



**HAL**  
open science

## Serological screening in animals combined with environmental surveys provides definite proof of the local establishment of *Burkholderia pseudomallei* in Guadeloupe

Mégane Gasqué, Vanina Guernier-Cambert, Gil Manuel, Rachid Aaziz, Jules Terret, Thomas Deshayes, Xavier Baudrimont, Sébastien Breurec, Emma Rochelle-Newall, Karine Laroucau

### ► To cite this version:

Mégane Gasqué, Vanina Guernier-Cambert, Gil Manuel, Rachid Aaziz, Jules Terret, et al.. Serological screening in animals combined with environmental surveys provides definite proof of the local establishment of *Burkholderia pseudomallei* in Guadeloupe. 2024. anses-04557601

**HAL Id: anses-04557601**

**<https://anses.hal.science/anses-04557601>**

Preprint submitted on 29 Apr 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

1 **Serological screening in animals combined with environmental surveys provides definite**  
2 **proof of the local establishment of *Burkholderia pseudomallei* in Guadeloupe**

3

4 Short title: *Burkholderia pseudomallei* in the French West Indies

5

6 Mégane Gasqué<sup>1,2</sup>, Vanina Guernier-Cambert<sup>2,3\*</sup>, Gil Manuel<sup>4</sup>, Rachid Aaziz<sup>1</sup>, Jules Terret<sup>1</sup>,

7 Thomas Deshayes<sup>1</sup>, Xavier Baudrimont<sup>5</sup>, Sébastien Breurec<sup>6</sup>, Emma Rochelle-Newall<sup>2</sup>, Karine

8 Laroucau<sup>1</sup>

9

10 <sup>1</sup> Animal health laboratory, Bacterial Zoonosis Laboratory, Anses, 947000 Maisons-Alfort,  
11 France

12 <sup>2</sup> Sorbonne Université, UPEC, IRD, CNRS, INRAE, Institute of Ecology and Environmental  
13 Sciences-Paris (iEES-Paris), 4 place Jussieu, 75005 Paris, France

14 <sup>3</sup> Faculty of Veterinary Technology, Kasetsart University, Bangkok, Thailand

15 <sup>4</sup> Veterinary clinic of Carmel, 97100 Basse Terre, Guadeloupe, France

16 <sup>5</sup> Unit for Animal and Plant Health and Protection Environment (SPAVE), Directorate for  
17 Environment, Agriculture, Food and Forestry (DEAAF), Veterinary and Phytosanitary  
18 Inspection Service (SIVEP)

19 <sup>6</sup> Pasteur Institute of Guadeloupe, Morne Jolivière, Les Abymes 97139, Guadeloupe, France

20

21 \*Corresponding author

22 E-mail: [vanina.guernier@ird.fr](mailto:vanina.guernier@ird.fr) (VG)

23

24

25

26 **Abstract**

27 **Background**

28 Melioidosis is an emerging infectious disease caused by the soil-dwelling bacterium  
29 *Burkholderia pseudomallei* that affects both humans and animals. It is endemic in South and  
30 Southeast Asia, and northern Australia, causing an estimated 165,000 human cases annually  
31 worldwide. Human cases have been reported in the French West Indies (Martinique and  
32 Guadeloupe) since the 1990s. Conversely, no human cases have been reported in French  
33 Guiana, a French territory in South America. Our study aimed to investigate whether *B.*  
34 *pseudomallei* is locally established in Guadeloupe and French Guiana. We assessed animal  
35 exposure by serology and examined the presence of *B. pseudomallei* in the environment of  
36 seropositive animals.

37 **Methodology/Principal findings**

38 Blood samples were collected from domestic animals in two goat farms in Les Saintes,  
39 Guadeloupe (n=31), and in 56 farms in French Guiana (n=670) and tested by ELISA.  
40 Serological follow up was performed on selected farms. Soil, water and goat rectal swabs  
41 were collected and analysed by culture and PCR. In French Guiana, the highest prevalence  
42 rates were observed in equids (24%) and cattle (16%), while in Les Saintes, a prevalence of  
43 39% was observed in goats. The longitudinal study in Les Saintes revealed consistent high  
44 seropositivity in goats. A *B. pseudomallei* strain was isolated from the soil from one of the  
45 farms and detected in goat rectal swabs from the other farm.

46 **Conclusions/Significance**

47 Our environmental investigation prompted by the serologic data confirms the presence of *B.*  
48 *pseudomallei* in Les Saintes, consistent with documented human cases of melioidosis on this  
49 island. In French Guiana, our serologic results call for environmental surveys and a re-

50 evaluation of human infections with melioidosis-like symptoms. The approach developed in  
51 this study may help to identify high-risk areas that warrant further investigation.

52

53 **Keywords:** serology, veterinary science, ELISA, environment, melioidosis, *Burkholderia*  
54 *pseudomallei*

55

### 56 **Author summary**

57 *Burkholderia pseudomallei*, an environmental bacterium, is the causative agent of melioidosis  
58 in humans and animals. If the disease has been historically reported to be endemic in South  
59 Asia and northern Australia, recent studies reveal its presence outside of these territories, both  
60 in the environment and among patients who have not travelled to endemic areas. Furthermore,  
61 the projected increase in extreme climatic events in the near future could increase the  
62 prevalence of the disease as well as cause its emergence in new territories. For these reasons,  
63 it is important to identify new areas at risk.

64 Our study aimed to investigate the presence of the pathogen in French West Indies. We  
65 combined surveys in domestic animals (cattle, goats, horses, sheep, and pigs) and in the  
66 environment. The identification of seropositive animals without clinical signs, together with  
67 the isolation of *B. pseudomallei* in the environment of a goat farm in Guadeloupe, underscores  
68 the importance of including melioidosis in animal surveillance programs. The use of serologic  
69 methods can help identify animal exposure to the pathogen, thereby helping to identify areas  
70 where the pathogen may be present in the environment.

## 71 **Introduction**

72 Melioidosis is an opportunistic infectious disease with a significant prevalence in South Asia,  
73 Southeast Asia, and northern Australia, primarily due to environmental exposure to  
74 *Burkholderia (B.) pseudomallei*. This telluric bacterium can affect both humans and animals  
75 through inhalation, ingestion, or skin contact [1]. It is listed as a Class 1 pathogen by the U.S.  
76 Centers for Disease Control and Prevention (CDC) and as a selected agent under the  
77 Microorganisms and Toxins (MOT) regulations by the French National Agency for the Safety  
78 of Medicines and Health Products (ANSM).

79 The global melioidosis burden was predicted to be 165,000 human cases in 2015 (2). Moreover,  
80 reports of human and, occasionally, animal cases are increasing along with environmental  
81 evidence of the bacteria worldwide, highlighting the probable presence of the bacterium in all  
82 continents, including Africa and the Americas [3–5].

83 The clinical presentation of the disease is highly variable between individuals and species,  
84 ranging from chronic forms to acute septicaemia [1,6]. Diagnosis and treatment of human  
85 melioidosis are complicated by the lack of pathognomonic clinical features and a long  
86 treatment duration. Several risk factors, such as diabetes, and chronic diseases, contribute to  
87 increased susceptibility [7]. In animals, melioidosis affects a variety of species with multiple  
88 cases documented in domestic animals such as cats, cattle, deer, dogs, goats, equids, sheep, and  
89 sporadic cases in other species, including marine mammals, birds and even crocodiles in zoos  
90 or wildlife parks [6].

91 Human melioidosis is a neglected disease and, as such, lacks accurate epidemiological data and  
92 diagnostic tools. These are even more limited for animal melioidosis, and the diagnostic  
93 approaches for animal melioidosis are generally the same as those in humans [6,8,9]. Although  
94 diagnostic methods such as indirect hemagglutination (IHA), complement fixation test (CFT)  
95 and enzyme-linked immunosorbent assay (ELISA) have been developed for commonly tested

96 species such as small ruminants, pigs, and equids [10–12], standardised tests for the diagnosis  
97 of melioidosis in all susceptible species are currently lacking. A recent study highlighted the  
98 effectiveness of serological tests developed for glanders (a disease caused by *B. mallei*, a  
99 bacterium closely related to *B. pseudomallei*) for accurately detecting melioidosis in equids.  
100 These tests include both a reference method (CFT) and alternative methods (ELISA, Western  
101 blot) [13].

102 The bacterium *B. pseudomallei* is primarily found in moist clay soils and in turbid waters  
103 [14,15]. Its distribution in soils is strongly influenced by climatic events, with more cases  
104 observed during the rainy season and sporadic contamination peaking during extreme weather  
105 events such as storms or floods [16,17]. Environmental detection of *B. pseudomallei* is  
106 challenging and specific culture protocols are required to isolate the bacterium [18]. Its  
107 detection could be harder in non-endemic areas where the bacterium may be present at lower  
108 levels. A new culture medium, with erythritol as a carbon source, was recently developed to  
109 improve culture isolation from soil samples [19]. It was successfully used to isolate *B.*  
110 *pseudomallei* from a rice farm in South-Central Ghana, Western Africa [20].

111 Cases of melioidosis have been reported in both South, Central and North America. This  
112 includes the French West Indies, i.e., Guadeloupe and Martinique, where 21 human cases have  
113 been recorded since 1993 [21–25], some of whom had no history of travel to endemic areas. In  
114 contrast, no human cases have been reported in French Guiana (Guiana Shield, South America),  
115 while human cases have been reported in neighboring Brazil [3], as well as in Venezuela and  
116 Colombia [26].

117 The objective of this study was to demonstrate the local establishment of *B. pseudomallei* in  
118 French Guiana and Guadeloupe. We aimed to document animal exposure through a serology  
119 study, to document their immune response over time, and to investigate the link between  
120 seropositive animals and the presence of *B. pseudomallei* in their close environment. The

121 serological analysis was performed with an ELISA kit – using a recombinant protein and  
122 double antigen technology – originally developed for the diagnosis of equine glanders [11] and  
123 was used in our study on different domestic species (goats, cattle, equids and sheep).

124

## 125 **Materials and Methods**

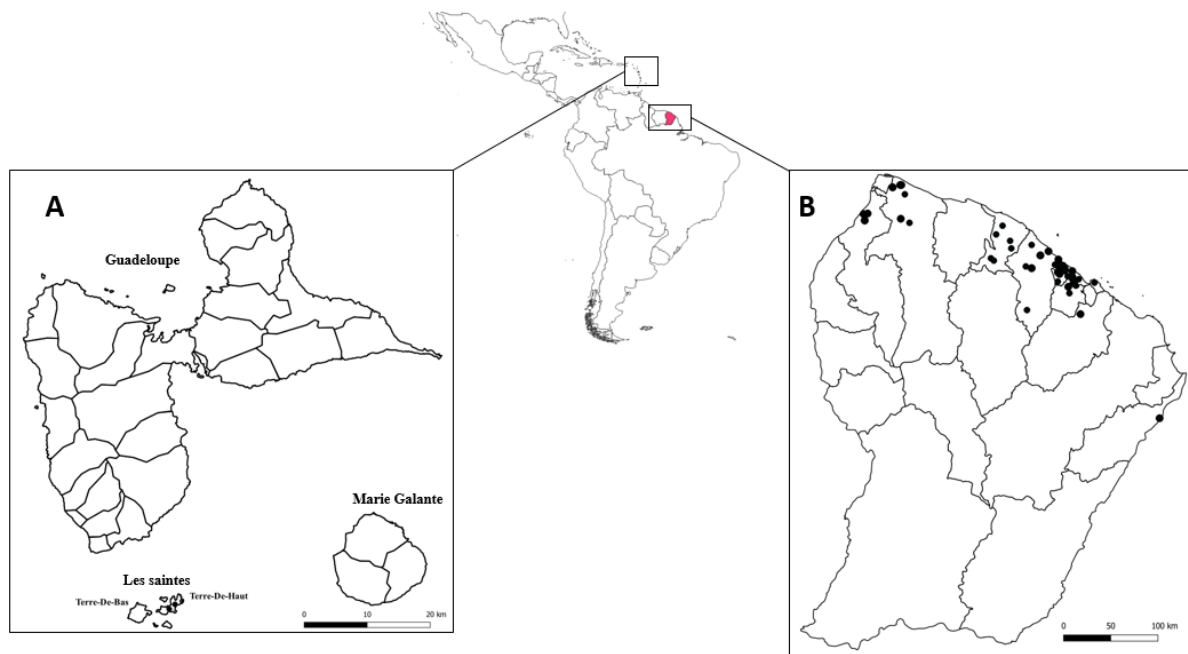
### 126 **Animal sampling in French Guiana and Guadeloupe**

127 In French Guiana, a total of 56 farms were surveyed, primarily along the northern coast where  
128 80% of the population resides (Fig. 1A). These farms included both single-species and  
129 multispecies farms, and 2 to 31 animals per farm were analysed. Among the different regions,  
130 24 farms were located in Macouria, 14 in Kourou, 9 in Mana, 7 in Saint Laurent du Maroni, 5  
131 in Sinnamary, 3 in Montsinery Tonnegrade, 2 in Saint George de l'Oyapack and Cayenne, and  
132 1 in Roura and Iracoubo. Notably, 10 of these farms had multiple species, including cattle,  
133 sheep and equids. In Les Saintes, part of the Guadeloupe archipelago, two goat farms were  
134 sampled, Farm A with approximately 40 goats and Farm B with approximately 60 goats on the  
135 island of Terre de Haut (Fig. 1B). On both farms, mixed-sex goats grazed freely in open  
136 pastures, consuming local vegetation, and no birth control measures were used, hence the  
137 approximate herd sizes reported by the farmers. The topography of the pastures was  
138 characterized by steep terrain and both were susceptible to significant surface water runoff.

