria. Local Nigeria
79 studies have described an abundance of what was believed to be *Ornithodoros*
80 *moubata*, the East African vector of TBRF, describing this tick as infesting up to 80%
81 of households/animal dwellings and markets (14). Others have reported presence of
82 soft ticks belonging to *Argas persicus*, or *A. walkerae* in addition to both *O. moubata*
83 and *O. savingyi* infesting poultry in Nigeria (15). Collectively, the presence of an
84 *Ornithodoros* vector, coupled with compatible clinical cases, prompted this study to
85 investigate whether TBRF was present in Nigeria.

Materials and Methods

Tick Samples:

Ticks were collected using sieving earth using standard kitchen food sieves, from around human and animal shelters and market areas in Gubio town, Gubio LGA in Borno state region (see figure 1a and 1b). Collected ticks were pooled by life stage with 47 containing 1-5 ticks each and the remaining two pools with unspecified number of nymphal ticks. These were surface cleansed with sterile saline prior to homogenisation using a pestle and mortar. Samples were vortexed and subjected to overnight digestion with proteinase K and DNeasy ATL buffer at 56°C (20 µl and 180 µl respectively; Qiagen). Samples were again vortexed and DNA extracted according to the DNeasy kit protocol (Qiagen). DNA extracts from fifty pooled tick samples were then shipped to University of East London for molecular analysis. One sample had leaked in transit, leaving 49 for analysis. Upon receipt, sample purity was checked using nanodrop (Thermo Scientific) revealing that extracts still contained excessive protein, consequently samples were re-extracted using DNeasy kit prior to analysis.

Tick identification:

As DNA was received, morphological identification of ticks was not possible, consequently, molecular approaches were used. Various primers against tick ribosomal genes 16S and 18S, COI, Cox1, and internal transcribed spacer ITS2 used previously to characterise tick identity were employed and used according to published methods (16-20). Details of primers and their use are given in table 1. Primers described by Dupraz and team (18) were used at a final concentration of

110 500mM together with $MgCl_2$ at 2.5mM whist those described by Lv and co-workers
111 (20) were used at 300nM together with $MgCl_2$ at 1.75mM . DNA extracted from an
112 *Ixodes ricinus* tick was used as a positive control whilst nucleotide free water served
113 as a negative control. Buffer, magnesium chloride, dNTPs and recombinant Taq were
114 all supplied by Invitrogen (Fisher Scientific, UK). Amplifications were done using
115 conventional PCR using (BioRad T100™) thermocyclers, with amplicons resolved on
116 1% agarose gels stained with SybrSafe (Invitrogen) and results captured by an
117 imaging system (BioRad ChemiDoc™). Amplicons were cleaned using PCR clean-up
118 kit (Qiagen) prior to being submitted for Sanger sequencing at DBS, Durham
119 University Sequencing Service. All amplicons were sequenced in both directions.
120
121 Screening and Identification of *Borrelia*:
122 Ticks DNA extracts were screened using a real-time genus-specific PCR targeting the
123 conserved 16S gene of Borreliae (21). Briefly, primers were used at 700nM whilst the
124 HEX and BHQ-labelled probe used at 100nM; dNTPs were used at 0.2mM each
125 whilst 5mM of $MgCl_2$ was used with single strength buffer and recombinant Taq
126 (Invitrogen). Reactions were made up to a final volume of 25µl which contained 2µl
127 of template DNA. Amplification was detected using an Aria Mx1.2 (Agilent)
128 thermocycler using a hot start of 95°C for 10 minutes and 40 cycles of 95°C for 15
129 seconds and annealing at 60°C for 30 seconds.
130
131 Reactive samples were subjected to conventional PCR
132 Following amplification, samples yielding positive results were subjected to
133 conventional PCR for 16S rRNA, flagellin *fla*B, *uvr*A and intragenic spacer assays
134 (IGS) were performed as previously described and summarised in supplementary

