1   **Highly reproducible murine model of oral *Salmonella* infection via inoculated**

2   **food**

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Abstract

Oral infection of mice with *Salmonella* Typhimurium is an important model system. In particular C57Bl/6 mice, which are susceptible to *Salmonella*, are used to study both systemic and gastrointestinal pathogenesis. Pretreatment with streptomycin disrupts the intestinal microbiota and results in colitis resembling human intestinal *Salmonellosis*. Oral gavage is typically used for delivery of both antibiotic and bacteria. Although convenient, this method requires a moderate level of expertise, can be stressful for experimental animals, and may lead to unwanted tracheal or systemic introduction of bacteria. Here, we demonstrate a simple method for oral infection of mice using small pieces of regular mouse chow inoculated with a known number of bacteria. Mice readily ate chow pieces containing up to 10^8 CFU *Salmonella*, allowing for a wide range of infectious doses. In mice pretreated with streptomycin, infection with inoculated chow resulted in less variability in numbers of bacteria recovered from tissues compared to oral gavage, and highly consistent infections even at doses as low as 10^3 *Salmonella*. Mice not treated with streptomycin, as well as resistant Nramp1 reconstituted C57Bl/6J mice, were also readily infected using this method. In summary, we show that foodborne infection of mice by feeding with pieces of chow inoculated with *Salmonella* results in infection comparable to oral gavage but represents a natural route of infection with fewer side effects and less variability among mice.

Introduction

38   Bacteria belonging to the genus *Salmonella enterica* subsp. *enterica* are one of the most

39   common causes of self-limiting foodborne diarrheal disease in humans and other animals [1]

40   and a leading cause of death due to foodborne pathogens globally [2] and in the US [3].

41   *Salmonella enterica* serovar Typhimurium (hereafter *Salmonella*) is one of the serovars most

42   commonly isolated from human gastrointestinal infections. *Salmonella* infects new hosts in a

43   fecal-to-oral manner and is often the cause of foodborne disease outbreaks when present in

44   contaminated food, such as fresh produce [4] and poultry and egg products [5]. *Salmonella* has

45   been thoroughly studied and is one of the best characterized human pathogens. This, combined

46   with its simple growth requirements, has led to its frequent use as a model organism for *in vivo*

47   studies of the pathogenesis of gastrointestinal infections.

48   The most widely used animal model for *Salmonella* is the mouse [6]. Strains of mice differ in

49   their susceptibility to *Salmonella* infection, with C57Bl/6J and BALB/c mice being highly

50   susceptible and 129/Sv strains of mice being very resistant [7-10]. Susceptibility is

51   multifactorial, but one important resistance factor is the Nramp1 protein encoded by the

52   *Slc11a1* gene [11]. Nramp1 is an ion transporter responsible for the transport of divalent

53   cations out of phagosomes, thus limiting the availability of iron and other ions for ingested

54   microbes and impairing their growth in phagocytes [12]. Many susceptible mouse strains,

55   including C57Bl/6J, harbor a point mutation in the *Slc11a1* gene resulting in a non-functional

56   Nramp1 protein [13, 14]. Oral infection of susceptible mouse strains eventually leads to a lethal

57   systemic infection but without diarrhea and only diffuse enteritis [15]. However, following

58   disruption of the intestinal microbiota by antibiotic treatment, mice develop intestinal

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59   inflammation more similar to human intestinal *Salmonellosis*, although *Salmonella* do still go

60   systemic [16, 17].

61   In the preferred murine model of oral *Salmonella* infection, C57Bl/6 mice are treated with

62   antibiotics and infected by oral gavage using blunt end gavage needles. This method of delivery

63   is intragastric rather than oral, since substances are delivered directly into the stomach. While

64   the use of gavage needles allows for the delivery of precise amounts of inoculum and timing of

65   delivery, there are several drawbacks to their use, which have been recognized mostly in

66   toxicological studies [18]. Performing oral gavage requires a moderate degree of technical

67   expertise and can induce stress, e.g. raising corticosteroid levels in the blood or increasing

68   blood pressure, which may affect study outcome [19-21]. Furthermore, mice may regurgitate

69   delivered substances or infectious agents following gavage, resulting in tracheal or nasal

70   administration [22, 23]. Lastly, gavage may induce pharyngeal or esophageal trauma, leading to

71   the inadvertent delivery of substances or infectious agents directly into the blood stream or, in

72   rare cases, death [23, 24].

73   Improvements to oral gavage have been suggested, such as precoating needles with sucrose,

74   which improved gavage success rate and reduced stress of animals [25]. An alternative method

75   to gavage would be ingestion of food or water containing a pathogen. This would circumvent

76   many of the drawbacks with oral gavage and mimics a natural route of infection for *Salmonella*.

77   Recently, a paper described a method of delivering *Salmonella* orally in drinking water [26], and

78   a similar approach was used for the oral delivery of a *Salmonella* vaccine to sheep [27].

79   Inoculated food (pieces of bread) has been used for *Listeria monocytogenes* infections [28].

80   However, to our knowledge, food as a vehicle of delivery for *Salmonella* infection has not been

81   reported.