139 Blood samples were collected between November 2021 and December 2022 from i) cattle (361  
140 animals from 37 farms), sheep (131 animals from 15 farms), goats (100 animals from 10 farms),  
141 equids (63 animals from six farms), and pigs (15 animals from one farm) in French Guiana,  
142 and ii) goats (31 animals from two farms) in Les Saintes.

143

144 **Fig 1. Map of sampling sites.** Farms where animals were tested in (A) Guadeloupe and (B)  
145 French Guiana are represented as black dots.



146

147 After blood clotting and centrifugation at 900 rpm for 10 min, sera were collected, heat-  
148 inactivated at 60°C for 30 min and stored at -20°C until analysis.

149 In some farms with GLANDA-ELISA positive animals (see § Serological analysis hereafter),  
150 serological longitudinal monitoring was conducted. In French Guiana, additional serological  
151 tests were conducted in one equid farm between October 2021 and January 2022. In Les  
152 Saintes, a more detailed longitudinal study was conducted in the two goat farms. Blood samples  
153 were collected between November 2021 and March 2023 (farm A) or December 2022 (farm  
154 B) (Fig 2). Rectal swabs were collected in duplicate for each animal between April 2022 and  
155 December 2022 (Fig 2). One swab was stored dry at -20°C until PCR analysis, while the second  
156 was stored in Luria-Bertani (LB)-20% glycerol at -20°C for culture.

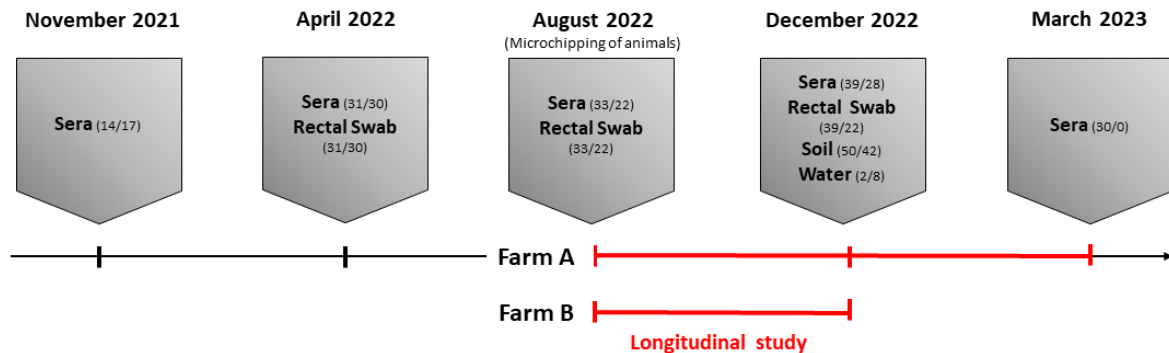
157 Animal sampling protocols were approved by the IRD Ethics Committee (21 April 2022).

158

159 **Fig 2. Sampling timeline in two goat farms in Les Saintes, Guadeloupe.** Sampling occurred  
160 between November 2021 and March 2023. The longitudinal serological study (indicated in red)  
161 took place between August 2022 and March 2023 for farm A, and between August 2022 and



162 December 2022 for farm B. The numbers in parentheses correspond to the number of samples  
163 collected from farms A and B, respectively.



164

### 165 Environmental sampling in Guadeloupe

166 Soil and water samples were collected from both goat pastures (farms A and B) in December  
167 2022 during the rainy season (Fig 2). Soil samples (n = 50 in farm A, n = 42 in farm B) were  
168 collected every 5 m along a series of transects perpendicular to the main slope of the pasture.  
169 Each transect was distant by at least 6 m from the previous transect. Samples were collected  
170 30 cm deep with a standard soil auger that was disinfected with absolute ethanol after each  
171 sampling. Approximately 200 g of soil was collected and placed in closed Ziploc bags to  
172 maintain moisture. Samples were stored at room temperature (approximately 25°C) and  
173 protected from light until shipped to France. Samples were analysed within two weeks of  
174 collection (see § Environmental sample processing). Water samples were collected in farm A  
175 (n = 2) and B (n = 8) in goat drinking troughs.

176 All samples (both animal and environmental) were sent to Anses, Maisons-Alfort, France, to  
177 be processed.

178

### 179 Serological analysis

180 The ID Screen<sup>®</sup>Glanders Double Antigen Multi-species ELISA test (Innovative Diagnostics,  
181 Grabels, France) (hereafter referred to as GLANDA-ELISA) was used according to the  
182 manufacturer's instructions. This test does not rely on the use of a specific conjugate and can

183 therefore be used for multiple animal species. The relative amounts of antibodies in serum  
184 samples were calculated by reference to the positive and the negative controls provided in the  
185 kit. The serological titer (expressed as the Sample to Positive ratio or S/P%) was calculated  
186 using the optical density (OD) reading at 450 nm following the formula:  $S/P\% = ((OD \text{ sample}$   
187  $- OD \text{ negative control}) / (OD \text{ positive control} - OD \text{ negative control})) \times 100$ . Serum samples  
188 with  $S/P\% < 70\%$  were considered negative and  $\geq 70\%$  were considered positive.

189

### 190 **Additional serological tests on equine sera, French Guiana**

191 The complement fixation test (CFT), the reference method for glanders in equids, was  
192 performed as previously described using the cold method with the Malleus CFT antigen  
193 (Bioveta, Czech Republic) [27]. Serum samples were initially tested at 1/5 dilution. Samples  
194 with 100% hemolysis were considered negative, those with 25–75% hemolysis were  
195 considered doubtful, and those with 100% inhibition of hemolysis were considered positive.  
196 All samples primarily identified as suspect or positive were retested over a range of dilutions.  
197 The “titer” is a six-digit barcode corresponding to the intensity of hemolysis inhibition (0 =  
198 0%; 1 = 25%; 2 = 50%; 3 = 75%; 4 = 100%) at the reciprocal dilutions (1/5, 1/10, 1/20, 1/40,  
199 1/80, 1/160) for each sample. Anti-complementary activity (due to incomplete elimination of  
200 complement proteins during the serum heat inactivation) was checked for each serum.  
201 Additionally, the Luminex assay, originally developed for the diagnosis of glanders in equids,  
202 was performed as previously described using the heat shock protein (GroEL) (BPSL2697) and  
203 hemolysin-coregulated protein (Hcp1) (BPSS1498) antigens [28]. MRI#1 was used as positive  
204 control to determine S/P%, which was calculated for each antigen using the same formula as  
205 in the GLANDA-ELISA. S/P% values greater than 45% for GroEL and 43% for Hcp1 were  
206 considered positive.

207

## 208 **Environmental sample processing**

209 Each soil sample was homogenized, and 10 g of soil was diluted in 10 mL of sterile water.  
210 After vigorous vortexing, the suspension was shaken on an orbital shaker at 160 rpm for one  
211 hour and then allowed to settle for 10 min. From the supernatant, 1 mL was centrifuged at  
212 8,000 rpm for 10 min and the resulting pellet was stored at -80°C until PCR analysis and 1 mL  
213 was used for a two-step enrichment. First, 1 mL of supernatant was mixed with 9 mL of liquid  
214 Ashdown medium [29] and incubated at 37°C for 48 h. Second, the Ashdown enrichment was  
215 mixed, and 1 mL was transferred to 9 mL of erythritol medium [19] and incubated at 37°C for  
216 96 h. One mL of erythritol enrichment was centrifuged at 8,000 rpm for 10 min and the  
217 resulting pellet was stored at -80°C until PCR analysis. The remaining 9 mL of enriched  
218 medium was stored at 4°C.

219 Within two hours of collection, 50 mL of each water sample were filtered sequentially through  
220 a 3 µm filter followed by a 0.2 µm filter. The 0.2 µm filters were folded in four, preserved in  
221 Lysogeny Broth (LB) (10 g peptone, 5 g yeast extract, 10 g NaCl for 1 L)-glycerol 20% and  
222 stored at -80°C until analysis. For analysis, the LB-glycerol 20% was discarded and the filters  
223 were aseptically cut in half. One half was placed in 10 mL of liquid Ashdown medium and  
224 followed the enrichment protocol described above, while the other half was stored at -80°C for  
225 PCR analysis.

226

## 227 **DNA extraction and PCR analysis**

228 The swab heads were cut and placed into the lysis buffer. The half filters, used for filtering the  
229 water samples, were ribolysed for three cycles of 20 s at 5,500 rpm (Lysing Matrix E two mL,  
230 MPBio, Eschwege, Germany) and DNA extraction was performed on 800 µL of the  
231 supernatant. Soil supernatants without pre-enrichment were extracted using the DNeasy  
232 PowerSoil Pro kit (QIAGEN, Hilden, Germany), while supernatants from enriched soil/water

233 samples and rectal swabs were extracted using the High Pure PCR Template Preparation kit  
234 (Roche, Mannheim, Germany) according to the manufacturer's instructions. To ensure  
235 extraction efficiency, an internal inhibition extraction control (Xeno™ DNA control or  
236 Diagenode IPC, Thermofisher) was added to each sample.

237 An initial PCR screening was performed using a real-time PCR assay targeting the *B.*  
238 *pseudomallei* complex and targeting *aroA* [30]. DNA samples that tested positive were further  
239 tested using real-time PCR assays targeting specific regions of the *B. pseudomallei* genome  
240 (*orf11*, BPSS0087, BPSS0745, and/or BPTT4176-4290) [31,32] and/or *B. thailandensis* (70  
241 kDa) [33]. Each PCR reaction contained 5 µL of DNA and 15 µL of reaction mix, 1X of  
242 Universal Mastermix TaqMan™ Fast Advanced 2X (Applied Biosystems™, Vilnius,  
243 Lithuania), 0.5 µM of each primer, 0.1 µM of probe, 1X of IPC Diagenode and 2.8 µL of sterile  
244 water. The amplification procedure included a 2-min incubation at 50°C followed by a  
245 denaturation step at 95°C for 20 sec, then 45 cycles at 95°C for 3 sec and 60°C for 30 sec.

246

#### 247 **Culture and screening of suspected colonies**

248 Enrichment broths stored at 4°C were homogenized and 100 µL were plated on both  
249 conventional Ashdown agar and modified *B. cepacia* CHROMagar™ agar (CHROMagar,  
250 Paris, France) supplemented with 4% glycerol, 500 mg/mL gentamicin and 130 mg/mL  
251 fosfomicin (CHR\_GGF). Plates were incubated at 37°C and monitored daily. Suspect colonies  
252 (purple on Ashdown, green on CHR\_GGF) were transferred to non-selective blood agar plates  
253 containing 5% horse serum (BA) and incubated at 37°C for 24 h. The bacterial suspensions  
254 were resuspended in sterile water, heated at 100°C for 20 min, and then centrifuged at 13,000  
255 g for 10 min. The supernatant was used for PCR analysis (*aroA* and *orf11* systems) to confirm  
256 colonies identification. Isolates confirmed to be *B. pseudomallei* by PCR were further  
257 characterized and their antimicrobial resistance pattern identified.

