

Mosquitoes' bites nuisance and the risk of infection transmission

1 Research article

2 Parasitology, Poverty and Prevention: is there any relationship 3 between the three P? Is it possible to eradicate Parasitic diseases 4 without eliminating Poverty?

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25 **Abstract**

26 **Context**

27 Talking about Poverty is not obvious without examples, I would like to understand the link
28 between Parasitology, Poverty and Prevention (the three P). I explain the three P by saying that
29 there is four level of knowledge in Parasitology and the fourth level is the integration with other
30 disciplines including virology with preventive measures, nutrition aspects with denutrition
31 leading by some parasites as Ascaris, economy involving patient's income and Poverty. As a
32 reminder, the first level in Parasitology is the knowledge of the parasitic cycle with an emphasis
33 on the mode of contamination, the second level is that of the implementation of technical or
34 diagnostic means to identify the parasite in the laboratory or the bench and the third level is that
35 of treating infected cases diagnosed in the laboratory.

36 **Objective**

37 The objective of this work is to contribute to reach the first sustainable development goal i.e. no
38 Poverty. Specifically, this manuscript aimed to evaluate poverty with the protective measures
39 against the harmful effects of mosquitoes that contribute to the quality of care given to patients of
40 the University Hospital of Kinshasa (UHK).

41 **Findings**

42 Residual mosquito capture, carried out in 31 randomly selected rooms per block and per level in
43 hospital departments, presented the number of 1,144 female mosquitoes (845 *Culex*, 207
44 *Anopheles* and 62 *Aedes*). Overall considered, the Mean Mosquito Density (MMD) was 36.2 /
45 mosquito per room (6.9 *Anopheles* / room, 29.1 *Culex* / room and 2.1 *Aedes* / room with an

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46 extreme between 0 and 144 mosquitoes / room. The lowest MMD (6.2 mosquitoes / room) was
47 observed in Block II (clinical biology and microbiology laboratories, delivery and private
48 hospitalization rooms) compared to other hospital blocks that had the highest MMD and
49 statistically identical (ranging between 29.2 and 45.5 mosquitoes / room).

50 Our observations give a good idea of Poverty inside this hospital and where to concentrate in the
51 prevention of malaria transmission within the hospital. Regardless of the block considered, it was
52 the ground floor with an MMD of 52.8 mosquitoes / room which were the most dangerous places
53 compared with the first and second floors with MMD respectively 17.6 and 25.6 mosquitoes /
54 room.

55 Conclusion

56 In conclusion, the insufficiency of the UHK anti-mosquito measures was obvious. These should
57 be applied without delay to prevent the risk of infection transmission by mosquitoes, even within
58 the hospital, of hepatitis B virus and strains of *Plasmodium falciparum*, sometimes highly
59 virulent, which may be concentrated there.

60 Limits

61 We were on the right track and this study needs more research because of its limitations: we
62 investigate and did not find if any of the mosquitoes collected were infected; we did not
63 investigate if the hospital had any patients with a mosquito transmitted disease in the rooms
64 where the mosquitoes were collected.

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67 **Recommendation**

68 The recommendation is if it is not possible to eradicate parasitic diseases as malaria without
69 eliminating poverty, then we need to eliminate them both.

70 **Keywords:** mosquito nuisance, University Hospital of Kinshasa, Democratic Republic of the
71 Congo.

72 **Résumé**

73 **Contexte**

74 Parler de la pauvreté n'est pas évident sans exemples, je voudrais comprendre le lien entre
75 parasitologie, pauvreté et prévention (les trois P). J'explique les trois P en disant qu'il existe
76 quatre niveaux de connaissance en parasitologie et que le quatrième niveau est l'intégration à
77 d'autres disciplines, notamment la virologie avec des mesures préventives, les aspects
78 nutritionnels avec la dénutrition observée avec certains parasites comme l'Ascaris, l'économie
79 impliquant le revenu des patients et la pauvreté. Pour rappel, le premier niveau en parasitologie
80 est la connaissance du cycle parasitaire en mettant l'accent sur le mode de contamination, le
81 second niveau est celui de la mise en œuvre de moyens techniques ou diagnostiques permettant
82 d'identifier le parasite en laboratoire ou sur le banc et le troisième niveau est celui du traitement
83 des cas infectés diagnostiqués en laboratoire.