135 table 1 (10, 22-24). Purified DNA from *Borrelia burgdorferi* sensu stricto (B31 strain)
136 was used as a positive control whilst nuclease free water served as the negative
137 control. These were subsequently prepared for Sanger sequencing as described above.
138
139 Analysis of data:
140 Resulting sequences were analysed using MEGA7 software to assess quality of
141 sequencing, produce multiple alignments and undertake phylogenetic comparisons
142 using the Neighbor-Joining algorithm with a 1000 bootstrap test of confidence (25).
143 Comparison of resulting sequences with other sequences from the GenBank
144 repository was performed by using the similarity search tool BLAST with its default
145 settings.
146
147 **Results**
148 Most assays used for tick identification remained negative, despite successful
149 amplification of the positive control, a UK-collected *I. ricinus* tick that produced an
150 amplicon of the expected size for all assays. Nanodrop assessment of samples
151 revealed poor purity that might account for variable amplification success. The
152 exception was the tick 16S assay described by Lv and co-workers which successfully
153 produced amplicons for 23 of the 49 pools [47%] (20).
154
155 Of these tick pools, a convenience sample of eight were prepared for 16S Sanger
156 sequencing. All were found to be identical over the 431 bp generated. Similarity
157 BLAST searches demonstrated that the tick identity was *Ornithodoros savingyi* the
158 "sand tampan". The compatibility of our sequences to that of *O. savingyi* are shown in

159 figure 1. A representative sequence has been deposited under accession number
160 MG256662.
161
162 In total, three of the 49 pooled tick DNA samples were positive for *Borrelia* [6.1%]
163 using the genus-specific 16S real-time screening PCR (21). Removing the two pools
164 with unspecified numbers of nymphs from analysis, based on actual numbers of ticks
165 within these pools, the minimum prevalence was 2.7% (95% confidence range -
166 0.36%-5.76%) whilst the maximum prevalence was 7.4% (95% confidence range
167 2.46%-12.34%). Attempts to further characterise this *Borrelia* by conventional PCR
168 generated amplicons from 16S rRNA (555 bp from 3 samples; representative
169 sequence MG255295; see figure 3); flagellin (749 bp from 2 samples; MG257488 and
170 MG257489; see figure 4 and figure 6); and IGS (726 bp from 2 samples;
171 representative sequence MG257909; see figure 5). A synonymous mutation was
172 detected between the two flagellin sequences at position 177 encoding lysine
173 (AAA/AAG). None of the *Borrelia*-positive DNA extracts produced an amplifiable
174 product for *uvr*A. BLAST searches *fla*B for similarity revealed that this species was
175 highly similar to *Ca*. B. kalaharica recently described in a German holiday maker
176 returning from South Africa (10). The IGS sequences were highly similar to three
177 sequences previously reported from *O. moubata* ticks in Tanzania DQ000284-
178 DQ000285 (tick extracts IM/16; IM/19 and IK/23; see figure 5) (23). These
179 observations were further corroborated by the notable similarity with flagellin
180 sequences from ticks and patients in Tanzania, figure 6 (26, 27). The 16S rRNA
181 sequences showed poor discriminatory values and were thus phylogenetically less
182 informative (see figure 3). The relationship of amplicons produced in this study to
183 those previously deposited is given in figures 2-6. Collectively, our results indicate

184 that the ticks were *O. savignyi* and that the *Borrelia* species found associated with

185 these was *Ca.* B. kalaharica.

186

187 **Discussion**

188 Epidemiological maps of *Ornithodoros* tick species overlaid with those carrying

189 borreliae indicate an absence of both soft ticks and relapsing fever causing

190 spirochaetes in Nigeria (13). Despite this, clinical presentations compatible with

191 TBRF occur. The ticks assessed were only amplified by one of the sets of primers

192 used, those for 16S rRNA (20) and only 47% of samples successfully amplified with

193 these primers. Whether the failure of other targets to amplify rests in sequence

194 heterogeneity or was a result of the poor DNA quality of samples received, remains to

195 be resolved. The positive control DNA extracted from a single *I. ricinus* tick

196 demonstrated that the reagents and cycling conditions were appropriate.