## 258 ***B. pseudomallei* strain characterization**

### 259 *Antibiogram*

260 Antibiotic susceptibility testing was performed using the disk diffusion method according to  
261 European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [34]. The  
262 antibiotics tested included: amoxicillin-clavulanate (20-10 µg), meropenem (10 µg),  
263 trimethoprim-sulfamethoxazole (1.25-23.75 µg), chloramphenicol (30 µg), imipenem (10 µg),  
264 ceftazidime (10 µg), and tetracycline (30 µg) (Bio-Rad, France). Fresh 0.5 McFarland  
265 suspensions were prepared and plated on Mueller-Hinton agar (Bio-Rad, France). Antibiotic  
266 discs were then placed on the agar and plates were incubated at 35°C for 18 h. EUCAST  
267 breakpoints were used for interpretation. Two reference strains, *Escherichia coli* ATCC 25922  
268 and *Staphylococcus aureus* ATCC 29213, were included as controls.

269

### 270 *Biochemical testing*

271 The API 20 NE system (Biomérieux, France) was used according to the manufacturer's  
272 instructions. Bacterial suspensions were adjusted to a 0.5 McFarland standard and the API  
273 cassette was then incubated at 29°C for 24 h. The internet-based APIWEB database (accessed  
274 7 April 2023, Biomérieux, France, <https://apiweb.biomerieux.com/>) was used to interpret the  
275 obtained profiles. Simultaneously, an oxidase test (oxidase reagent 50 x 0.75 mL, Biomérieux,  
276 France) was performed on each colony.

277

### 278 *Multilocus sequence typing (MLST)*

279 MLST was performed as previously described [35]. Amplified fragments were sent to Eurofins  
280 in Germany for sequencing. The sequences were compared to the *Burkholderia pseudomallei*  
281 PubMLST database for MLST profiling

282 (<https://pubmlst.org/https://pubmlst.org/organisms/burkholderia-pseudomallei>, accessed 17  
283 February 2023) [36].

284

## 285 **Results**

286

### 287 **Serological survey in French Guiana and Guadeloupe**

288 In French Guiana, seropositivity rates were 16% (58/361) in cattle, 2% (2/100) in goats, 3%  
289 (4/131) in sheep, 24% (15/63) in equids and 0% (0/15) in pigs (Table 1). The highest S/P%  
290 were observed in cattle, sheep and equids. In Les Saintes, 39% (12/31) of goats were  
291 seropositive (Table 1) and distributed over two farms (5/14 in farm A; 7/17 in farm B (Table  
292 2)), with S/P% ranging from 0 to 503%.

293

### 294 **Table 1. Summary of the serology (GLANDA-ELISA) results in livestock in French** 295 **Guiana (n = 670) and Les Saintes, Guadeloupe (n = 31)**

296 S/P% serological titer expressed as the Sample to Positive ratio (calculated using a formula,  
297 see § Materials and Methods). Serum samples with S/P%  $\geq 70\%$  were considered positive.  
298 Farms where at least one animal tested GLANDA-ELISA positive were considered positive.

Sampling site	French Guiana					Guadeloupe
	Cattle	Goats	Sheep	Equids	Pigs	Goats
Number of positive animals/total (%)	58/361 (16)	2/100 (2)	4/131 (3)	15/63 (24)	0/15 (0)	12/31 (39)
[Min - Max] S/P%	[0-507]	[0-99]	[0-413]	[0-435]	[0-22]	[0-503]
Number of positive farms/total (%)*	17/37 (45)	2/10 (20)	2/15 (13)	4/6 (66)	0/1 (0)	2/2 (100)

\* The number of farms in the table is higher than the total number of farms tested, as 10 farms included more than one

animal species, i.e., appear in several columns.

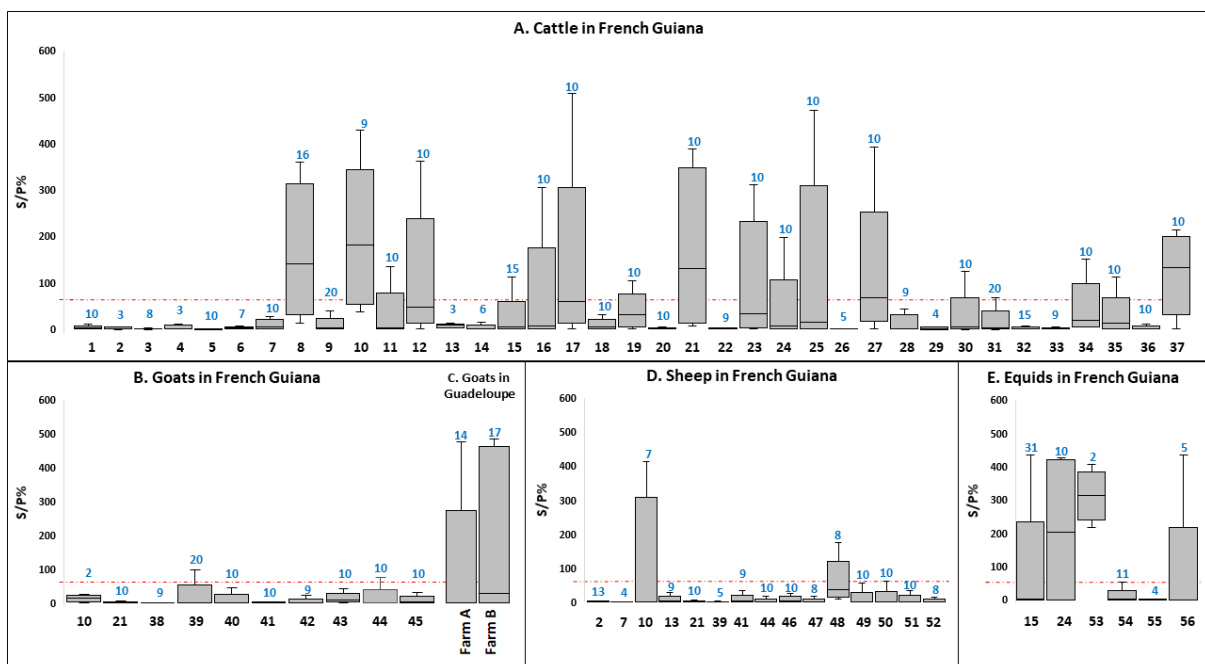
299

300 In French Guiana, 25 out of 56 farms were tested positive, of which 10 included multiple  
301 species. Sixteen were in three townships: Macouria (n = 8), Kourou (n = 4), Sinnamary (n =

302 4). The serological results showed heterogeneity both between farms, differentiating positive  
 303 and negative farms, as well as within positive farms, where both positive and negative animals  
 304 were detected. Positive animals showed variable S/P% (**Fig 3, S1 Table**). For example, among  
 305 the 25 ELISA-positive farms in French Guiana, 10 farms included animals with S/P% > 200.  
 306

307 **Fig 3. Summary of GLANDA-ELISA results per farm per animal species**

308 (A) cattle (n = 37), (B) goats in French Guiana (n = 10), (C) goats in Les Saintes (n = 2), (D)  
 309 sheep (n = 15), (E) equids (n = 6). S/P%: serological titer expressed as the Sample to Positive  
 310 ratio. The x-axis shows the farm identification numbers. Farms are numbered from 1 to 56,  
 311 when several species were on the same farm, the same number is used. The total number of  
 312 animals tested per farm is shown in blue above each box plot. The red dotted line represents  
 313 the S/P% threshold (70%). The bar above and below the box plot indicates the minimum and  
 314 maximum S/P%. The top and bottom of the box indicate the 1<sup>st</sup> and 3<sup>rd</sup> quartiles of S/P%. The  
 315 bar in the box indicates the median. Due to the small number of pig farms tested, we have  
 316 excluded these data from the following figure.



317

318

319 **Additional analysis on equids from French Guiana and follow-up on farm #24**

320 In French Guiana, equids originating from six farms out of 56 (2 to 31 equids per farm; n=63)  
 321 were further tested by two additional methods developed for the diagnosis of glanders: the  
 322 reference CFT method and the newly developed Luminex method using GroEL and Hcp1  
 323 antigens [28]. All equids with ELISA-positive results were confirmed positive with Hcp1 by  
 324 Luminex, but negative with CFT and with GroEL by Luminex except for farm 24 (**Table 2**).  
 325 In this farm, some of the horses/equids were tested positive with CFT.

326

327 **Table 2. Serology results from 63 equids from six farms in French Guiana**

328 GLANDA-ELISA: enzyme-linked immunosorbent assay with the ID Screen® Glanders  
 329 Double Antigen Multi-species ELISA test (Innovative Diagnostics, Grabels, France). CFT:  
 330 complement fixation test (see § Materiel and methods; Additional serological tests on equine  
 331 sera, French Guiana). S/P%: serological titer expressed as the Sample to Positive ratio.  
 332 Luminex: bead-based assay targeting recombinant proteins Hcp1 or GroEL. Id: animal  
 333 identifier; P: positive; D: doubtful; N: negative; AC: anti-complementarity (interpretation not  
 334 possible); na: not analysed; ns: not sampled.

Id	Farm (sampling date)	GLANDA-ELISA	CFT	Luminex - Hcp1	Luminex - GroEL
		(P≥70%)		(P≥43%)	(P≥45%)
		Result (S/P%)	Result <sup>a</sup>	Result (S/P%)	Result (S/P%)
03684		P (411%)	N	P (103%)	N (27%)
03685		N (0%)	N	N (19%)	N (5%)
03686		P (424%)	N	P (107%)	N (7%)
03687		N (0%)	N	N (1%)	N (2%)
03688		N (0%)	N	N (31%)	N (4%)
03689		N (1%)	N	N (5%)	N (9%)
03690	Farm 15	N (0%)	N	N (8%)	N (3%)
03691	(Mar-21)	N (0%)	N	N (3%)	N (5%)
03692		N (0%)	N	N (3%)	N (29%)
03693		N (1%)	N	N (4%)	N (6%)
03694		P (137%)	N	P (107%)	N (1%)
03695		N (0%)	N	N (2%)	N (4%)
03696		N (44%)	N	P (98%)	N (2%)



03697		P (144%)	N	P (66%)	N (4%)
03698		N (1%)	N	N (28%)	N (15%)
03699		N (0%)	N	N (0%)	N (2%)
03700		N (0%)	N	N (4%)	N (10%)
03701		N (5%)	N	N (17%)	N (8%)
03702		N (20%)	N	N (2%)	N (14%)
03703		P (419%)	N	P (99%)	N (11%)
03704		P (435%)	N	P (108%)	N (4%)
03705		N (0%)	N	N (5%)	N (6%)
03706		N (0%)	AC	N (5%)	N (17%)
03707		N (0%)	N	N (1%)	N (1%)
03708		N (11%)	N	N (17%)	N (3%)
03709		N (25%)	N	N (17%)	N (10%)
03710		N (5%)	N	P (76%)	N (10%)
03711		N (0%)	N	N (19%)	N (5%)
03712		N (0%)	N	N (1%)	N (0%)
03713		N (0%)	AC	N (2%)	P (58%)
03714		P (203%)	N	P (68%)	N (3%)
<hr/>					
11786		N (0%) / N (0%)	N / N	N (2%) / N (2%)	N (16%) / N (12%)
11787		P (382%) / P (413%)	P <sup>1</sup> / P <sup>2</sup>	P (104%) / P (101%)	N (2%) / N (2%)
11788		P (296%) / P (387%)	N / N	P (90%) / P (58%)	N (2%) / N (1%)
11789		P (420%) / P (406%)	P <sup>3</sup> / P <sup>4</sup>	P (101%) / P (111%)	N (3%) / N (4%)
11790	Farm 24	N (0%) / N (0%)	P <sup>5</sup> / P <sup>6</sup>	N (1%) / N (8%)	N (19%) / N (12%)
11791	(Oct-21 / Jan-22)	N (0%) / ns	D <sup>7</sup> / ns	N (3%) / ns	N (29%) / ns
01034		ns / N (0%)	ns / P <sup>8</sup>	na	na
01036		ns / N (56%)	ns / N	na	na
01038		ns / P (427%)	ns / N	ns / P (96%)	ns / N (2%)
01040		ns / P (408%)	ns / D <sup>9</sup>	ns / P (106%)	ns / N (3%)
<hr/>					
03671	Farm 53	P (407%)	N	P (96%)	N (11%)
03672	(Mar-21)	P (217%)	N	P (70%)	N (6%)
<hr/>					
03673		N (0%)	AC	N (1%)	N (15%)
03674		N (3%)	N	N (1%)	N (3%)
03675		N (2%)	N	N (4%)	N (3%)
03676		N (1%)	N	N (11%)	N (12%)
03677		N (0%)	AC	N (1%)	N (5%)
03678	Farm 54	N (51%)	N	N (38%)	N (3%)
03679	(Mar-21)	N (1%)	N	N (14%)	N (15%)
03680		N (0%)	N	N (2%)	N (2%)
03681		N (0%)	N	N (3%)	N (5%)
03682		N (1%)	N	N (2%)	N (3%)
03683		N (3%)	AC	N (7%)	N (3%)
<hr/>					
04240		N (2%)	N	N (8%)	N (5%)
04241	Farm 55	N (3%)	N	N (6%)	N (3%)
04242	(Mar-21)	N (0%)	AC	N (1%)	N (0%)
04243		N (0%)	N	N (0%)	N (0%)
<hr/>					
05946	Farm 56	N (0%)	N	N (8%)	N (17%)

05947	(Apr-21)	P (435%)	N	P (99%)	N (4%)
05948		N (0%)	N	N (7%)	N (0%)
05949		N (0%)	N	N (1%)	N (6%)
05950		N (1%)	N	N (2%)	N (3%)

\*CFT titer mentioned in the footnote. <sup>1</sup>444310, <sup>2</sup>431000, <sup>3</sup>444431, <sup>4</sup>421000, <sup>5</sup>444431, <sup>6</sup>421000, <sup>7</sup>222100, <sup>8</sup>431000, <sup>9</sup>200000.