84 **Objectif**

85 L'objectif de ce travail est de contribuer à la réalisation du premier objectif de développement
86 durable, c'est-à-dire l'absence de pauvreté. Plus précisément, ce manuscrit visait à évaluer la
87 pauvreté grâce à des mesures de protection contre les effets nocifs des moustiques qui contribuent
88 à la qualité des soins prodigués aux patients des Cliniques Universitaires de Kinshasa (CUK).

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89 **Résultats**

90 La capture résiduelle de moustiques, effectuée dans 31 locaux choisis au hasard par bloc et par
91 niveau dans les services hospitaliers, a présenté le nombre de 1 144 moustiques femelles (845
92 *Culex*, 207 *Anopheles* et 62 *Aedes*). Dans l'ensemble, la densité moyenne des moustiques (DMM)
93 était de 36,2 / moustiques par local (6,9 *Anopheles* / local, 29,1 *Culex* / local et 2,1 *Aedes* / local
94 avec une extrême entre 0 et 144 moustiques / local) ont été observés dans le bloc II (laboratoires
95 de biologie clinique et de microbiologie, salles d'accouchement et d'hospitalisation privée) par
96 rapport aux autres blocs hospitaliers qui avaient la plus haute DMM statistiquement identiques
97 (allant de 29,2 à 45,5 moustiques / local).

98 Nos observations donnent une bonne idée de la pauvreté à l'intérieur de cet hôpital et du lieu où
99 se concentrer dans la prévention contre le paludisme. Quel que soit le bloc considéré, c'étaient les
100 rez-de-chaussée avec une DMM de 52,8 moustiques / local qui étaient les endroits les plus
101 dangereux par rapport aux premiers et deuxièmes étages avec DMM respectivement 17,6 et 25,6
102 moustiques / local.

103 **Conclusion**

104 En conclusion, l'insuffisance des mesures anti-moustiques des CUK était évidente. Celles-ci
105 doivent être appliquées sans délai pour prévenir le risque de transmission par les moustiques,
106 même à l'intérieur de l'hôpital, du virus de l'hépatite B et de souches de *Plasmodium falciparum*,
107 parfois extrêmement virulentes, qui peuvent s'y concentrer.

108 **Limites**

109 Nous étions sur la bonne voie avec la notion de risque qui n'était pas confirmé donc cette étude
110 qui nécessite davantage de recherche en raison de ses limites: nous avons enquêté et n'avons

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111 trouvé aucun des moustiques collectés infectés; aussi nous n'avons pas cherché à savoir si
112 l'hôpital avait des patients atteints d'une maladie transmissible par les moustiques dans les salles
113 où les moustiques avaient été collectés.

114 **Recommandation**

115 La recommandation est la suivante: s'il n'est pas possible d'éradiquer les maladies parasitaires
116 comme le paludisme sans éliminer la pauvreté, nous devons éliminer tous les deux.

117 **Mots-clés:** nuisance culicidienne, Cliniques Universitaires de Kinshasa, République
118 Démocratique du Congo.

119 120 **Introduction**

121 At the UN Summit of September 2015, the Member States of the United Nations adopted the
122 2030 Agenda for Sustainable Development with a set of Sustainable Development Goals (SDGs)
123 that **aim to end extreme poverty, promote equity and opportunity for all, and protect the**
124 **planet** (<https://www.un.org/sustainabledevelopment/development-agenda/>). SDGs are universal
125 goals accepted by all countries and applicable to all – both rich and poor, taking into account
126 different national realities, capacities and levels of development and respecting national policies
127 and priorities (<https://www.connectyet.org/sdg/category/about-sdg>) worldwide and the
128 Democratic Republic of the Congo cannot miss this opportunity to open itself to development
129 also the University Hospital of Kinshasa (UHK), located on the hill of Mount Amba, in the
130 southern suburbs of the city of Kinshasa, that occupies the top of the health system of the
131 Democratic Republic of the Congo (DRC). Compared to the rest of the city, hospital
132 environments are a real reactor that concentrates most pathogens at a very high-density level. As

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133 a result, the biosecurity problem is acute. Conventional prophylactic measures to prevent
134 nosocomial infections do not usually include those intended for protection against mosquito's
135 nuisance, when they should. This work set itself the objective of assessing the level of mosquito's
136 nuisance that reigns in this important care institution in the Democratic Republic of the Congo.