82   In this paper, we describe an oral infection method using pieces of regular mouse chow

83   inoculated with *Salmonella*. Preparation of inoculated chow is simple and mice readily consume

84   chow containing high numbers of bacteria. This mode of infection leads to a very consistent

85   disease progression among mice, less variability in bacterial load, in both systemic and

86   gastrointestinal tissues, and eliminates many of the possible drawbacks with oral gavage.

87   Importantly, this method represents a natural route of infection with *Salmonella*.

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Results and Discussion

Salmonella remains viable on mouse chow

91   We hypothesized that regular mouse chow could be used to inoculate mice with *Salmonella* to

92   establish a simple, stress-free and natural route of *Salmonella* infection. At our facility, mice

93   typically receive 2016 Teklad Global 16% Protein Rodent Diet (Envigo, Madison, Wisconsin

94   USA), chow commonly used by research institutions (see e.g. [29]). To confirm that *Salmonella*

95   does not lose viability on chow, pieces of chow (approximately 5 mm in diameter) were

96   prepared from pellets (Fig 1A) and inoculated with known numbers of bacteria. Following

97   incubation at room temperature for 1 or 3 h, the chow pieces were homogenized, diluted and

98   plated to enumerate colony forming units (CFUs). As a comparison, bacteria were also diluted

99   and inoculated in sterile pharmaceutical grade saline (SPGS). *Salmonella* showed no decrease in

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100   viability in food or saline over the course of 3 h (Figs 1B and C), irrespective of the initial

101   numbers of bacteria.

102   **Fig 1: Salmonella survives on pieces of mouse chow.** (A) Representative image of structure and

103   size of a regular mouse chow pellet and prepared chow pieces. (B, C) Survival of *Salmonella* on

104   pieces of chow and in SPGS, 1 and 3 h after inoculation. Original inoculum added as a

105   comparison. Data represent the mean ± standard deviation of three independent experiments

106   (n=1 per experiment).

Delivery of *Salmonella* using chow results in infection similar to gavage delivery and has less variability

109   Gavage is the method traditionally used to deliver *Salmonella* in oral infection of mice and for

110   streptomycin treatment. To investigate how oral infection with chow compared to the

111   commonly used procedure for infection by gavage, streptomycin treated (hereafter referred to

112   as strep+) C57Bl/6J mice were infected with 10^4 CFU *Salmonella* using either method (Fig 2A).

113   For oral gavage, mice were gavaged with streptomycin (20 mg) 24 h prior to being gavaged with

114   *Salmonella* diluted in SPGS (100 µl). Mice were fasted for 4 h prior to each gavage. For mice

115   infected with chow, to avoid gavage altogether, streptomycin was added to the drinking water

116   (final dilution of 5 mg/ml) for 24 h [30]. C57Bl/6J mice drink approximately 6 ml of water per 24

117   h [31], which results in an approximate total dose of 30 mg streptomycin. After 24 h normal

118   drinking water was returned, mice were fasted for approximately 20 h, then fed pieces of chow

119   inoculated with *Salmonella* diluted in SPGS. Since we were concerned that high levels of

120   *Salmonella* might affect the palatability of chow, we initially used a low inoculum of *Salmonella*,

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121   although the dose most frequently used is approximately 10^8 *Salmonella* [16, 29, 32]. Mice

122   readily ate chow pieces inoculated with approximately 10^4 CFU. At 3 days post infection (p.i.),

123   mice displayed very mild, if any, clinical signs of disease, although feces was frequently found

124   on the walls of the cage, indicating wet stool. Despite the low inoculum, all mice were infected

125   although those infected with inoculated chow had higher bacterial loads in tissues, especially in

126   the intestines, compared to those infected by gavage (Fig 2B). While this could be a result of

127   the inoculation method itself, it could also be due to either the prolonged streptomycin

128   treatment in the drinking water or the prolonged fasting period [33, 34]. Bacterial numbers

129   were more consistent between the mice in the chow infection group when compared to mice in

130   the gavage group. This consistency was most notable in the feces, where bacterial numbers in

131   gavaged mice ranged from 0 (below the limit of detection) to 3.4×10^8 and in chow infected

132   mice from 1.6×10^8 to 1.3×10^{10}. The variability in infection due to oral gavage has been

133   observed previously [26]. Since oral gavage may induce regurgitation and subsequent

134   unintended tracheal delivery of substances [22] we also examined the lungs for *Salmonella*.

135   Mice infected by either method contained low levels of *Salmonella* in the lungs, again with

136   more variability in mice infected with gavage (0 to 8.7×10^3) compared to those infected via

137   chow (8.9×10^1 to 5.4×10^2) (Fig 2B). The presence of bacteria in the lungs is not overly surprising

138   considering the susceptibility of strep+ C57Bl/6J mice to disseminated *Salmonella* infection,

139   however, two mice infected by oral gavage contained considerably (>1 log) higher bacterial

140   numbers in the lungs than the rest of the mice. This may indicate unintended tracheal

141   administration of *Salmonella* as a complication of the gavage process. Altogether, these

142   findings indicate that infection with inoculated chow results in more consistent disease with

143   less complications compared to the traditional gavage method.