335

### 336 **Focused study in two goat farms in Les Saintes, Guadeloupe**

337

#### 338 *Serological and molecular biology study*

339 To investigate the temporal evolution of the serological response in two goat farms that initially  
340 tested ELISA-positive in November 2021, additional blood samples were collected in April  
341 2022, August 2022, and December 2022. Farm A was also sampled again in March 2023.

342 Seropositive animals were consistently detected in both farms at all-time points, although the  
343 percentage of positive animals varied over time. It ranged from 13% to 36% in farm A and  
344 from 23% to 41% in farm B. The highest number of ELISA-positive animals was observed in  
345 both farms in November 2021 (Table 3).

346 Rectal swabs were collected in parallel with blood samples to test for the fecal shedding of *B.*  
347 *pseudomallei*. In total, six swabs tested positive by PCR for the *B. pseudomallei* complex, of  
348 which two were positive for *B. pseudomallei* species, one collected in April 2022 and one in  
349 December 2022 in farm A (Table 3).

350

### 351 **Table 3. Serological and molecular results from two goat farms in Les Saintes,** 352 **Guadeloupe**

353 The ratio of real-time PCR-positive rectal swabs samples to the total number of samples tested  
354 is provided for six gene targets. The PCR cycle threshold (Ct) is provided for positive samples.

355 ELISA: enzyme-linked immunosorbent assay with the ID Screen® Glanders Double Antigen  
356 Multi-species ELISA test (Innovative Diagnostics, Grabels, France).

---

GLANDA-ELISA

Real-time PCR

Farm	Date of sampling	pos/tot (%)	[min - max] S/P%	<i>Bpm</i> complex	<i>Bpm</i>				<i>Bt</i>
				<i>aroA</i>	<i>orf11</i>	BPSS087	BPSS0745	BPSS4208	70 kDa
Farm A	Nov-21	5/14 (36)	[0 - 494]	ns	ns	ns	ns	ns	ns
	Apr-22	4/31 (13)	[0 - 373]	2/31 <sup>(Ct 43;38)</sup>	1/2 <sup>(Ct 37)</sup>	0/2	0/2	0/2	0/2
	Aug-22	8/33 (24)	[0 - 289]	0/33	0/33	na	na	na	na
	Dec-22	11/39 (28)	[0 - 395]	1/39 <sup>(Ct 41)</sup>	1/1 <sup>(Ct 39)</sup>	1/1 <sup>(Ct 38)</sup>	1/1 <sup>(Ct 41)</sup>	0/1	0/1
	Mar-23	7/30 (23)	[0 - 396]	0/30	na	na	na	na	na
Farm B	Nov-21	7/17 (41)	[0 - 503]	ns	ns	ns	ns	ns	ns
	Apr-22	7/30 (23)	[0 - 351]	3/30 <sup>(Ct 39;40;37)</sup>	0/3	na	na	na	na
	Aug-22	5/22 (23)	[0 - 343]	0/22	0/22	na	na	na	na
	Dec-22	7/25 (28)	[0 - 455]	0/25	0/25	na	na	na	na

357 *Bpm*: *Burkholderia pseudomallei*, *Bt*: *Burkholderia thailandensis*. Apr: April; Aug: August; Mar: March; Nov:  
358 November; Dec: December. ns: not sampled; na: not analysed (samples for which *aroA* and *orf11* PCR analysis  
359 were negative). Pos/tot (%): number of positive animals/total (percentage), S/P%: serological titer expressed as  
360 the Sample to Positive ratio.  
361

362 The longitudinal serological study started in August 2022, when as many goats as possible  
363 were implanted with a microchip to identify individual goats. In farm A, 20 goats were sampled  
364 three times: 13 were consistently ELISA-negative, five were consistently ELISA-positive and  
365 two changed status during the course of the 8-month study (**Table 4**). Of note, six goats had  
366 S/P% greater than 200% on at least two sampling events. One goat with the lowest S/P%  
367 ( $\approx 150\%$ ) later tested negative in March 2023. Conversely, two goats with initially low S/P%  
368 later had higher values. Of the seven goats that were seropositive at some point, five were over  
369 one year of age. The two identified mother-daughter pairs were consistently negative.  
370 In farm B, 11 animals were sampled in August and December 2022. Eight tested ELISA-  
371 negative twice, one tested ELISA-positive twice, and two changed status during the course of  
372 the study (seroconversion). All three animals that were seropositive at some points were over  
373 one year of age and had S/P% values greater than 200%.

374

375 **Table 4. Longitudinal serological study in two goat farms in Les Saintes, Guadeloupe**  
376 GLANDA-ELISA: enzyme-linked immunosorbent assay with the ID Screen® Glanders  
377 Double Antigen Multi-species ELISA test (Innovative Diagnostics, Grabels, France). S/P%:

378 serological titer expressed as the Sample to Positive ratio, N: negative (S/P% <70), P: positive  
 379 (S/P% ≥70%), F: female, M: male, m: month old, y: year-old, ns: not sampled (farm B was not  
 380 sampled in March 2023).  
 381 \* 5483 is the mother of 5482; \*\* 5574 is the mother of 5465.

				<b>GLANDA-ELISA result (S/P%)</b>		
<b>Farm</b>	<b>Id Animal</b>	<b>Sex</b>	<b>Age</b>	<b>Aug-22</b>	<b>Nov-22</b>	<b>Mar-23</b>
<b>Farm A</b>	<b>1625</b>	F	4 m	N (0%)	N (0%)	N (0%)
	<b>5456</b>	F	4 m	N (0%)	N (0%)	N (0%)
	<b>5482*</b>	F	4 m	N (3%)	N (3%)	ns
	<b>5465**</b>	F	4 m	N (0%)	N (0%)	ns
	<b>5578</b>	F	4 m	N (0%)	N (0%)	N (0%)
	<b>5486</b>	F	6 m	P (140%)	P (377%)	P (385%)
	<b>5554</b>	F	6 m	N (1%)	N (1%)	N (0%)
	<b>5464</b>	F	1 y	N (1%)	P (389%)	P (393%)
	<b>5626</b>	F	1 y	N (1%)	N (1%)	N (3%)
	<b>5469</b>	F	2 y	P (147%)	P (164%)	N (55%)
	<b>5473</b>	F	3 y	P (289%)	P (395%)	P (326%)
	<b>5472</b>	F	4 y	P (265%)	P (385%)	P (396%)
	<b>5478</b>	F	4 y	P (240%)	P (109%)	P (247%)
	<b>5571</b>	F	4 y	N (1%)	N (3%)	N (0%)
	<b>5483*</b>	F	5 y	N (1%)	N (0%)	N (0%)
	<b>5574**</b>	F	5 y	N (0%)	N (0%)	N (0%)
	<b>5460</b>	F	8 y	N (2%)	N (6%)	N (2%)
	<b>5546</b>	M	6 m	N (1%)	N (0%)	N (0%)
	<b>5560</b>	M	6 m	N (2%)	N (10%)	N (1%)
	<b>5476</b>	M	6 m	P (263%)	P (384%)	P (384%)
<b>5477</b>	M	3 y	N (1%)	N (1%)	N (0%)	
<b>5461</b>	M	6 y	N (7%)	N (4%)	N (1%)	
Total positive / total tested				6/20	7/20	7/20
<b>Farm B</b>	<b>5496</b>	F	1 y	N (0%)	N (7%)	
	<b>5492</b>	F	3 y	N (2%)	N (1%)	
	<b>5652</b>	F	4 y	N (0%)	N (0%)	
	<b>5435</b>	F	5 y	P (272%)	P (455%)	
	<b>5646</b>	F	6 y	N (32%)	P (398%)	
	<b>5506</b>	M	2 y	N (1%)	N (7%)	ns
	<b>5562</b>	M	2 y	N (2%)	N (67%)	
	<b>5606</b>	M	2 y	N (34%)	N (59%)	
	<b>5610</b>	M	2 y	N (14%)	P (379%)	
	<b>5640</b>	M	2 y	N (0%)	N (2%)	
<b>5690</b>	M	2 y	N (0%)	N (3%)		

Total positive / total  
tested 1/11 3/11

382 ***Environmental sampling in goat pastures***

383 In December 2022, 50 soil samples and two water samples (from the drinking troughs) were  
384 collected from farm A; and 42 soil samples and eight water samples (from the drinking troughs)  
385 were collected from farm B. All samples were analysed by PCR and bacteriological culture.  
386 Samples from farm A were all PCR-negative, pre- or post-enrichment. In farm B, two out of  
387 42 soil samples (#313 and #336) were PCR-positive for the *B. pseudomallei* complex post-  
388 enrichment, but only sample #313 tested PCR-positive for *B. pseudomallei* (all four specific  
389 targets) (Table 5).

390

391 **Table 5. Results from molecular testing of environmental samples from two goat farms**  
392 **in Les Saintes, Guadeloupe**

393 Soil and water samples collected in December 2022 were tested by real-time PCR for the  
394 presence of different *Burkholderia* spp. Results are provided as the ratio of positive samples  
395 against the total number of tested samples tested for each target. For PCR-positive results, Ct  
396 values are provided. na: not analysed.

Farm	Type of sample	Number of samples	<i>Bpm</i> complex	<i>Bpm</i>				<i>Bt</i>
			<i>Bpm</i> aroA	orf11	BPSS087	BPSS0745	BPSS4208	70 kDa
Farm A	soil	50	0/50*	na	na	na	na	na
	water	2	0/2	na	na	na	na	na
Farm B	soil	42	2/42* (Ct 34 ;34)	1/2*(Ct 40)	1/2*(Ct 38)	1/2*(Ct 37)	1/2*(Ct 39)	0/2*
	water	8	0/8	na	na	na	na	na

397 *Bpm*: *Burkholderia pseudomallei*, *Bt*: *Burkholderia thailandensis*. \* PCR results were obtained post-enrichment  
398 (see § Material and Methods).