197

198 Analysis of eight samples clearly demonstrated the tick identity to be *O. savignyi*, an

199 aggressive rapid-feeding multi-host tick species with a wide distribution in arid areas

200 of Africa, Egypt, Saudi Arabia, the Persian Gulf, India and Sri Lanka. Five of these

201 samples were derived from single ticks, whereas the remaining three were pools of 3-

202 4 ticks, thus it is not inconceivable for these to have contained mixed species. The

203 sequence reads were of good quality and gave no evidence of mixed species,

204 suggesting our conclusions regarding identity to be valid.

205

206 This tick frequents areas where livestock and humans seek shade, often hidden in the

207 sandy earth under trees, or cracks and crevices of animal housing or surrounding areas

208 where livestock congregate such as markets. This tick is believed to have a lifespan of

209 some 15-20 years and survive periods of starvation of 5-6 years (28). To date, the tick
210 *O. savignyi* has been largely over-looked as a vector of pathogens, with focus instead
211 being placed on its toxic potential. This tick species has major impact upon animal
212 husbandry with reports of mortality particularly amongst lambs and calves. More
213 recently, it has been proposed as a potential vector for Alkhurma haemorrhagic fever
214 virus (29).
215
216 The question pertaining to the role of *O. savignyi* as a vector of relapsing fever is old
217 with prior reports believed to have erroneously attributed *O. savignyi* to be the vector
218 of TBRF in Africa. Investigation of 2000 ticks and use of animal inoculation failed to
219 substantiate these early claims, with *O. moubata* subsequently being established as the
220 vector for TBRF (30). Interestingly, elegant infection studies of *O. savignyi* with *B.*
221 *crocidurae* demonstrated successful transstadial transmission, but not transovarial,
222 unlike *O. erraticus* which was also able to show efficient transovarial infection rates
223 (31).
224
225 More recently, a few reports of *Borrelia* associated with *O. savignyi* have emerged
226 from Egypt (32-34). Elegant studies by Shanbaky and Helmy demonstrated that the
227 *Borrelia* species present in *O. savignyi* showed both successful transstadial and
228 transovarial transmission analogous to that seen with the sympatric tick species in
229 Egypt, *O. erraticus* and its *B. crocidurae* (33). Cross infection of *B. crocidurae* into
230 *O. savignyi* and the *Borrelia* species of *O. savignyi* showed the ability to survive
231 transstadially and to be infectious to hamsters, but less efficiently than the naturally
232 associated tick-spirochaete relationship and failed to demonstrate transovarial
233 transmission. These studies did not further characterise the *Borrelia*. A later study

234 suggested somewhat surprisingly that the *Borrelia* present in *O. savignyi* belonged to
235 the *B. burgdroferi* sensu lato complex (34). This has not been subsequently confirmed
236 by others.
237
238 During the current study, we similarly detected *Borrelia* in *O. savignyi* ticks, albeit at
239 a much lower prevalence [6.1% of pools with potential range of 2.7-7.4% amongst
240 individual ticks]. This might have reflected a genuine low infection prevalence from
241 the collection site, or be a consequence of the poor quality of DNA received for
242 analysis with only 23 of the 49 samples yielding tick 16S rRNA amplicons. The three
243 samples positive for *Borrelia* were also ones demonstrating amplicons for tick 16S
244 rRNA, thus our prevalence amongst these samples would be 13% [3/23]. Analysis of
245 16S rRNA highlighted the similarity to Nearctic species of *Borrelia*, but was poorly
246 discriminatory demonstrated by low bootstrap values and thus not able to accurately
247 speciate. Others have reported the poor discriminatory value of 16S rRNA for
248 borreliae (35). Both flagellin *fla*B and intragenic spacer IGS sequencing were more
249 informative. Flagellin sequencing revealed a single polymorphism between the two
250 sequences, but both were most like the proposed *Ca*. B. kalaharica detected in a
251 tourist returning from a holiday in South Africa (10). This case was bitten by a "mite-
252 like" arthropod from an area known to be endemic for *O. savignyi*, thus it is entirely
253 possible that this tick species might have been the un-documented culprit in this case.
254 Our sequence analysis, as in the report of *Ca*. B. kalaharica, highlighted the similarity
255 with Nearctic species and *B. anserina* known to be present in the widely distributed
256 Argas tick species (36). This closer link to *B. anserina* and Nearctic species was
257 further confirmed by IGS sequencing. Surprisingly, this showed greatest similarity
258 with sequences found amongst presumed *O. moubata* ticks from Ikombolinga and