144   **Fig 2: Infection via inoculated chow or oral gavage results in similar disease progression.** (A)

145   Schematic representation of the gavage and chow infections performed in this study. (B)

146   Bacterial numbers in tissues from mice infected either by oral gavage or pieces of chow 3 days

147   p.i. n=10 mice. Symbols represent individual mice.

Mice readily eat chow pieces inoculated with high loads of *Salmonella* and show consistent infection

150   Having shown that chow inoculated with a low dose (10^4 CFU) of *Salmonella* results in

151   consistent infection we next compared a dose range. Pieces of chow were inoculated with 10^3

152   to 10^6 CFU and fed to strep+ C57Bl/6J mice. For doses of 10^5 bacteria and higher, the bacteria

153   were pelleted by centrifugation and then diluted in SPGS (washed) before dilution since mice

154   were hesitant to eat chow inoculated with these high doses if they were not washed (data not

155   shown). This may be due to the intrinsic smell or taste of the growth medium or the presence

156   of high amounts of bacterial products. After introducing the wash step, mice readily ate chow

157   pieces containing up to 10^8 CFU. By 3 days p.i. all mice had developed systemic infection even

158   at the lowest dose (10^3) and bacterial loads were strikingly similar (Fig 3A), indicating no dose

159   dependence for the development of disseminated systemic disease, correlating with previous

160   reports [30]. Bacterial loads were consistent in systemic tissues but showed more variability in

161   gastrointestinal tissues with the greatest variability seen in the ileum (3.0×10^5 to 5×10^8 CFU/g).

162   Bacterial loads were highest in the cecum (approximately 10^9 CFU/g) and lowest in the blood

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163   (approximately 10^2 CFU/ml). To investigate if chow infection works in a resistant mouse strain,

164   we used C57Bl/6J mice reconstituted with a functional Nramp1 (hereafter referred to as

165   Nramp1$^{+/+}$) [11, 13, 14]. Mice with a functional Nramp1 protein are more resistant to oral

166   *Salmonella* infection both with and without prior streptomycin treatment [8, 17]. For the

167   Nramp1$^{+/+}$ mice we tested doses of 10^4 and 10^5 bacteria. A dose of 10^4 resulted in inconsistent

168   infection in systemic tissues, while 10^5 resulted in a consistent systemic infection, indicating

169   that 10^5 is the minimum dose required for 100% systemic infection. As expected, Nramp1$^{+/+}$

170   mice were substantially more resistant to infection, compared to C57Bl/6J mice, with lower

171   bacterial loads in all tissues but particularly in systemic organs (Fig 3B). Nonetheless, bacterial

172   loads in Nramp1$^{+/+}$ mice followed the same trends as in C57Bl/6J mice with the highest number

173   of bacteria in the feces followed by the cecum, ileum, spleen, liver and blood. Interestingly,

174   bacterial loads were also lower in the feces of Nramp1$^{+/+}$ mice, indicating that functional

175   Nramp1 is important for limiting bacterial numbers in intestinal luminal contents as well as

176   systemically.

177   **Fig 3: Pieces of chow containing a wide range of inoculums can be fed to strep+ mice.** (A)

178   Bacterial numbers in tissues 3 days p.i. in strep+ C57Bl/6J mice infected with different doses. (B)

179   Bacterial numbers in tissues 3 days p.i. in strep+ Nramp1$^{+/+}$ mice infected with different doses.

180   n=5 mice and symbols represent individual mice. Tissues where bacterial load was below the

181   level of detection are given a value of "1" for visualization purposes.

182   Next we infected non-streptomycin treated (hereafter referred to as strep-) C57Bl/6J and

183   Nramp1$^{+/+}$ mice using pieces of chow. Since these mice are much less susceptible to oral

184   infection, we used a dose of 10^8 bacteria, the standard dose used in oral infections of mice with

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185   *Salmonella* (see e.g. [16, 32, 35]). After washing the bacteria, mice readily ate pieces of chow

186   containing this high inoculum. C57Bl/6J mice had consistent bacterial loads in intestinal tissues,

187   while Nramp1$^{+/+}$ mice showed more variability in loads which were also sometimes below the

188   level of detection (Fig 4). In systemic tissues, no bacteria were detected in any of the Nramp1$^{+/+}$

189   mice and bacterial numbers were variable in C57Bl/6J mice. In the intestinal tract, the

190   differences in bacterial loads between C57Bl/6J and Nramp1$^{+/+}$ mice were statistically

191   significant in the ileum and feces.

192   **Fig 4: Infection of strep- mice results in more variability in organ loads of *Salmonella*.** Bacterial

193   numbers in tissues 3 days p.i. in strep- C57Bl/6J and Nramp1$^{+/+}$ mice infected with 10^8 CFU

194   *Salmonella*. n=5 mice and symbols represent individual mice. Tissues where bacterial load was

195   below the level of detection are given a value of "1" for visualization purposes. Asterix indicates

196   statistical significance; * p < 0.05, ** p < 0.01, ns, not statistically different, two-tailed Mann-

197   Whitney U test.

198   In summary, infections of strep+ and strep- C57Bl/6J and Nramp1$^{+/+}$ mice with pieces of chow

199   inoculated with *Salmonella* are consistent with studies using oral gavage. While it is difficult to

200   determine the dose of nontyphoidal *Salmonella* required to cause gastroenteritis in humans,

201   reports indicate doses as low as 10 organisms [36, 37]. In this infection model we achieved

202   consistent infection of strep+ mice using only 10^3 organisms, indicating that this model can be

203   used to investigate a range of relevant infection doses.