399

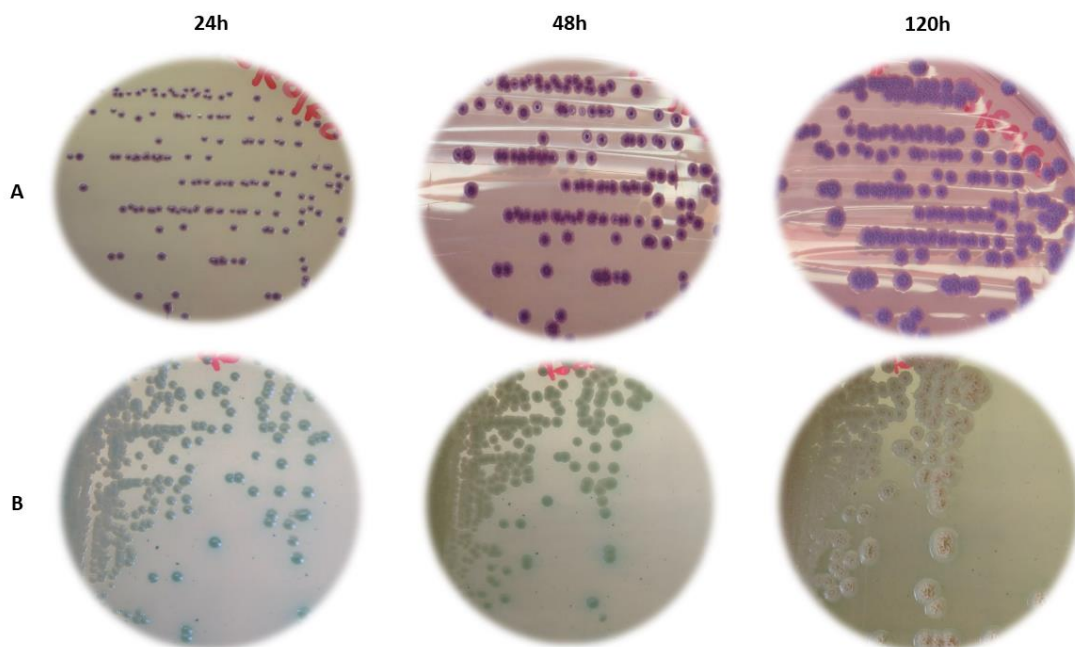
400 PCR-positive soil samples #313 and #336 were plated post-enrichment on both Ashdown and  
401 CHR\_GGF agar media for isolation. A total of 46 suspect colonies were isolated from soil  
402 #313. Of these, 30 were PCR-positive for the *B. pseudomallei* complex and all four *B.*  
403 *pseudomallei* specific targets. Fifteen suspect colonies were isolated from soil #336 and tested

404 PCR-positive for the *B. pseudomallei* complex but PCR-negative for *B. pseudomallei* and *B.*  
405 *thailandensis*.

406 *B. pseudomallei* colonies on Ashdown initially appeared purple and smooth after 24 h at 37°C  
407 and then changed to a rough, dry texture after 48 h (**Fig 4A**). On CHR\_GGF, the same  
408 morphological changes were observed: colonies were green and smooth after 24 h of culture,  
409 but changed to a white, rough, and dry texture after 120 h (**Fig 4B**).

410

411 **Fig 4. *Burkholderia pseudomallei* strain D22-10884\_313#20 cultures.** Photographs from  
412 culture plates (A) on Ashdown and (B) on CHR\_GGF (modified *B. cepacia* CHROMagar™  
413 agar supplemented with 4% glycerol, 500 mg/mL gentamicin and 130 mg/mL fosfomycin)  
414 after a 24, 48 and 120 h incubation at 37°C. The strain was isolated from soil #313 collected  
415 from farm B in Les Saintes, Guadeloupe.



416

417

418 One *B. pseudomallei* colony (D22-10884\_S313#20) isolated from soil #313 was selected for  
419 further analysis. The strain showed the API 20 NE profile 1-456-574 together with a positive  
420 result for the oxidase test. When compared with the profiles listed in APIWEB, the strain had

421 the highest identification scores with *B. pseudomallei* (47.9%) and *B. cepacia* (45.3%). The  
422 antibiotic susceptibility profile of strain D22-10884\_S313#20 showed sensitivity to all  
423 antibiotics tested (commonly used for human melioidosis treatment): amoxicillin-clavulanate,  
424 ceftazidime, tetracycline, imipenem, meropenem, trimethoprim-sulfamethoxazole and  
425 chloramphenicol (**S2 Table**). The MLST sequence type of strain D22-10884\_S313#20 was  
426 ST92.

427

## 428 **Discussion**

429

430 By interpreting positive ELISA results in animals as a sign of previous environmental exposure  
431 to *B. pseudomallei*, our serological screening showed variable results between regions but also  
432 between species. In French Guiana, the seroprevalence was 2% in sheep and goats, 15% in  
433 cattle and 22% in equids; in Les Saintes, where only goats were tested, the seroprevalence was  
434 39%. In comparison, studies carried out in endemic countries using different serological  
435 methods showed seroprevalence of 6% and 13.6% in sheep, 0.3% and 2.6% in goats, and 2%  
436 and 7.6% in cattle, in Thailand (using IHA) [37] and Malaysia (using CFT) [38], respectively.  
437 Due to different levels of endemicity and methodological strategies, the data are not easily  
438 comparable. Our serological screening also showed within-farm variability. The longitudinal  
439 study conducted in two ELISA-positive farms in Les Saintes showed that most goats remained  
440 ELISA-positive over an 8-month period. Further studies are needed to better understand the  
441 long-term immune response dynamics after exposure to *B. pseudomallei*. This should help to  
442 explain the within-farm heterogeneity, i.e., why animals living in farms with similar  
443 environmental exposure do not all show the same serological result.

444

445 Melioidosis has been studied primarily in well-established endemic regions such as Southeast  
446 Asia and Australia with limited research in Central and South America [26,39]. In the  
447 Caribbean, human cases have been documented for over 30 years in Guadeloupe and  
448 Martinique [21–23,25] but the local presence of *B. pseudomallei* in the environment has never  
449 been established. Isolating the strain from the environment is challenging, as demonstrated by  
450 a recent environmental survey in Puerto Rico where only three out of 500 soil samples from 60  
451 sites tested positive for *B. pseudomallei* using PCR, with only one sample being culture positive  
452 [40]. Previous exposure to *B. pseudomallei* promotes an immune response in animals that can  
453 be detected serologically. Hence, one major objective of our study was to confirm the benefit  
454 of an initial serological screening in animals to increase the probability of detecting *B.*  
455 *pseudomallei* in the environment.

456 The GLANDA-ELISA kit, originally developed for the diagnosis of equine glanders [11], was  
457 used on a goat farm in New Caledonia where one case of melioidosis was confirmed. The  
458 serological survey allowed to confirm exposure in 35% of goats on this farm, while those from  
459 three other farms with no clinical case were 100% seronegative (Laroucau *et al.*, en révision).  
460 However, the validation of this ELISA kit in detecting clinical melioidosis and/or previous  
461 exposure to *B. pseudomallei* called for further data. The application of the GLANDA-ELISA  
462 kit in our multi-species study yielded heterogeneous results between individuals and between  
463 farms, with some farms fully seronegative. Overall, serology results were consistent with the  
464 absence (in French Guiana) or limited number (in Les Saintes) of human melioidosis cases.

465 Our study supports the idea that animals could be used as sentinels of *B. pseudomallei* presence  
466 in the environment and that the GLANDA-ELISA kit is a relevant tool to do so. Further  
467 research is now necessary to establish the specificity of this kit in a context different from its  
468 original design and validation. The high diversity and prevalence of *Burkholderia* species in  
469 the environment, including closely related species within the *B. pseudomallei* complex, raises



470 concerns about the possibility of cross-reactivity. Since the identity of the protein used in the  
471 GLANDA-ELISA kit is not disclosed, it is not possible to assess its degree of conservation in  
472 environmental *Burkholderia* strains, if any, and thus the risk of cross-reactivity when using this  
473 kit. In addition, the cutoff used in our study was originally established for equine glanders in a  
474 clinical context and not for the assessment of environmental exposure to *B. pseudomallei*. A  
475 reevaluation of the cutoff for our specific goal and in different host species may help to refine  
476 the data obtained with this kit.

477 While quantitative data on *B. pseudomallei* in the environment are scarce, it is plausible that in  
478 non-endemic areas the bacteria are present at lower concentrations which might decrease the  
479 probability of detection. In anticipation of this additional challenge, we conducted our  
480 environmental sampling in Les Saintes during the rainy season (June to November), a period  
481 identified as the most favorable for the presence of *B. pseudomallei* in the soil [15]. We also  
482 optimized the protocol from existing guidelines [18], adding two new media to the  
483 conventionally recommended Ashdown medium [29]. First, an erythritol-based medium was  
484 used as the sole carbon source during the second step of a two-step enrichment to select for *B.*  
485 *pseudomallei* [19]. A similar two-step enrichment protocol (TBSS-C50 + erythritol) recently  
486 allowed the isolation of environmental *B. pseudomallei* strains in Ghana, West Africa [20].  
487 Second, we used a chromogenic medium originally developed for the detection of *B. cepacia*  
488 in clinical samples as green colonies. To increase its selectivity for *B. pseudomallei*, we  
489 modified the medium by incorporating new antibiotics and confirmed that reference strains of  
490 *B. pseudomallei* grew well on this medium (S1 Figure). To our knowledge, this is the first use  
491 of a chromogenic medium to improve the selection of *B. pseudomallei* colonies from poly-  
492 contaminated environmental matrices. Despite these protocol improvements, in our study only  
493 one soil sample (out of 92 samples collected from seropositive farms) and a single *B.*  
494 *pseudomallei* strain was isolated. Although the use of media CHROMagar™ *B. cepacia* seems

495 promising, further protocol optimization (e.g., sample volume, enrichment time, chromogenic  
496 and/or selective medium for environmental matrices) will be needed to facilitate the detection  
497 and isolation of environmental strains of *B. pseudomallei*. Of note, an additional soil sample  
498 tested PCR-positive for the *B. pseudomallei* complex, but not for *B. pseudomallei* or *B.*  
499 *thailandensis* species. Further characterization of all non-*B. pseudomallei* strains isolated in  
500 this study will help identify all the *Burkholderia* species to which animals can be exposed and  
501 to assess their cross-reactivity with the GLANDA-ELISA kit.

502 Differentiating glanders (caused by *B. mallei*) from melioidosis (caused by *B. pseudomallei*) is  
503 challenging due to their high antigenic similarity [13], with *B. mallei* being a monophyletic  
504 clade within *B. pseudomallei* [41]. Considering that (i) the GLANDA-ELISA kit has been  
505 designed for the diagnosis of equine glanders, and that (ii) glanders is frequently reported in  
506 Brazil [42], a country sharing a border with French Guiana, the detection of GLANDA-ELISA  
507 positive horses in French Guiana warranted further testing. Clinically healthy horses that tested  
508 ELISA-positive were all Hcp1-positive by Luminex but GroEL-negative by Luminex, both  
509 proteins being key markers for glanders diagnosis [28]. When adding CFT (the reference  
510 method for glanders diagnosis) as a fourth test, all ELISA-positive horses were CFT-negative,  
511 except for horses on farm #24. In this farm, the detection of horses positive for three out of four  
512 tests relevant to glanders diagnosis prompted close surveillance. A follow-up examination three  
513 months later confirmed the initial serological results as well as the absence of clinical signs  
514 typical of acute glanders (e.g., respiratory distress or abscesses). Overall, the variability in test  
515 results among animals in farm #24 may indicate different exposure and/or infection dynamics  
516 and/or test characteristics. Despite the absence of previously reported cases of glanders or  
517 melioidosis in French Guiana, the final health status of these equids remains uncertain given  
518 the chronic potential of these two diseases [43–45].

519

520 Although the serological approach is generally not favored for the epidemiology of melioidosis  
521 in endemic areas, due to more frequent environmental exposure of humans and animals and the  
522 resulting challenges of interpreting positive results outside of a clinical context [46,47], this  
523 approach can be promising for non-endemic areas. The GLANDA-ELISA kit may be  
524 particularly valuable for identifying past exposure in animals and conducting targeted  
525 environmental surveys around exposed animals. This is particularly relevant for livestock that  
526 are often confined to restricted areas.

527 Our serological and molecular screening on two goat farms in Les Saintes, followed by an  
528 environmental survey, confirmed fecal shedding of *B. pseudomallei* in one farm and  
529 successfully led to the isolation of an environmental strain of *B. pseudomallei* in the other farm.  
530 These results provide the first definite confirmation of the local establishment of *B.*  
531 *pseudomallei* in Les Saintes, Guadeloupe. They also support the hypothesis of a local  
532 contamination of two cases of human melioidosis reported on the island in 1997 (a tourist that  
533 visited Les Saintes for 3 weeks) [25] and in 2016 (a local resident) [23]. Importantly, the MLST  
534 profile of the strain isolated from our study (ST92) was identical to that of the 1997 human  
535 case [23]. In the *Burkholderia pseudomallei* PubMLST database, ST92 was identified in nine  
536 human strains: one from a Swiss traveler to Martinique [21], one from Puerto Rico, two from  
537 Mexico [48], and five from Brazil [49], illustrating the restricted spatial distribution of this  
538 sequence type.