259 Iringa Mvumi, Dodoma Rural District in Tanzania (23). These ticks were collected
260 from dwellings heavily infested by *O. moubata* and with a high infection prevalence
261 of *B. duttonii*. Identity of ticks collected during this study were not confirmed by
262 molecular methods or by entomological keys, however similar strains were reported
263 by *fla*B sequencing whereby the ticks were confirmed as *O. porcinus* complex (which
264 includes *O. moubata*) (37). Significantly, these *Borrelia* species have been
265 documented from human cases in Tanzania (26, 27).
266
267 In conclusion, we have demonstrated *O. savignyi* in Nigeria, with evidence of
268 *Borrelia*. This spirochaete is highly related to *Ca*. B. kalaharica suggesting that *O.*
269 *savignyi* ticks are the vector for this species. The similarity to species present in *O.*
270 *moubata* ticks in Tanzania is intriguing as *Borrelia* generally show strict vector
271 associations. Whether both tick vectors were sympatric in this region or if indeed
272 these *Borrelia* are less vector specific than previously appreciated, remains to be
273 addressed. Importantly, this *Borrelia* is capable of producing TBRF and has a
274 geographically wide distribution from Africa through the Middle East, and possibly
275 beyond. Consequently, greater consideration of TBRF as part of the differential
276 diagnosis among febrile patients from regions where *O. savingyi* ticks are present is
277 essential to diagnose this treatable infection.
278
279 **Acknowledgements**
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281
282 **Disclaimers**
283 The authors have no conflicts of interest to declare.

284 **References**

285 1. Nordstrand A, Bunikis I, Larsson C, Tsogbe K, Schwan TG, Nilsson M,
286 Bergström S. 2007. Tickborne relapsing fever diagnosis obscured by Malaria,
287 Togo. Emerging Infectious Diseases 13:117-123.
288 2. Vial L, Diatta G, Tall A, Hadj Ba E, Bouganali H, Durand P, Sokhna C,
289 Rogier C, Renaud F, Trape JF. 2006. Incidence of tick-borne relapsing fever in
290 west Africa: longitudinal study. Lancet 368:37-43.
291 3. Cutler SJ, Ruzic-Sabljic E, Potkonjak A. 2017. Emerging borreliae –
292 Expanding beyond Lyme borreliosis. Molecular and Cellular Probes 31:22-27.
293 4. Schwan TG, Raffel SJ, Schrumpf ME, Gill JS, Piesman J. 2009.
294 Characterization of a novel relapsing fever spirochete in the midgut, coxal
295 fluid, and salivary glands of the bat tick *Carios kelleyi*. Vector Borne and
296 Zoonotic Diseases 9:643-647.
297 5. Socolovschi C, Kernif T, Raoult D, Parola P. 2012. *Borrelia, Rickettsia,* and
298 *Ehrlichia* species in bat ticks, France, 2010. Emerging Infectious Diseases
299 18:1966-1975.
300 6. Yabsley MJ, Parsons NJ, Horne EC, Shock BC, Purdee M. 2012. Novel
301 relapsing fever *Borrelia* detected in African penguins (*Spheniscus demersu*s)
302 admitted to two rehabilitation centers in South Africa. Parasitology Research
303 110:1125-1130.
304 7. Wagemakers A, Staarink PJ, Sprong H, Hovius JWR. 2015. *Borrelia*
305 *miyamotoi:* A widespread tick-borne relapsing fever spirochete. Trends in
306 Parasitology.