Mice infected using pieces of chow succumb to *Salmonella* infection

similar to mice infected by oral gavage

206   Next, we sought to compare the development of clinical disease of C57Bl/6J and Nramp1$^{+/+}$

207   mice infected using inoculated chow. Strep+ and strep- mice were inoculated with 10^4 or 10^8

208   CFU of *Salmonella,* respectively, and monitored for overt clinical signs at which point they were

209   euthanized. As expected, strep+ mice succumbed faster to disease compared to strep- mice

210   (Figs 5A and B). Only one strep- Nramp1$^{+/+}$ mouse developed clinical signs and was euthanized

211   15 days p.i.; all others were euthanized at the experimental end point of 21 days p.i. This is

212   consistent with an intact microbiota and the ability of Nramp1$^{+/+}$ mice to effectively combat

213   infection. The mouse euthanized 15 days p.i. showed very high bacterial loads in all tissues

214   except the liver and blood (open symbols in Fig 5F). Strep+ mice developed very high bacterial

215   loads in the intestinal tissues, especially C57Bl/6J mice, which also developed high loads in

216   systemic tissues (Figs 5C and D). This indicates that Nramp1 contributes to controlling

217   *Salmonella* infection both in systemic and intestinal sites. At the time of euthanasia, strep-

218   C57Bl/6J mice had higher and more consistent bacterial loads in all tissues compared to strep-

219   Nramp1$^{+/+}$ (Figs 5E and F). It is interesting to note that although strep- C57Bl/6J mice have

220   lower intestinal bacterial loads compared to strep+ mice, they do go on to develop higher

221   systemic bacterial loads. This is probably a result of the prolonged survival of strep- mice

222   allowing additional time for the bacteria to replicate at systemic sites. These findings correlate

223   with other studies of oral infection of strep- susceptible and resistant mouse strains [8, 35, 38],

224   and show the reduced survival of strep+ mice.

225   **Fig 5: Strep- mice survive longer than strep+ mice following chow infection and show more**

226   **variability in organ loads.** (A) Survival of strep+ and strep- C57Bl/6J mice orally infected with

227   10^4 and 10^8 bacteria, respectively. (B) Survival of strep+ and strep- Nramp1$^{+/+}$ mice orally

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228   infected with 10^4 and 10^8 bacteria, respectively. (C) Bacterial loads of strep+ C57Bl/6J mice in

229   (A) at time of euthanasia. (D) Bacterial loads of strep+ Nramp1$^{+/+}$ mice in (B) at time of

230   euthanasia. (E) Bacterial loads of strep- C57Bl/6J mice in (A) at time of euthanasia. (F) Bacterial

231   loads of strep- Nramp1$^{+/+}$ mice in (B) at time of euthanasia. n=5 mice in each experiment and

232   symbols represent individual mice. One strep- C57Bl/6J mouse died, leaving only 4 mice for

233   tissue collection (Fig 5E). Open symbols in Fig 5F indicate the one Nramp1$^{+/+}$ mouse that was

234   euthanized 15 days p.i. Tissues where bacterial load was below the level of detection are given

235   a value of "1" for visualization purposes.

Shorter periods of fasting increases hesitancy of mice to eat

inoculated chow

238   For practical reasons, it would be advantageous to have the option of varying the fasting time

239   although mice fasted for shorter times are less likely to eat inoculated chow. Therefore, to

240   determine how changing the duration of fasting would impact the method, we fasted mice for

241   4, 8 or 14 h and then measured the time taken to completely consume inoculated pieces of

242   chow. These mice were not streptomycin treated. After 14 h (o/n) of fasting, mice consumed

243   chow within 2 min, while shorter fasting resulted in longer consumption times, up to 23 min

244   (Fig 6A). The inoculum did not seem to affect consumption time, since mice fasted for 4 h and

245   offered chow inoculated with 10^8 CFU consumed the chow in a time frame similar to mice fed

246   10^4 CFU (Fig 6A). The very low consumption time after 14 h may be due to fasting taking place

247   overnight when mice are more active. Shorter fasting times required a modification to the

248   feeding procedure, where mice were moved into individual clean cages, offered a piece of chow

249   and then left undisturbed until consuming the whole chow piece. When comparing the organ

250   loads 3 days p.i. of mice fed 10^8 CFU after fasting for 4 h (Fig 6B) or 20 h (Fig 4), the shorter

251   fasting period led to lower bacterial loads in systemic tissues and in some of the intestinal

252   tissues. This indicates that shorter fasting times results in a delayed, or less efficient,

253   dissemination of *Salmonella*. In a study describing oral infection of mice with *Listeria*

254   *monocytogenes* the authors noted that mice sometimes had to be left undisturbed for up to 2 h

255   to eat the offered food, even after 24 h of fasting [28]. The reason for the mice more readily

256   eating the inoculum in this study may be due to the inoculated pieces of chow are derived from

257   their regular chow, a type of food they are already familiar with.