539 Even though human melioidosis has not been reported so far in French Guiana, our results call  
540 for the implementation of environmental surveys around the ELISA-positive farms identified  
541 in this study, as well as a retrospective re-evaluation of human infections with melioidosis-like  
542 symptoms. In Guadeloupe and Martinique, where several human cases have been diagnosed in  
543 recent years [23,24], we suggest adding melioidosis to existing animal surveillance programs.

544

545 **Supporting information**

546 **S1 Table.** Serological results of animals in French Guiana and Les Saintes (Guadeloupe)

547 **S2 Table.** Antibioqram results of 22-10884\_313#20 strains

548 **S1 Figure.** Evaluation of CHROMagar™ *B. cepacia* as a relevant media for other *Burkholderia*  
549 strains.

550

551

552 **Acknowledgements**

553 This study was supported by the Ile de France Region (Dim1Health) and the European  
554 Commission Directorate-General for Health and Consumers (EC no.180/2008). We thank the  
555 French Institut de Recherche pour le Développement (IRD) and the French Agence nationale  
556 de sécurité sanitaire de l'alimentation, de l'environnement et du travail “ANSES” for their  
557 financial support. We also thank all the livestock farmers, particularly the owners of the two  
558 goat farms in Les Saintes, who allowed repeated sampling to complete the longitudinal study.  
559 Special thanks to CHROMagar™ who supplied the culture media and Dr. Sophie Granier for  
560 her valuable advice on the implementation of the antibiogram test. This work is part of the  
561 Doctoral requirements of the first author (MG).

562

563 **Conflicts of Interest**

564 None of the authors have any financial or personal relationships with any individuals or  
565 organizations that could inappropriately influence or bias this paper.

566

567 **Author Contributions**

568 Conceptualization: Karine Laroucau (KL), Emma Rochelle-Newall (ERN), Vanina Guernier-  
569 Cambert (VGC), Sébastien Breurec (SB)

570 Resources: Gil Manuel (GM), Xavier Baudrimont (XB), Sébastien Breurec (SB)  
571 Methodology: Mégane Gasqué (MG), Karine Laroucau (KL)  
572 Investigation: Mégane Gasqué (MG), Gil Manuel (GM), Xavier Baudrimont (XB), Rachid  
573 Aaziz (RA), Thomas Deshayes (TD), Jules Terret (JT)  
574 Supervision: Karine Laroucau (KL), Emma Rochelle-Newall (ERN), Vanina Guernier-  
575 Cambert (VGC)  
576 Writing –original draft: Mégane Gasqué (MG), Karine Laroucau (KL)  
577 Writing –review & editing: Mégane Gasqué (MG), Karine Laroucau (KL) , Emma Rochelle-  
578 Newall (ERN), Vanina Guernier-Cambert (VGC), Gil Manuel (GM), Xavier Baudrimont (XB),  
579 Rachid Aaziz (RA), Thomas Deshayes (TD), Jules Terret (JT), Sébastien Breurec (SB)

580

## 581 Reference

- 582 1. Meumann EM, Limmathurotsakul D, Dunachie SJ, Wiersinga WJ, Currie BJ.  
583 *Burkholderia pseudomallei* and melioidosis. Nat Rev Microbiol. 2023.  
584 doi:10.1038/s41579-023-00972-5
- 585 2. Limmathurotsakul D, Golding N, Dance D, Messina J, Pigott D, Moyes C, et al. Predicted  
586 global distribution of *Burkholderia pseudomallei* and burden of melioidosis. Nat  
587 Microbiol. 2016. doi:10.1038/nmicrobiol.2015.8
- 588 3. Brilhante RSN, Bandeira TJPG, Cordeiro RA, Grangeiro TB, Lima RAC, Ribeiro JF, et  
589 al. Clinical-epidemiological features of 13 cases of melioidosis in Brazil. J Clin  
590 Microbiol. 2012;50: 3349–3352. doi:10.1128/JCM.01577-12
- 591 4. Renou A, Miltgen G, Blondé R, Jean F, Allyn J, Allou N. Case report: Six new cases of  
592 Melioidosis in the Indian Ocean Region: Comoros, Madagascar, Reunion Island. Am J  
593 Trop Med Hyg. 2020;103: 1844–1845. doi:10.4269/ajtmh.20-0497
- 594 5. Taetzsch SJ, Swaney EM, Gee JE, Hidalgo PM, Broussard KR, Martines RB, et al.  
595 Melioidosis in *Cynomolgus* Macaques (*Macaca Fascicularis* ) imported to the United  
596 States from Cambodia. Comp Med. 2022;72: 394–402. doi:10.30802/AALAS-CM-22-  
597 000024
- 598 6. Sprague LD, Neubauer H. Melioidosis in Animals: A review on epizootiology, diagnosis  
599 and clinical presentation. J Vet Med Ser B. 2004;51: 305–320. doi:10.1111/j.1439-  
600 0450.2004.00797.x
- 601 7. Gassiep I, Armstrong M, Norton R. Human melioidosis. Clin Microbiol Rev. 2020;33:  
602 e00006-19. doi:10.1128/CMR.00006-19

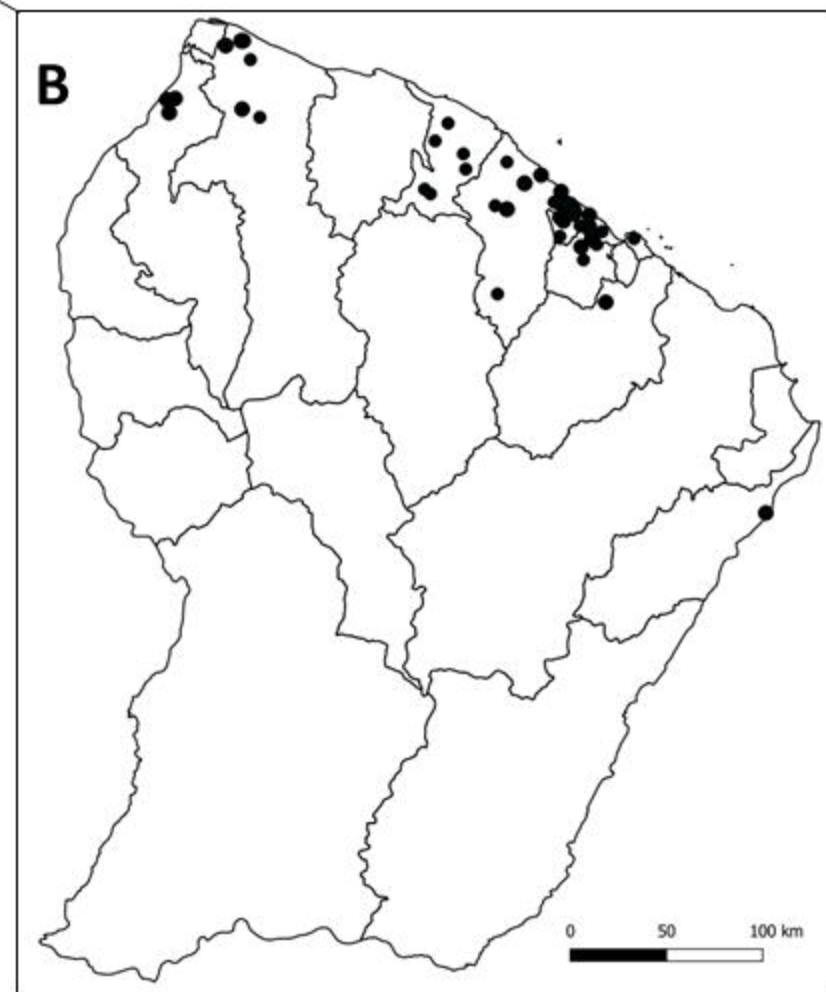
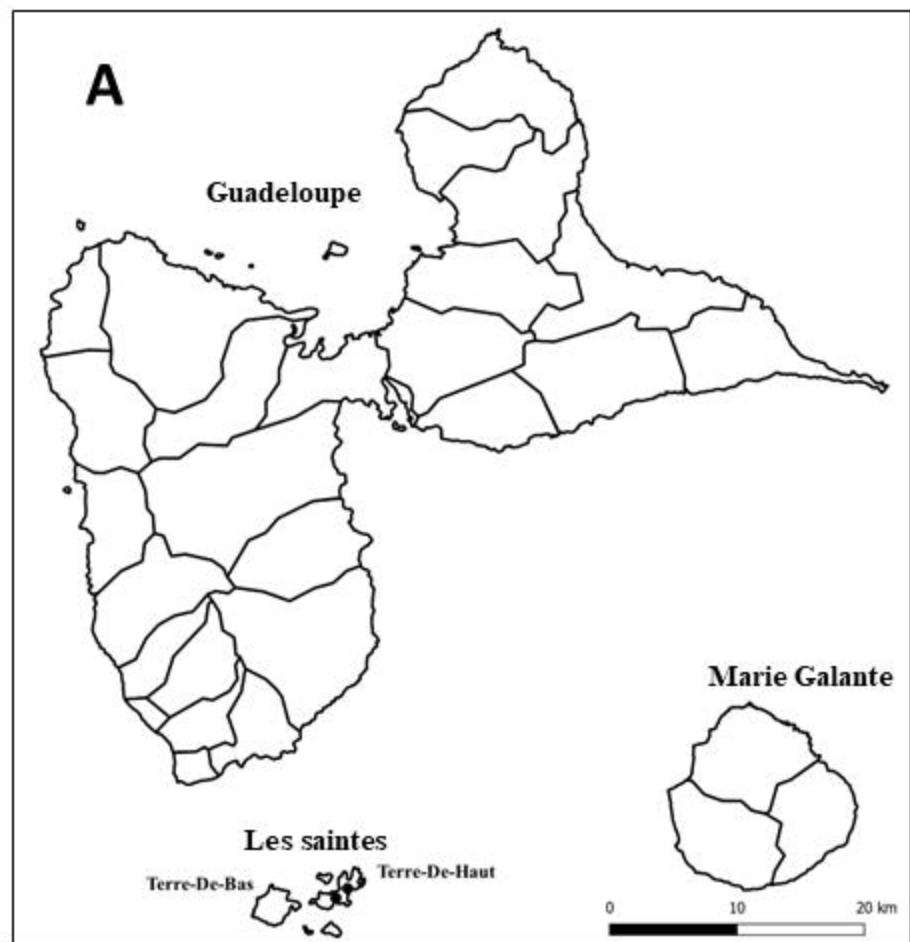
- 603 8. Hambali I, Abba Y, Bitrus A, Peter I, Jesse F, Balakrishnan T, et al. Seroprevalence of  
604 melioidosis in sheep and goats from selected small ruminants in Selangor, Malaysia. *Adv*  
605 *Anim Vet Sci*. 2018;2: 88–94. doi:10.17582/journal.aavs/2018/6.2.88.94
- 606 9. Musa HI, Hassan L, Rachmat R, Chandrawthani P, Zunita Z, Saleha A. Seroprevalence  
607 of melioidosis among livestock in Malaysia from 2000-2009. *Malays J Vet Res*. 2012;3:  
608 41–46.
- 609 10. Duval BD, Elrod MG, Gee JE, Chantratita N, Tandhavanant S, Limmathurotsakul D, et  
610 al. Evaluation of a latex agglutination assay for the identification of *Burkholderia*  
611 *pseudomallei* and *Burkholderia mallei*. *Am J Trop Med Hyg*. 2014;90: 1043–1046.  
612 doi:10.4269/ajtmh.14-0025
- 613 11. Elschner MC, Melzer F, Singha H, Muhammad S, Gardner I, Neubauer H. Validation of  
614 a commercial glanders ELISA as an alternative to the CFT in international trade of  
615 equidae. *Front Vet Sci*. 2021;8: 628389. doi:10.3389/fvets.2021.628389
- 616 12. Elschner MC, Laroucau K, Singha H, Tripathi BN, Saqib M, Gardner I, et al. Evaluation  
617 of the comparative accuracy of the complement fixation test, Western blot and five  
618 enzyme-linked immunosorbent assays for serodiagnosis of glanders. *Plos One*. 2019;14:  
619 e0214963. doi:10.1371/journal.pone.0214963
- 620 13. Wernery U, Caveney M, Wernery R, Raghavan R, Laroucau K, Syriac G, et al. Evaluation  
621 of serological responses in horses challenged with *Burkholderia pseudomallei* using  
622 current diagnostic tests for glanders. *Vet Ital*. 2019;55: 261–267.  
623 doi:10.12834/VetIt.1701.9026.2
- 624 14. Cheng A, Currie B. Melioidosis: epidemiology, pathophysiology, and management. *Clin*  
625 *Microbiol Rev*. 2005;18: 383–416. doi:10.1128/CMR.18.2.383-416.2005
- 626 15. Zimmermann RE, Ribolzi O, Pierret A, Rattanavong S, Robinson MT, Newton PN, et al.  
627 Rivers as carriers and potential sentinels for *Burkholderia pseudomallei* in Laos. *Sci Rep*.  
628 2018;8: 8674. doi:10.1038/s41598-018-26684-y
- 629 16. Canales R, Sanchez-Okrucky R, Bustamante L, Vences M, Dennis M. Melioidosis in a  
630 bottlenose dolphin (*Tursiops truncatus*) after a hurricane in the Caribbean islands. *J Zoo*  
631 *Wildl Med*. 2020;51: 443–447. doi:10.1638/2019-0050
- 632 17. Guendel I, Ekpo LL, Hinkle MK, Harrison CJ, Blaney DD, Gee JE, et al. Melioidosis  
633 after Hurricanes Irma and Maria, St. Thomas/St. John District, US Virgin Islands, October  
634 2017. *Emerg Infect Dis*. 2019;25: 1952–1955. doi:10.3201/eid2510.180959
- 635 18. Limmathurotsakul D, Dance DAB, Wuthiekanun V, Kaestli M, Mayo M, Warner J, et al.  
636 Systematic review and consensus guidelines for environmental sampling of *Burkholderia*  
637 *pseudomallei*. *PLoS Negl Trop Dis*. 2013;7: e2105. doi:10.1371/journal.pntd.0002105
- 638 19. Trinh TT, Assig K, Tran QTL, Goehler A, Bui LNH, Wiede C, et al. Erythritol as a single  
639 carbon source improves cultural isolation of *Burkholderia pseudomallei* from rice paddy  
640 soils. *PLoS Negl Trop Dis*. 2019;13. doi:10.1371/journal.pntd.0007821