307 8. Naddaf SR, Ghazinezhad B, Bahramali G, Cutler SJ. 2012. Phylogenetic
308 analysis of the spirochete *Borrelia microti*, a potential agent of relapsing fever
309 in Iran. Journal of Clinical Microbiology 50:2873-2876.
310 9. Naddaf SR, Ghazinezhad B, Sedaghat MM, Asl HM, Cutler SJ. 2015.
311 Tickborne relapsing fever in Southern Iran, 2011–2013. Emerging Infectious
312 Diseases 21:1078-1080.
313 10. Fingerle V, Pritsch M, Wachtler M, Margos G, Ruske S, Jung J, Loscher T,
314 Wendtner C, Wieser A. 2016. "*Candidatus* Borrelia kalaharica" detected from
315 a febrile traveller returning to Germany from vacation in Southern Africa.
316 PLoS Neglected Tropical Diseases 10:e0004559.
317 11. Hoch H, Wieser M, Löscher T, Margos G, Pürner F, Zühl J, Seilmaier M,
318 Balzer L, Guggemos W, Rack-Hoch A, von Both U, Hauptvogel K,
319 Schönberger K, Hautmann W, Sing A, Fingerle V. 2015. Louse-borne
320 relapsing fever *(Borrelia recurrentis)* diagnosed in 15 refugees from North-
321 Eastern Africa in Bavaria, Germany: Diagnostics, epidemiology and
322 preventive control measures Eurosurveillance 20.
323 12. Cutler SJ. 2016. Refugee crisis and re-emergence of forgotten infections in
324 Europe. Clinical Microbiology and Infection 22:8-9.
325 13. Trape JF, Diatta G, Arnathau C, Bitam I, Sarih M, Belghyti D, Bouattour A,
326 Elguero E, Vial L, Mané Y, Baldé C, Pugnolle F, Chauvancy G, Mahé G,
327 Granjon L, Duplantier JM, Durand P, Renaud F. 2013. The epidemiology and
328 geographic distribution of relapsing fever borreliosis in West and North
329 Africa, with a review of the *Ornithodoros erraticus* complex (Acari: Ixodida).
330 PLoS ONE 8.

331 14. Manu IJ. 2012. Socio-demographic features and risk factors associated with
332 suspected cases of tick-borne relapsing fever in northern Borno State Nigeria.
333 Master in Public Health. Ahmadu Bello University Zaria.
334 15. Bunza M, Yahaya M, Muhammad A, Saidu A. 2008. A survey on tick species
335 infesting domestic birds sold at Sakata central market, Nigeria. Sokoto Journal
336 of Veterinary Sciences 7:56-58.
337 16. Black WC, Klompen JSH, Keirans JE. 1997. Phylogenetic Relationships
338 among Tick Subfamilies (Ixodida: Ixodidae: Argasidae) Based on the 18S
339 Nuclear rDNA Gene. Molecular Phylogenetics and Evolution 7:129-144.
340 17. Black WC, Piesman J. 1994. Phylogeny of hard- and soft-tick taxa (Acari:
341 Ixodida) based on mitochondrial 16S rDNA sequences. Proceedings of the
342 National Academy of Sciences of the United States of America 91:10034-
343 10038.
344 18. Dupraz M, Toty C, Noël V, Estrada-Peňa A, González-Solís J, Boulinier T,
345 Dujardin J-P, McCoy KD. 2016. Linking morphometric and genetic
346 divergence with host use in the tick complex, Ornithodoros capensis sensu
347 lato. Infection, Genetics and Evolution 46:12-22.
348 19. Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P. 1994. Evolution,
349 Weighting, and Phylogenetic Utility of Mitochondrial Gene Sequences and a
350 Compilation of Conserved Polymerase Chain Reaction Primers. Annals of the
351 Entomological Society of America 87:651-701.
352 20. Lv J, Wu S, Zhang Y, Chen Y, Feng C, Yuan X, Jia G, Deng J, Wang C,
353 Wang Q, Mei L, Lin X. 2014. Assessment of four DNA fragments (COI, 16S
354 rDNA, ITS2, 12S rDNA) for species identification of the Ixodida (Acari:
355 Ixodida). Parasites and Vectors 7.