258   **Fig 6: Fasting time affects the time taken to eat inoculated chow and the progression of**

259   **infection.** (A) Time required for mice to completely consume pieces of chow with the indicated

260   inoculum and fasting time. Symbols represent individual mice and horizonatal line represents

261   the mean. 5 mice per experiment. (B) Bacterial loads in tissues 3 days p.i. following 4 h of

262   fasting and an inoculum of 10^8 CFU *Salmonella*. n=5 mice, symbols represent individual mice.

263   In summary, oral infection of mice using pieces of their regular chow inoculated with

264   *Salmonella* mimics the natural route of infection, is technically very simple and results in

265   reproducible infection while avoiding the stress and potential adverse side effects of oral

266   gavage. Further refinements to the method are possible, such as; adjusting fasting times; the

267   concentration of streptomycin in drinking water; and the time allowed for mice to access water

268   containing streptomycin. We expect that this approach would work for other strains of mice

269   and intestinal pathogens.

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Materials and Methods

272 *Ethics statement.* All animal studies were carried out following the recommendations in the

273 Guide for the Care and Use of Laboratory Animals, 8th Edition (National Research Council), and

274 the animal study protocol was approved by the Rocky Mountain Laboratories Animal Care and

275 Use Committee. Protocol number 2017-021-E. Animals were either euthanized before the

276 development of clinical disease, at specified time points, or at the defined humane endpoint

277 (development of clinical disease: ruffled fur, hunched posture, lethargy).

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279 *Bacterial strains and growth conditions*. *Salmonella* Typhimurium strain SL1344 was used for all

280 experiments. For infections, bacteria were grown in a 125 ml Erlenmeyer flask in 10 ml LB-

281 Miller containing 100 µg/ml streptomycin for 18 h at 37°C, with shaking at 225 RPM, and

282 diluted in sterile SPGS to get the correct inoculum in 10 µl (e.g. to get 10^4 CFU an overnight

283 culture was diluted 1:5000), the volume added to pieces of chow. For inoculum of 10^5 CFU and

284 higher, a wash step was included prior to dilution. 1 ml of the overnight culture was centrifuged

285 at 8000 G for 2 min, the supernatant was aspirated, and the bacterial pellet resuspended in 1

286 ml SPGS. In order to achieve a final concentration of 10^8 CFU in 10 µl, the bacterial pellet was

287 resuspended in 0.5 ml SPGS.

288 *Preparation of chow pieces for infection*. Mouse chow pellets (2016 Teklad Global 16% Protein

289 Rodent Diet, Envigo, Madison, Wisconsin USA), were broken into smaller pieces of about 4-5

290 mm in diameter by gentle tapping with a small hammer followed by trimming with forceps.

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291   Selected pieces were gently tested for physical integrity, by dropping from a height of 4-5

292   inches, before 10 µl of inoculum was pipetted onto the surface. Prepared pieces were kept

293   separated in a petri dish during transport to the animal facility. One piece of inoculated chow

294   was retained for estimation of the CFU by plating.

295   *Mouse chow infections*. Except where specified, mice had unlimited access to food and water.

296   For Streptomycin pretreatment the antibiotic (5 mg/ml) was added to drinking water 42 – 46 h

297   prior to infection for 24 h. Mice were then moved to a clean cage (to limit coprophagy and

298   access to cached food), containing normal drinking water but no chow. After a period of 18 – 22

299   h (typically 20) individual mice were put in a clean empty cage (without bedding material) and

300   were then offered a piece of chow. Typically, mice ate the piece of chow immediately or within

301   a couple of min. For short fasting times (4 and 8 h) mice were left undisturbed until the chow

302   was eaten. Immediately after the inoculated chow was consumed, mice were returned to their

303   cage with unlimited access to food and water.

304   *Mouse oral gavage infections*. Mice were streptomycin treated 24 h before infection, using a

305   blunt end gavage needle with 100 µl SPGS containing 200 mg/ml streptomycin. For *Salmonella*

306   infection, mice were gavaged with bacteria in 100 µl SPGS. Mice were fasted for 4 h prior to all

307   gavages. For infections without streptomycin treatment, mice were only fasted prior to feeding.

308   *Tissue collection and processing*. Mice were euthanized by isoflurane inhalation followed by

309   exsanguination. Tissues were collected in screwcap tubes containing 500 µl SPGS and 3-4 2.0

310   mm zirconia beads (BioSpec Products) and homogenized using a Bead Mill 24 (Fisher Scientific,

311   4.85 m/s for 20 seconds). Tubes were weighed before and after organ collection. CFUs were

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312   estimated by 10 µl spot plating of 10-fold dilutions on LB agar plates containing the appropriate

313   antibiotic.