137 Materials and methods

138 Inclusion and exclusion criteria

139 To be eligible, a block should be accessible to the survey in all its parts. At each level of the
140 selected block, to be eligible, a room should be accessible to the investigators. The UHK have 4
141 hospital blocks, 1 consultation block (block C) and 1 technical block. In its hospital part, each
142 block has 4 levels: 1 basement, 1 ground floor and 2 floors. Block C has only 2 levels. Three of
143 the four hospital blocks (blocks I, II and IV) and the block of consultations (block C) met this
144 condition. Block III, which has at the last level a hospitalization service, was not retained because
145 of the ineligibility of these lower levels which contain sensitive services: radiology and nuclear
146 medicine on the ground floor, the resuscitation and operating rooms on the 1st floor. Also
147 excluded: bedridden patients' rooms, operating rooms, delivery rooms, laboratories, imaging
148 department, intensive care units, stores, basements and pharmacy.

149 Sampling

150 Showing the documents authorized by the UHK authorities, we proceeded with the identification
151 of the places and the census of the premises in which the mosquitoes capture was to take place.
152 Out of a total of 343 local respondents considered eligible for our survey, one in ten rooms was
153 selected using the random number table by level and block. Some additional premises were

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154 drawn in anticipation of the possible replacement of premises that would have lost their eligibility
155 in the meantime.

156 **Mosquito capture**

157 The capture of mosquitoes, carried out in 6 sessions, took place in 31 premises instead of 34
158 expected. We used insecticide bombs charged permethrin spray, a synthetic pyrethroid. Before
159 spraying insecticide into some rooms, the following precautions were taken: the occupants were
160 asked to leave, taking away their valuables, all the windows were closed, dishes, food and drinks,
161 were evacuated. Furniture and tiles were covered with white sheets.

162 Masked and gloved, we started spraying from the outside to the inside, first to the open door that
163 we close behind us while progressing on the bottom of the room to spray every possible corner.
164 Spraying a room, depending on its size, lasted from 20 to 40 seconds. We left the room closing
165 the door behind us. After waiting 10 minutes, the door and windows were opened again for 5
166 minutes to ventilate the treated room. Then, using pliers, we proceeded to collect mosquitoes
167 fallen on the sheets. Those fallen under the furniture were searched with a flashlight.

168 **Entomological examination**

169 Captured mosquitoes were brought back to the laboratory for identification. After exclusion of
170 males, females were sorted and enumerated by genus.

171 **Statistical analyzes**

172 The comparison of mean mosquitoes' densities (MMD) by local was done by means of the
173 analysis of variance (Snedecor test F) following a general linear model in which the blocks, the

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174 levels played the role of fixed factors, and, no random draw, while the premises were taken as a
175 random factor because drawn.

176 Given the large variability of the data (mean-proportional dispersion), this analysis was
177 performed on the logarithms of the data to which "1" was added to account for zeros. In this
178 comparison of MMD, *Anopheles*, a vector of malaria, was opposed to other *Culex* and *Aedes*
179 taken together, given their small size.

180 The significance of the results was assessed at the 5% probability level of significance.

181 **Results**

182 **Comparison of the nuisance between the blocks**

183 The collection, carried out in 31 rooms, brought back 1,144 female mosquitoes including 207
184 *Anopheles*, 845 *Culex* and 62 *Aedes*, respectively 19.2; 74.9 and 5.9% of the workforce. Overall,
185 MMD per local was 6.9 for *Anopheles* and 29.1 for *Culex* and 2.1 for *Aedes* (Table 1).