- 641 20. Oduro G, Robberts FJL, Dartey PKA, Owusu-Ofori A, Oppong C, Gyampomah TK, et  
642 al. On the Environmental Presence of *Burkholderia pseudomallei* in South-Central  
643 Ghana. *Appl Environ Microbiol.* 2022;88: e00600-22. doi:10.1128/aem.00600-22
- 644 21. Gétaz L, Michel Y, Merlani P, Emonet S. Fatal acute melioidosis in a tourist returning  
645 from Martinique Island, November 2010. 2010. Available from :  
646 <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19758>
- 647 22. Meckenstock R, Therby A, Marque-Juillet S, Sebastian Monnier S, David Khau D,  
648 Pangon, et al. Cutaneous melioidosis in adolescent returning from Guadeloupe. 2012.  
649 doi:<http://dx.doi.org/10.3201/eid1802111603>
- 650 23. Melot B, Bastian S, Dournon N, Valade E, Gorgé O, Le Fleche A, et al. Three new cases  
651 of melioidosis, Guadeloupe, French West Indies. *Emerg Infect Dis.* 2020;26: 617–619.  
652 doi:10.3201/eid2603.190718
- 653 24. Pasquier J, Olive C, Hurtrel G, Turmel J-M, Cabié A. Melioidosis. *Lancet Infect Dis.*  
654 2019;19: 1057. doi:10.1016/S1473-3099(19)30481-5
- 655 25. Pérez J, Petiot A, Adjidé C, Gerry F, Goursaud R, Juminer B. First case report of  
656 melioidosis in Guadeloupe, a French West Indies archipelago. *Clin Infect Dis.* 1997;25:  
657 164–165. doi:10.1086/516896
- 658 26. Rolim DB, Lima RXR, Ribeiro AKC, Colares RM, Lima LDQ, Rodríguez-Morales AJ,  
659 et al. Melioidosis in South America. *Trop Med Infect Dis.* 2018;3: 60.  
660 doi:10.3390/tropicalmed3020060
- 661 27. Laroucau K, Colaneri C, Jaÿ M, Corde Y, Drapeau A, Durand B, et al. Interlaboratory  
662 ring trial to evaluate CFT proficiency of European laboratories for diagnosis of glanders  
663 in equids. *Vet Rec.* 2016;178: 632–632. doi:10.1136/vr.103617
- 664 28. Laroucau K, Saqib M, Martin B, Deshayes T, Bertin C, Wernery U, et al. Development  
665 of a microsphere-based immunoassay for the serological detection of glanders in equids.  
666 *Acta Trop.* 2020;207: 105463. doi:10.1016/j.actatropica.2020.105463
- 667 29. Ashdown LR, Clarke SG. Evaluation of culture techniques for isolation of  
668 *Pseudomonas pseudomallei* from soil. *Appl Environ Microbiol.* 1992;58: 4011–4015.  
669 Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC183218/>  
670
- 671 30. Laroucau K, Aaziz R, Vorimore F, Varghese K, Deshayes T, Bertin C, et al. A genetic  
672 variant of *B. mallei* detected in Kuwait: consequences for the PCR diagnosis of glanders.  
673 *Transbound Emerg Dis.* 2020; 1–4. doi:10.1111/tbed.13777
- 674 31. Göhler A, Trung TT, Hopf V, Kohler C, Hartleib J, Wuthiekanun V, et al. Multitarget  
675 quantitative PCR improves detection and predicts cultivability of the pathogen  
676 *Burkholderia pseudomallei*. *Appl Environ Microbiol.* 2017;83: e03212-16.  
677 doi:10.1128/AEM.03212-16
- 678 32. Thibault FM, Valade E, Vidal DR. Identification and discrimination of *Burkholderia*  
679 *pseudomallei*, *B. mallei*, and *B. thailandensis* by real-time PCR targeting type III secretion

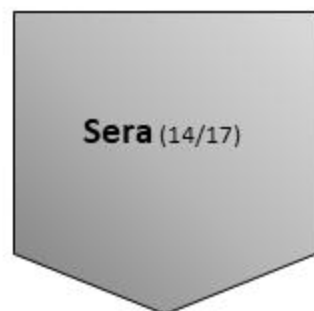
- 680 system genes. *J Clin Microbiol.* 2004;42: 5871–5874. doi:10.1128/JCM.42.12.5871-  
681 5874.2004
- 682 33. Lowe C-W, Satterfield BA, Nelson DB, Thiriot JD, Heder MJ, March JK, et al. A  
683 quadruplex real-time PCR assay for the rapid detection and differentiation of the most  
684 relevant members of the *B. pseudomallei* complex: *B. mallei*, *B. pseudomallei*, and *B.*  
685 *thailandensis*. *Plos One.* 2016;11: e0164006. doi:10.1371/journal.pone.0164006
- 686 34. Matuschek E, Brown DFJ, Kahlmeter G. Development of the EUCAST disk diffusion  
687 antimicrobial susceptibility testing method and its implementation in routine  
688 microbiology laboratories. *Clin Microbiol Infect.* 2014;20: O255–O266.  
689 doi:10.1111/1469-0691.12373
- 690 35. Godoy D, Randle G, Simpson A, Aanensen D, Pitt T, Kinoshita R, et al. Multilocus  
691 sequence typing and evolutionary relationships among the causative agents of melioidosis  
692 and glanders, *Burkholderia pseudomallei* and *Burkholderia mallei*. *J Clin Microbiol.*  
693 2003;41: 2068–2079. doi:10.1128/JCM.41.5.2068-2079.2003
- 694 36. Jolley KA, Bray JE, Maiden MCJ. Open-access bacterial population genomics: BIGSdb  
695 software, the PubMLST.org website and their applications. *Wellcome Open Res.* 2018;3:  
696 124. doi:10.12688/wellcomeopenres.14826.1
- 697 37. Srikawkheaw N, Lawhavinit O. Detection of antibodies against melioidosis from animal  
698 sera in Thailand by indirect haemagglutination test. *Kasetsart J - Nat Sci.* 2007;41: 81–  
699 85. Available from: <https://www.thaiscience.info/journals/Article/TKJN/10471514.pdf>
- 700 38. Masrin A, Fatiha A, Fakhrulisham, Sharil Azwan M. Geographical mapping on  
701 seropositive status of melioidosis among livestock in Malaysia from 2012 to 2016. *Malays*  
702 *J Vet Res.* 2018;9: 44–52. Available from: 20193004779.pdf (cabdirect.org)
- 703 39. Sanchez-Villamil J, Torres A. Melioidosis in Mexico, Central America, and the  
704 Caribbean. *Trop Med Infect Dis.* 2018;3: 24. doi:10.3390/tropicalmed3010024
- 705 40. Hall C, Jaramillo S, Jimenez R, Stone N, Centner H, Busch J, et al. *Burkholderia*  
706 *pseudomallei*, the causative agent of melioidosis, is rare but ecologically established and  
707 widely dispersed in the environment in Puerto Rico. *PLoS Negl Trop Dis.* 2019;13.  
708 doi:10.1371/journal.pntd.0007727
- 709 41. Losada L, Ronning CM, DeShazer D, Woods D, Fedorova N, Stanley Kim H, et al.  
710 Continuing evolution of *burkholderia mallei* through genome reduction and large-scale  
711 rearrangements. *Genome Biol Evol.* 2010;2: 102–116. doi:10.1093/gbe/evq003
- 712 42. Mota RA, Junior JWP. Current status of glanders in Brazil: recent advances and  
713 challenges. *Braz J Microbiol.* 2022;53: 2273–2285. doi:10.1007/s42770-022-00814-1
- 714 43. Bourrier M. Equine melioidosis in Mayenne in a saddle horse stable. *Bulletin de la Société*  
715 *vétérinaire pratique de France.* 1978. pp. 673–679.
- 716 44. Singha H, Shanmugasundaram K, Tripathi BN, Saini S, Khurana SK, Kanani A, et al.  
717 Serological surveillance and clinical investigation of glanders among indigenous equines  
718 in India from 2015 to 2018. *Transbound Emerg Dis.* 2020;67: 1336–1348.  
719 doi:10.1111/tbed.13475



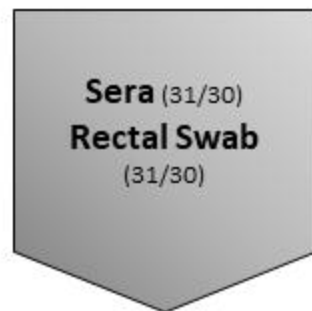
- 720 45. Hall CM, Baker AL, Sahl JW, Mayo M, Scholz HC, Kaestli M, et al. Expanding the  
721 *Burkholderia pseudomallei* complex with the addition of two novel species: *Burkholderia*  
722 *mayonis* sp. nov. and *Burkholderia savannae* sp. nov. Semrau JD, editor. Appl Environ  
723 Microbiol. 2022;88: e01583-21. doi:10.1128/AEM.01583-21
- 724 46. Hadi F, Ghazali S, Ahmad N, Ramli S. Trend and pattern of melioidosis seropositivity  
725 among suspected patients in Malaysia 2015-2019. Trop Biomed. 2021;38: 561–567.  
726 doi:10.47665/tb.38.4.099
- 727 47. Hii SYF, Kee CC, Ahmad N. Melioidosis: Overview of seropositivity in Malaysia. Trop  
728 Biomed. 2016;33: 697–701. Available from: <https://pubmed.ncbi.nlm.nih.gov/33579066/>
- 729 48. Cheng JW, Hayden MK, Singh K, Heimler I, Gee JE, Proia L, et al. *Burkholderia*  
730 *pseudomallei* infection in US traveler returning from Mexico, 2014. Emerg Infect Dis.  
731 2015;21: 1884–1885. doi:10.3201/eid2110.150815
- 732 49. Gee JE, Gulvik CA, Castelo-Branco DSCM, Sidrim JJC, Rocha MFG, Cordeiro RA, et  
733 al. Genomic diversity of *Burkholderia pseudomallei* in Ceara, Brazil. mSphere. 2021;6:  
734 e01259-20, /msphere/6/1/mSph.01259-20.atom. doi:10.1128/mSphere.01259-20
- 735