356 21. Diatta G, Mediannikov O, Boyer S, Sokhna C, Bassène H, Fenollar F,
357 Chauvancy G, Ndiaye AA, Diene F, Parola P, Raoult D. 2016. An alternative
358 strategy of preventive control of tick-borne relapsing fever in rural areas of
359 Sine-Saloum, Senegal. The American journal of tropical medicine and hygiene
360 95:537-545.
361 22. Bunikis J, Garpmo U, Tsao J, Berglund J, Fish D, Barbour AG. 2004.
362 Sequence typing reveals extensive strain diversity of the Lyme borreliosis
363 agents *Borrelia burgdorferi* in North America and *Borrelia afzelii* in Europe.
364 Microbiology 150:1741-1755.
365 23. Scott JC, Wright DJM, Cutler SJ. 2005. Typing African relapsing fever
366 spirochetes. Emerging Infectious Diseases 11:1722-1729.
367 24. Assous M, Wilamowski A, Bercovier H, Marva E. 2006. Molecular
368 Characterization of Tickborne Relapsing Fever Borrelia, Israel. Emerging
369 Infectious Diseases 12:1740-1743.
370 25. Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular evolutionary
371 genetics analysis version 7.0 for bigger datasets. Molecular Evolutionary
372 Genetics Analysis 33:1870-1874.
373 26. Kisinza WN, McCall PJ, Mitani H, Talbert A, Fukunaga M. 2003. A newly
374 identified tick-borne *Borrelia* species and relapsing fever in Tanzania. Lancet
375 362:1283-1284.
376 27. Mitani H, Talbert A, Fukunaga M. 2004. New World relapsing fever *Borrelia*
377 found in *Ornithodoros porcinus* ticks in central Tanzania. Microbiology and
378 Immunology 48:501-505.

379 28. Mans BJ, Neitz AWH. 2004. The sand tampan, *Ornithodoros savignyi,* as a
380 model for tick-host interactions. South African Journal of Science 100:283-
381 288.
382 29. Charrel RN, Fagbo S, Moureau G, Alqahtani MH, Temmam S, De
383 Lamballerie X. 2007. Alkhurma hemorrhagic fever virus in *Ornithodoros*
384 *savignyi* ticks. Emerging Infectious Diseases 13:153-155.
385 30. Heisch RB, Harvey AEC. 1960. Is *Ornithodoros savignyi* (Audouin) a vector
386 of relapsing fever in Africa? Annals of Tropical Medicine and Parasitology
387 54:205-207.
388 31. Gaber MS, Aboul-Nasr AE. 1984. *Borrelia crocidurae* localization and
389 transmission in *Ornithodoros erraticus* and *O. savignyi*. Parasitology 88:403-
390 413.
391 32. Helmy N. 2000. Seasonal abundance of *Ornithodoros (O.) savignyi* and
392 prevalence of infection with *Borrelia* spirochetes in Egypt. Journal of the
393 Egyptian Society of Parasitology 30:607-619.
394 33. Shanbaky NM, Helmy N. 2000. First record of natural infection with *Borrelia*
395 in *ornithodoros (Ornithodoros) savignyi*. Reservoir potential and specificity of
396 the tick to *Borrelia*. Journal of the Egyptian Society of Parasitology 30:765-
397 780.
398 34. Adham FK, El-Samie-Abd EM, Gabre RM, El Hussein H. 2010. Detection of
399 tick blood parasites in Egypt using PCR assay II- *Borrelia burgdorferi* sensu
400 lato. Journal of the Egyptian Society of Parasitology 40:553-564.
401 35. Ras NM, Lascola B, Postic D, Cutler SJ, Rodhain F, Baranton G, Raoult D.
402 1996. Phylogenesis of relapsing fever *Borrelia* spp. International Journal of
403 Systematic Bacteriology 46:859-865.