186 The comparison of the MMD between the blocks did not show a significant difference ($p =$
187 0.327). However, orthogonal contrast analysis showed that Block II had the lowest MMD
188 compared to the others ($p = 0.001$).

189 (Tables 1 and 2).

190 **Comparison of mosquito's density by level**

191 Overall, there was no significant difference between levels ($p = 0.437$). However, orthogonal
192 contrast analysis showed that the ground floors had a significantly higher MMD than those
193 observed in the floors ($p = 0.047$). Between floors, the difference was not significant ($p = 0.091$);

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194 (Table 4) The ground floor with an average of 52.8 mosquitoes / local, while the first and second
195 level had respective values: 17.6 and 25.6 mosquitoes / local. The interaction analysis showed
196 that the difference in MMD between the levels was the same from one block to another ($p =$
197 0.425).

198 Comparison of MMD by local

199 Overall, there was no significant difference in MMD between premises ($p = 0.926$). However, the
200 study of interactions showed the difference between premises was not the same, nor from one
201 block to another ($p < 0.0001$) nor from one level to another ($p = 0.0001$). The difference observed
202 between the species was identical whatever the place ($p = 0.712$), but not from one block to
203 another ($p = 0.001$) (Table 3).

204 Discussion

205 The work reports findings with implications for public health. Given the implication for patient
206 care or public health, among many causes, I think poor countries deserve to be affected by
207 parasitic diseases because of illiteracy, health negligence. It is observed that parasitology-
208 virology diseases incidence is more common in poor regions of the world [1-5]. Any
209 relationships in mechanisms of those diseases and poverty? On one hand malaria is often referred
210 to as the epidemic of the poor whilst the disease is in large part determined mainly by climate and
211 ecology [1], and not poverty, on the other hand, the impact of malaria takes its toll on the poorest
212 [1], those least able to afford preventative measures and medical treatment and those living in
213 suboptimal and poor hygiene and sanitation conditions that are indeed the root causes that define
214 the ascariasis [2]. The risk of HIV transmission in theory is also involved in the poor settings [3]
215 where when the international aid stops, disease prevalence become more prevalent as the case of

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216 trypanosomiasis [4]. In this context, MMD at CUK was 36.2 female / local with extremes
217 mosquitoes ranging from 0 to 144 mosquitoes / local. This number represents potentially as many
218 bites to which all people sharing the same premises were exposed.

219 If we consider, for example, an average of 10 people per room (patients and nurses included),
220 each of them will be exposed to receive an average of 3.6 bites per person per night (PPN), or
221 5,114 PPN annually. This nuisance was mainly due to *Culex*, which alone accounted for 75.9% of
222 the mosquito population caught. Compared with *Anopheles*, *Culex* was 4.1 times more numerous.
223 The average number of PPN due to *Anopheles* would be 0.7 per local. This number should be
224 increased to 5.4 in the extreme situations observed on the ground floor.

225 Thus, as evidenced by the results of this survey, the risk of malaria transmission within the UHK
226 itself can not be neglected [5]. Of course, we investigated our capture, and we did not find any of
227 the mosquitoes collected were infected; but ethically we had not the right to investigate if the
228 hospital had any patients with a mosquito-transmitted disease in the rooms where the mosquitoes
229 were collected. We know that the risk of transmission of parasitic infections (HIV, yellow fever,
230 dengue fever, Japanese encephalitis, viral hepatitis, and filariasis), is very real for some patients,
231 this study highlighted that the risk of infection remains for others purely theoretical [5].

232 As mosquitoes can transmit yellow fever, dengue fever and Japanese encephalitis, there is no
233 evidence that they are capable of transmitting HIV. Indeed, it is established experimentally, by
234 gene amplification techniques, that this virus disappears from the body of the mosquito after 1 to
235 2 days, time required for digestion of the blood meal. Consequently, since the virus can not
236 survive the time required to invade the salivary glands and multiply due to the absence of CD4

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237 receptors in arthropods, its cyclic transmission is therefore not possible [6]. There remains only
238 the possibility of a mechanical transmission.