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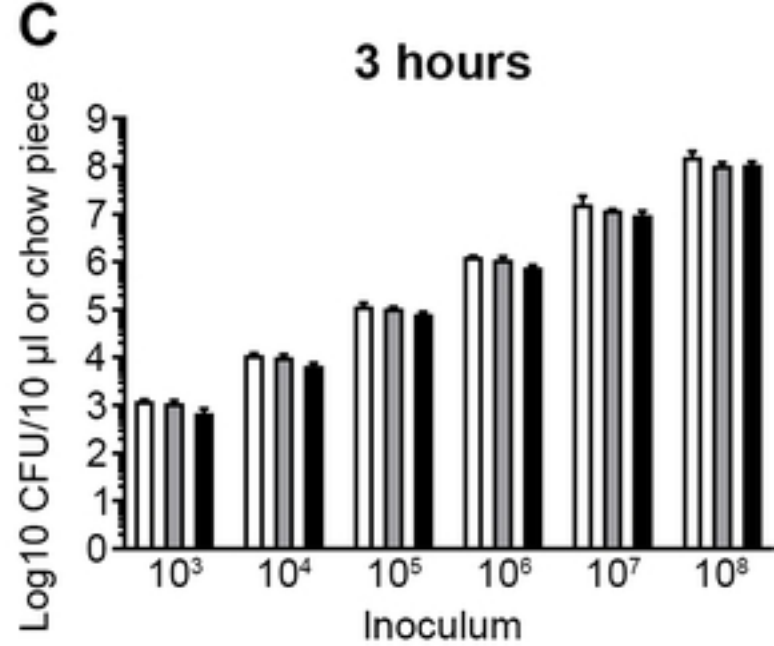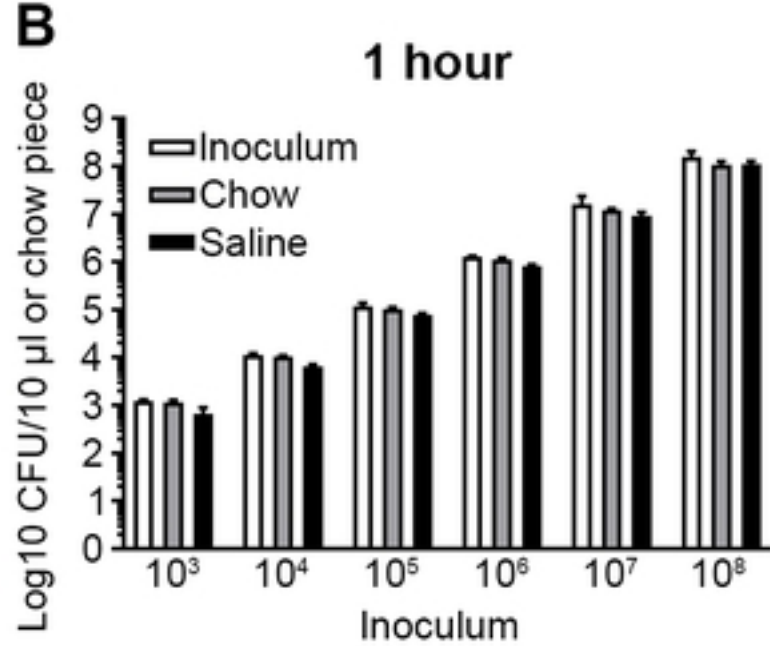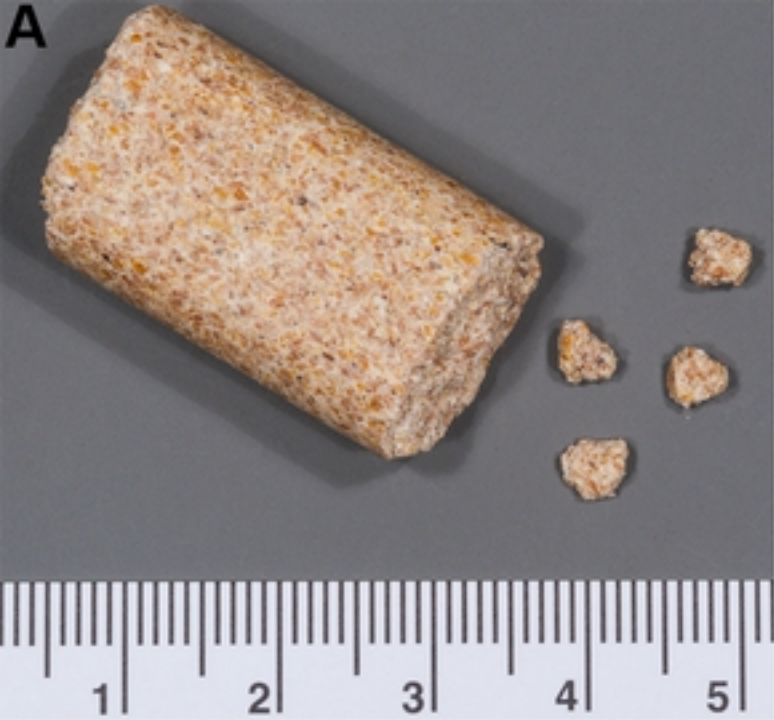412   