404 36. Cutler S, Abdissa A, Adamu H, Tolosa T, Gashaw A. 2012. *Borrelia* in
405 Ethiopian ticks. Ticks and Tick-borne Diseases 3:14-17.
406 37. Fukunaga M, Ushijima Y, Aoki LY, Talbert A. 2001. Detection of *Borrelia*
407 *duttonii*, a tick-borne relapsing fever agent in central Tanzania, within ticks by
408 flagellin gene-based nested polymerase chain reaction. Vector Borne Zoonotic
409 Dis 1:331-338.
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414 Figure legends:

415 FIG 1: Plate A shows the study locations in Borno State Nigeria (Gubio) whilst Plate
416 B shows the dorsal and ventral view of ticks collected during this study.

417

418 FIG 2: Neighbor joining tree of tick derived DNA sequences for 16S rRNA (431 bp)
419 comparing the Nigerian-collected ticks with other *Ornithodoros* species. The optimal
420 tree with the sum branch length = 1.74331329 is shown. Bootstrap value was set to
421 1000 replicates. Evolutionary distance were computed using the Maximum Composite
422 Likelihood method within MEGA7. The diamond identifies the Nigerian tick
423 sequence.

424

425 FIG 3: Neighbor-Joining phylogenetic analysis of *Borrelia* 16S rRNA nucleotide
426 sequence (475 bp). The optimal tree with the sum branch length =0.07300324 is
427 shown. Bootstrap value was conducted using 1000 replicates. Evolutionary distances
428 were computed using the Maximum Composite Likelihood method within MEGA7.
429 The diamond identifies the Nigerian *Borrelia* sequence. *= *Borrelia* species deposited
430 in GenBank as *B. duttonii* in error.

431

432 FIG 4: Neighbor Joining phylogenetic analysis of *Borrelia* flagellin (flaB) DNA
433 sequence (655 bp). The optimal tree with the sum branch length =0.09695016 is
434 shown. Bootstrap validation was conducted using 1000 replicates. The diamond
435 identifies the Nigerian *Borrelia* sequence.

436

437 FIG 5: Neighbor Joining phylogenetic analysis of *Borrelia* intragenic spacer (IGS)
438 DNA sequence (622 bp). The optimal tree with the sum branch length =18.18022296

439 is shown. Bootstrap validation was conducted using 1000 replicates. Evolutionary
440 distances were computed using the Maximum Composite Likelihood method within
441 MEGA7. The diamond identifies the Nigerian *Borrelia* sequences.
442
443 FIG 6: Neighbor Joining analysis of flagellin DNA sequence of the Nigerian *Borrelia*
444 trimmed to 287 bp for comparison with newly described variant strains from Africa.
445 The optimal tree with the sum branch length = 0.26956810 is shown. Bootstrap
446 validation was conducted using 1000 replicates. Evolutionary distances were
447 computed using the Maximum Composite Likelihood method within MEGA7. The
448 diamond identifies the Nigerian *Borrelia* sequences. *= *Borrelia* species deposited in
449 GenBank as *B. duttonii* in error.
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454 Table 1: Primers, probes and thermocycling conditions used during the study.