239 A mosquito that interrupted his meal on an HIV-positive patient, can he, when he immediately
240 restarted on another subject, transmit the infection? Here again, it is established that HIV is
241 inactivated within 20 minutes during its stay in the fallopian tubes. However, a mosquito that has
242 interrupted its meal can not statistically restart it before 20 minutes [7]. Moreover, even if some
243 viruses remain in the trunk, it has been calculated that for a mechanical transmission to be
244 possible, the healthy subject must receive an inoculum containing at least 10 million virus
245 particles. Due to a hypothetical inoculation of 10 copies of viral RNA per sting, it would take 1
246 million bites that can be expected to receive an individual during his stay, even extended to
247 several years, UHK.

248 Regarding yellow fever, the mere presence of *Aedes aegypti*, which accounts for 5.6% of CUK's
249 culicidae fauna, is not enough to ensure the transmission of this disease. Indeed, the cycle of the
250 yellow fever virus requires the interlocking of three cycles to pass the virus from the forest
251 (monkeys) to the city (man). The biotope of Kinshasa, far away from the forests, is not conducive
252 for the development of such a chain of transmission. On the other hand, the intrusion of a single
253 case of yellow fever into the hospital is potentially dangerous for all the people who live there.
254 Given the presence of the potential vector, draconian biosecurity measures must be taken without
255 delay.

256 Regarding the transmission of hepatitis B (HBV) and (HBC) viruses by mosquitoes, there is a
257 few studies that have focused on this issue. Regarding the transmission of HBV, the work of
258 Blow et al [8] showed that this virus could be transmitted to humans through the droppings of

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259 mosquitoes, especially when they are forced to interrupt their meal on a subject infected and then
260 resume immediately on another subject. In addition, the work of Zhang et al [9] on monkeys
261 confirmed the possibility of cyclic transmission of HBV by mosquito saliva. Moreover, the work
262 of Fouché et al [10], conducted on mosquitoes of the *Culex quinquefasciatus* species,
263 demonstrated the existence of a vertical (transovarian) transmission of HBV infection from one
264 generation to the next other.

265 Regarding the transmission of HCV, both mechanically and cyclically, the results of various
266 studies remain divergent on this question. Indeed, there are those who support this idea, this is the
267 case of Hassan et al [11] and Washenberger et al [12], while, for their part, Yeung et al [13] reject
268 it. The risk of transmission of the Japanese encephalitis virus is theoretically possible even if no
269 case has been reported so far. Indeed, the available vectors (*Aedes*, *Culex*) are legion in our
270 environment and the UHK. As for the dengue, although the DRC is included in the distribution
271 area of *Aedes aegypti*, dengue cases, which they are indigenous or imported, have never been
272 reported to date. Dengue is a tropical pathology caused by 4 species of virus whose cycle
273 involves humans and *Aedes aegypti*, an anthropophilic mosquito with diurnal activity. It must be
274 admitted, however, that the hospitalization of a single case of dengue at UHK is justifiable for
275 quarantine measures, especially for the haemorrhagic form, given the availability of the vector.
276 What about *Wuchereria bancrofti*, the only filariasis to be transmitted by culicidae? Although the
277 city of Kinshasa is located outside the area of its transmission, it is not excluded that local
278 transmission may take place there.

279 Thus, as evidenced by the results of this survey, the risk of malaria transmission within the UHK
280 itself can not be neglected. We investigated our capture in laboratory of the UHK Unit of
281 Parasitology, and we did not find any of the mosquitoes collected were infected mainly female