immunity against *Salmonella* independent of antibody secretion. J Immunol. 2012;189(12):5503-7.
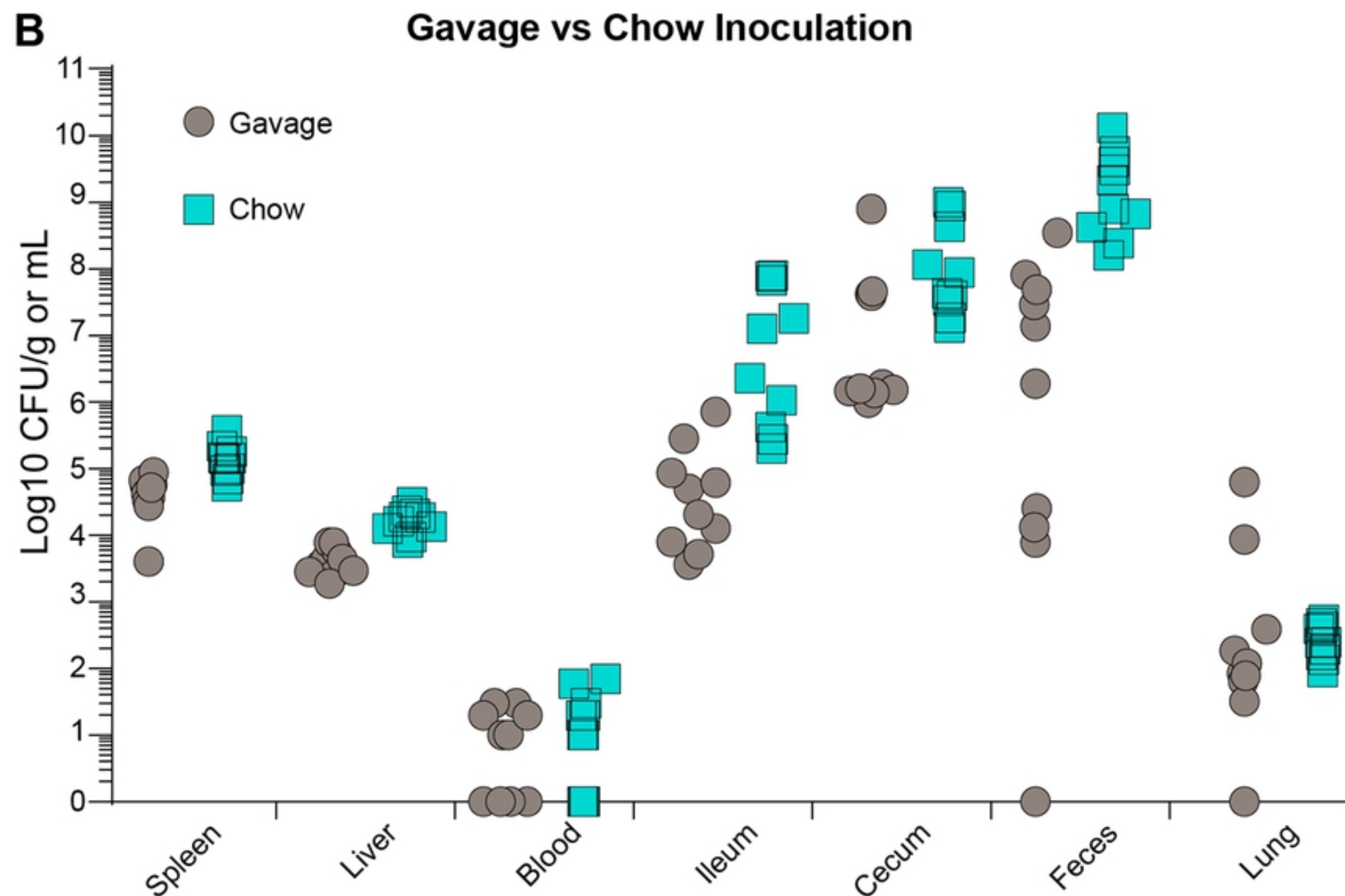
413