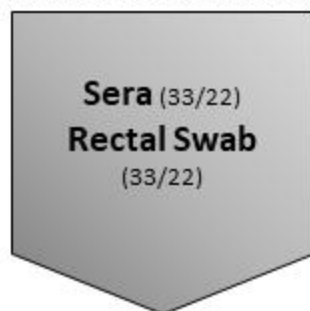
November 2021



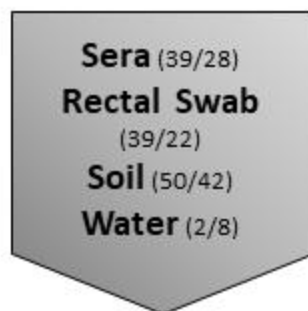
April 2022



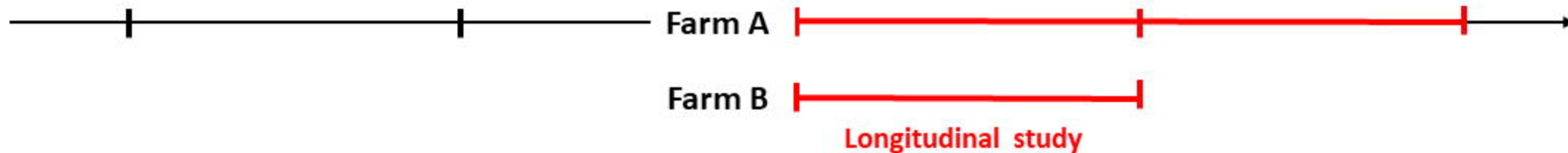
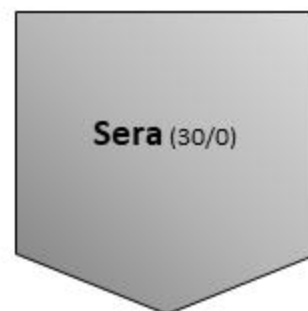
August 2022  
(Microchipping of animals)



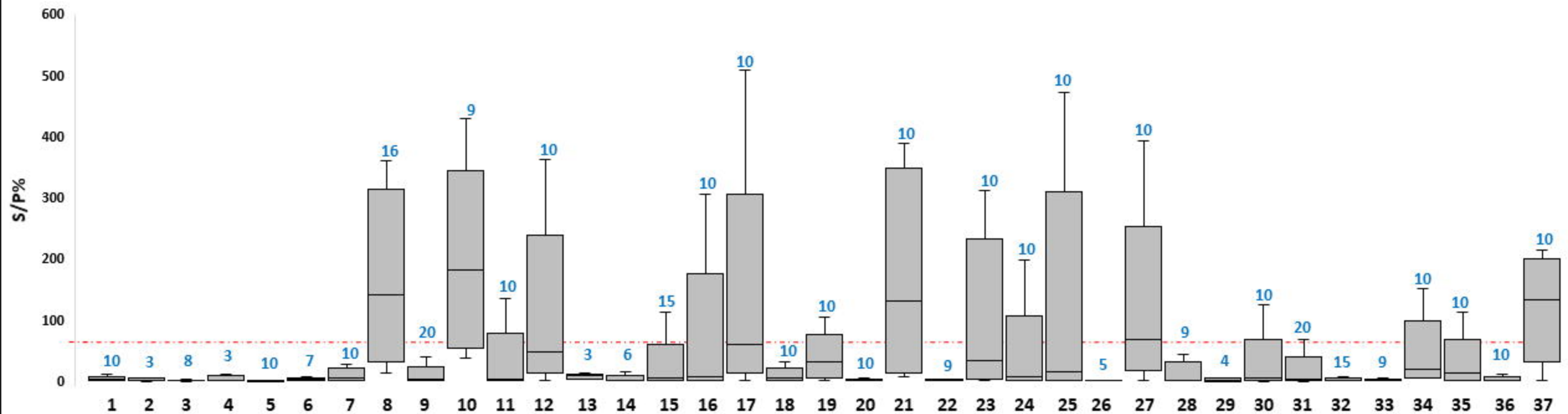
December 2022



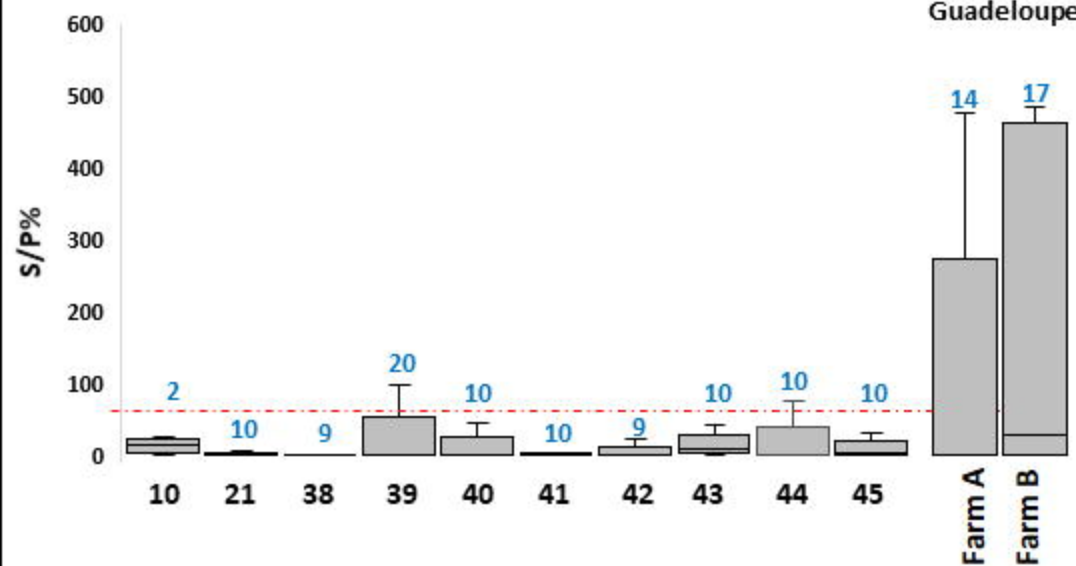
March 2023



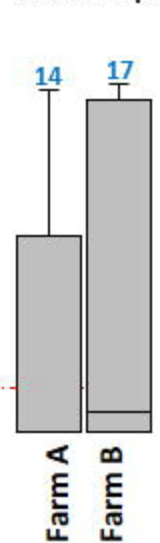
### A. Cattle in French Guiana



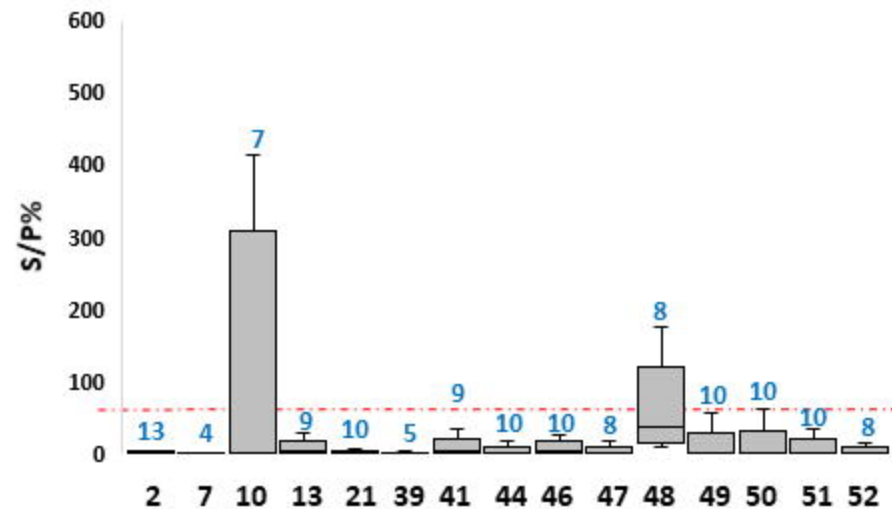
### B. Goats in French Guiana



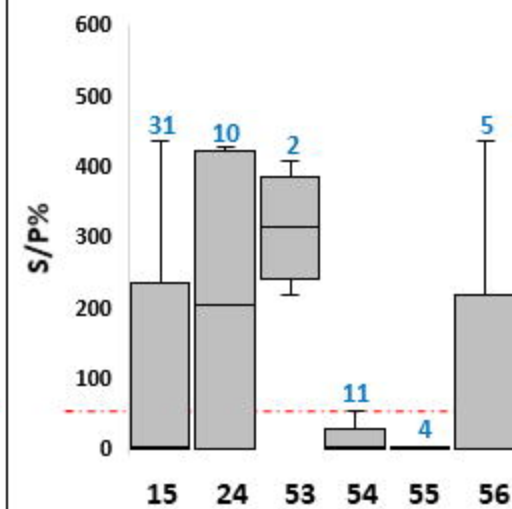
### C. Goats in Guadeloupe



### D. Sheep in French Guiana



### E. Equids in French Guiana

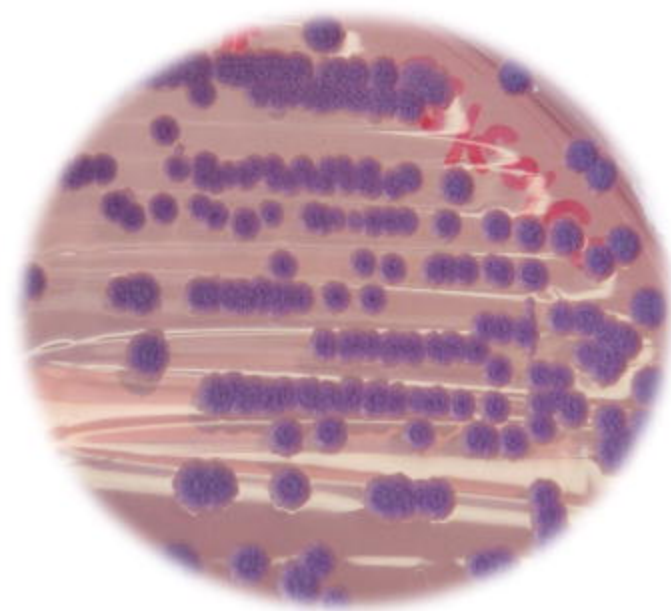
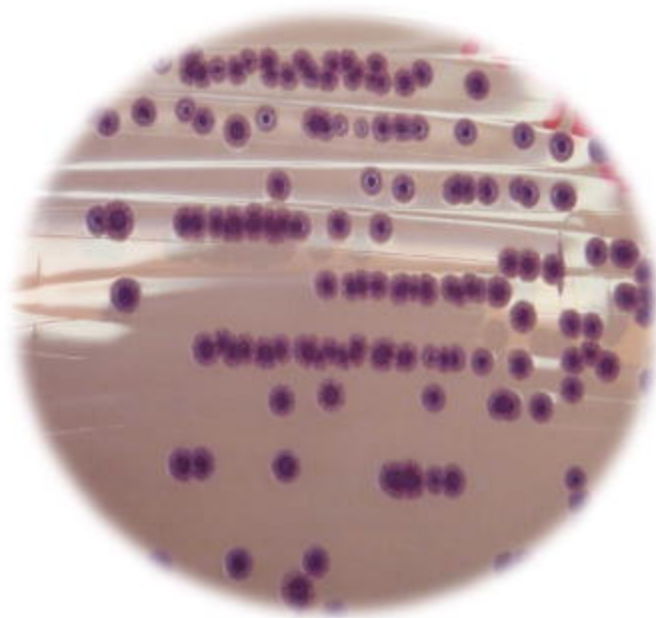
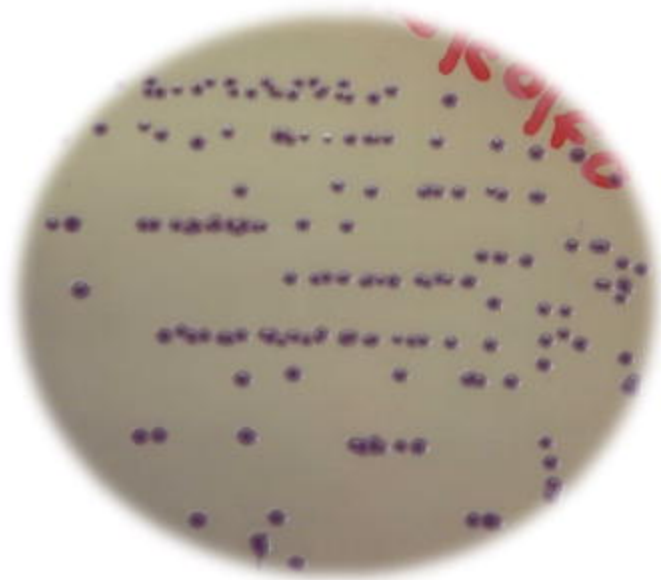


24h

48h

120h

A



B