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282 *Anopheles* by examining the presence of sporozoites in their salivary glands. Another study can
283 investigate if the hospital had any patients with a mosquito-transmitted disease in the rooms
284 where the mosquitoes were collected because it was not the aim of our study. We know by
285 literature review that the risk of transmission of parasitic infections (yellow fever, dengue fever,
286 Japanese encephalitis, filariasis), viral hepatitis and HIV, is very real for some patients, but that
287 the risk of infection remains for others purely theoretical: As mosquitoes can transmit yellow
288 fever, dengue fever and Japanese encephalitis, there is no evidence that they are capable of
289 transmitting HIV. The level of mosquito's nuisance found at UHK has highlighted the
290 inadequacy of mosquito control measures in this important health facility in the DRC. Adequate
291 mosquito control measures (drying up of stagnant water collections in and around the hospital,
292 periodic spraying of insecticides, use of mosquito nets and other insecticide-treated materials,
293 placement of mosquito nets in windows, etc.) should be deployed. They will not only improve the
294 quality of sleep of patients, an important element of the quality of care, but will contribute to
295 strengthening biosecurity measures to prevent the spread within the hospital of all germs
296 transmitted by the mosquitoes. We are thinking particularly of the hepatitis B virus and
297 dangerous strains of *Plasmodium falciparum* probably concentrated in the hospital.

298 Conclusion

299 The level of mosquito's nuisance found at UHK has highlighted the inadequacy of mosquito
300 control measures in this important health facility in the DRC. Adequate mosquito control
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306 mosquitoes. We are thinking here particularly of the hepatitis B virus and dangerous strains of
307 *Plasmodium falciparum* probably concentrated in the hospital.

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352 Table 1. MMD study by block and by type (arithmetic mean \pm 1 standard deviation)

BLOCK	GENUS		TOTAL
	<i>Anopheles</i>	<i>Culex+Aedes</i>	
I	7.7 \pm 15.1 (13)	37.8 \pm 38.1 (13)	45.5 \pm 64.4 (13)
II	2.0 \pm 1.8(4)	4.3 \pm 2.2 (4)	6.2 \pm 4.4 (4)
VI	7.7 \pm 5.5 (10)	32.8 \pm 31.7 (10)	40.4 \pm 51.2 (10)
C	9.5 \pm 5.7 (4)	19.8 \pm 14.9 (4)	29.2 \pm 23.6 (4)
TOTAL	7.1\pm10.3 (31)	29.1\pm31.9 (31)	36.9\pm52.0 (31)

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354 Legend:

355 • Block I: Ground floor (Pediatrics), 1st floor (Maternity, Neonatology), 2nd floor (child surgery,
356 specialties and gynecological hospitalization)

357 • Block II: Ground floor (clinical biology, microbiology); 1st floor (delivery rooms, gynecology
358 operating rooms); 2nd floor (private hospitalization).

359 • Block IV: Ground Floor (Hospitalization Internal Medicine, Intensive Care); 1st stage
360 (hospitalization surgery, hospitalization internal medicine); 2nd floor (hospitalization surgery,
361 hospitalization internal medicine).

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362 • Block C: Ground floor (pharmacy, administration, blood bank and emergencies); 1st floor
363 (outpatient).

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368 Table 2. Study of MMD between blocks (orthogonal contrast analysis)

CONTRAST	PROBABILITY
(Block II) vs (Others)	0.001
(Block I) vs (Blocks IV and C)	0.457
(Block IV) vs (Block C)	0.854

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399 Table 3. MMD study according to the levels (arithmetic average \pm 1 standard deviation), (the
400 number of mosquitoes is in parentheses)

	CULICIDIAN GENUS		
LEVEL	<i>Anopheles</i>	<i>Culex+Aedes</i>	TOTAL
Ground floor	10.1 \pm 14.0 (16)	42.7 \pm 39.6 (16)	52.8 \pm 67.2 (16)
1st floor	3.2 \pm 3.6 (9)	14.3 \pm 14.3 (9)	17.6 \pm 24.0 (9)
2 nd floor	6.0 \pm 4.1 (6)	19.7 \pm 15.5 (6)	25.6 \pm 26.0 (6)
TOTAL	7.1\pm10.3 (31)	29.1\pm31.9 (31)	36.2\pm52.0 (31)

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Mosquitoes' bites nuisance and the risk of infection transmission

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422 Table 4. Comparison of MMD between Blocks (Orthogonal Contrast Analysis)

CONTRAST	PROBABILITY
Ground Floor vs (1 st et 2 nd floor)	0.047
1 st floor vs 2 nd floor	0.091

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