Gene Target	Forward 5'-3'	Reverse 5'-3'	Thermocycling	Reference
Tick 16S	CTG CTC AAT GAT TTT TTA AAT TGC	CCG GTC TGA ACT CAG ATC ATG TA	94°C for 3 min, 35 cycles of denaturation at 94°C for 45s, 50°C for 45s and 72°C for 45s, with a 72°C 10 min final extension	(17, 18)
Tick 18S	GCA AGT CTG GTG CCA GCA GCC	CTT CCG TCA ATT CCT TTA AG	94°C for 3 min, 35 cycles at 94°C for 45s, 50°C for 45s and 72°C for 45s, with a 72°C 10 min final extension	(16, 18)
Tick COI	GGA GGA TTT GGA AAT TGA TTA GTT CC	ACT GTA AAT ATA TGAT GAG CTC A	94°C for 3 min, 35 cycles at 94°C for 45s, 50°C for 45s and 72°C for 45s, with a 72°C 10 min final extension	(18, 19)
Tick ITS2	ACA TTG CGG CCT TGG GTC TT	TCG CCT GAT CTG AGG TCG AC	94◦C for 5 min, 35 cycles of denaturation at 94◦C for 30 s, annealing at 55◦C for 30 s and elongation at 68◦C for 120 s with a 68◦C for 7 min final extension	(20)
Tick Cox1	GGAACAATATATTTAATTTTTGG	ATCTATCCCTACTGTAAATATATG	94◦C for 5 min, 35 cycles of denaturation at 94◦C for 30 s, annealing at 50◦C for 30 s and elongation at 68◦C for 60 s with a 68◦C for 7 min final extension	(20)
Tick COI	ATC ATA AAK AYH TTG G	GGG TGA CCR AAR AAH CA	94◦C for 5 min, 5 cycles of 94°C for 30 s, 52°C for 30 s, and 68°C for 30 s; 5 cycles of 94°C for 30 s, 50°C for 30 s,	(20)

			and 68°C for 30s; 5 cycles of 94°C for 30 s, 48°C for 30 s, and 68°C for 30s; 25 cycles of 94°C for 30 s, 46°C for 30 s, and 68°C for 30s; final extension step of 68°C for 7 min.	
Tick 16S	TTA AAT TGC TGT RGT ATT	CCG GTC TGA ACT CAS AWC	94◦C for 5 min, 5 cycles of 94°C for 30 s, 49°C for 30 s, and 68°C for 30 s; 5 cycles of 94°C for 30 s, 47°C for 30 s, and 68°C for 30s; 5 cycles of 94°C for 30 s, 45°C for 30 s, and 68°C for 30s; 25 cycles of 94°C for 30 s, 43°C for 30 s, and 68°C for 30s; final extension step of 68°C for 7 min.	(20)
***Borrelia* 16S**	AGC CTT TAA AGC TTC GCT TGT AG	GCC TCC CGT AGG AGT CTG G	95◦C for 10 min, 40 cycles of denaturation at 95◦C for 15 s, annealing at 60◦C for 30 s	(21)
Probe	HEX-CCG GCC TGA GAG GGT GAA CGG-BHQ			
***Borrelia* FlaB (Bor1) & (Bor2)**	TAA TAC GTC AGC CAT AAA TGC	GCT CTT TGA TCA GTT ATC ATT C	94◦C for 3 min, 40 cycles of denaturation at 94◦C for 30 s, annealing at 56◦C for 30 s and elongation at 72◦C for 30 s with a 72◦C for 7 min final extension	(24)

Borrelia ***uvr*A**	GCG TTA TCT TWC AAC TGA ATC	TCT AGA CTC TGG AAG CTT	94◦C for 3 min, 40 cycles of denaturation at 94◦C for 30 s, annealing at 50◦C for 45 s and elongation at 72◦C for 90 s with a 72◦C for 7 min final extension	(10)
***Borrelia* IGS First round of nested PCR**	GTA TGT TTA GTG AGG GGG GTG	GGA TCA TAG CTC AGG TGG TTA G	94◦C for 3 min, 35 cycles of denaturation at 94◦C for 30 s, annealing at 55◦C for 30 s and elongation at 72◦C for 60 s with a 72◦C for 7 min final extension	(22, 23)
***Borrelia* IGS Second round of nested PCR**	AGG GGG GTG AAG TCG TAA CAA G	GTC TGA TAA ACC TGA GGT CGG A	94◦C for 3 min, 35 cycles of denaturation at 94◦C for 30 s, annealing at 60◦C for 30 s and elongation at 72◦C for 60 s with a 72◦C for 7 min final extension	(22, 23)

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