Fig 1

A

Fasting

Oral gavage
Streptomycin

Oral gavage
infection

-28h   -24h

Fasting

-4h   0h

Sample Collection

-44h

Streptomycin
in water

Fasting

-20h   0h

**Early dissemination
(day 3)**

**Clinical disease
(day x)**

Chow infection

B   **Gavage vs Chow Inoculation**

● Gavage

■ Chow

Log10 CFU/g or mL

Spleen   Liver   Blood   Ileum   Cecum   Feces   Lung
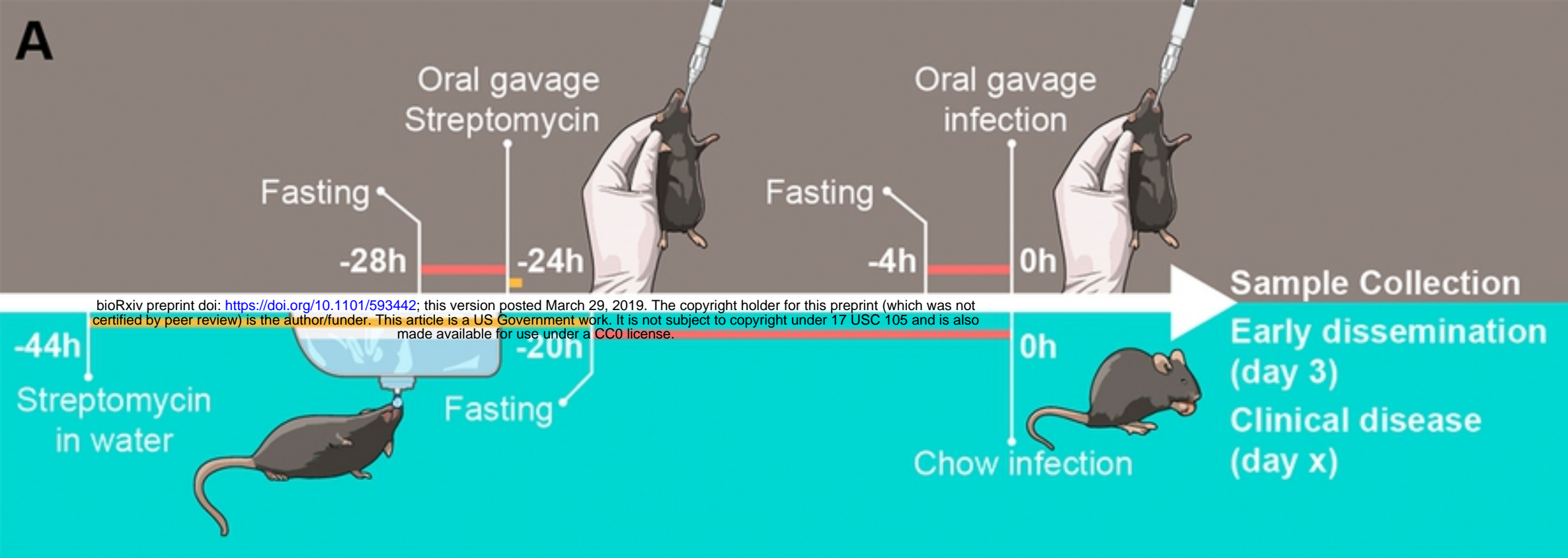
Fig 2
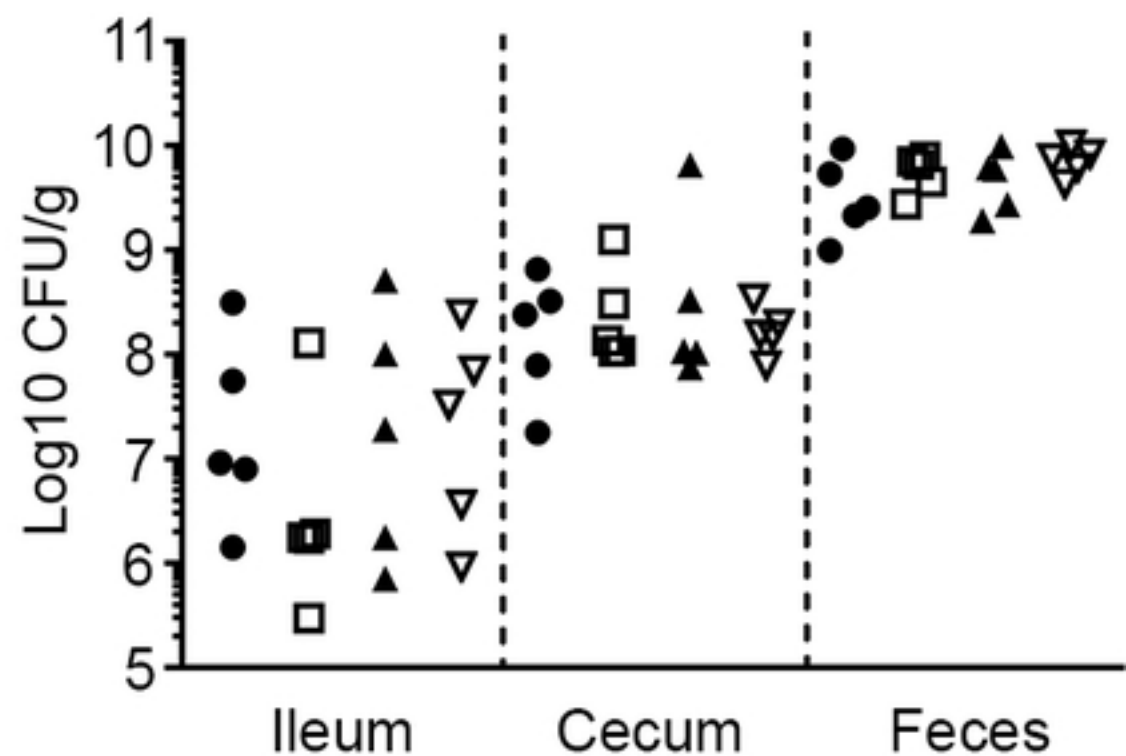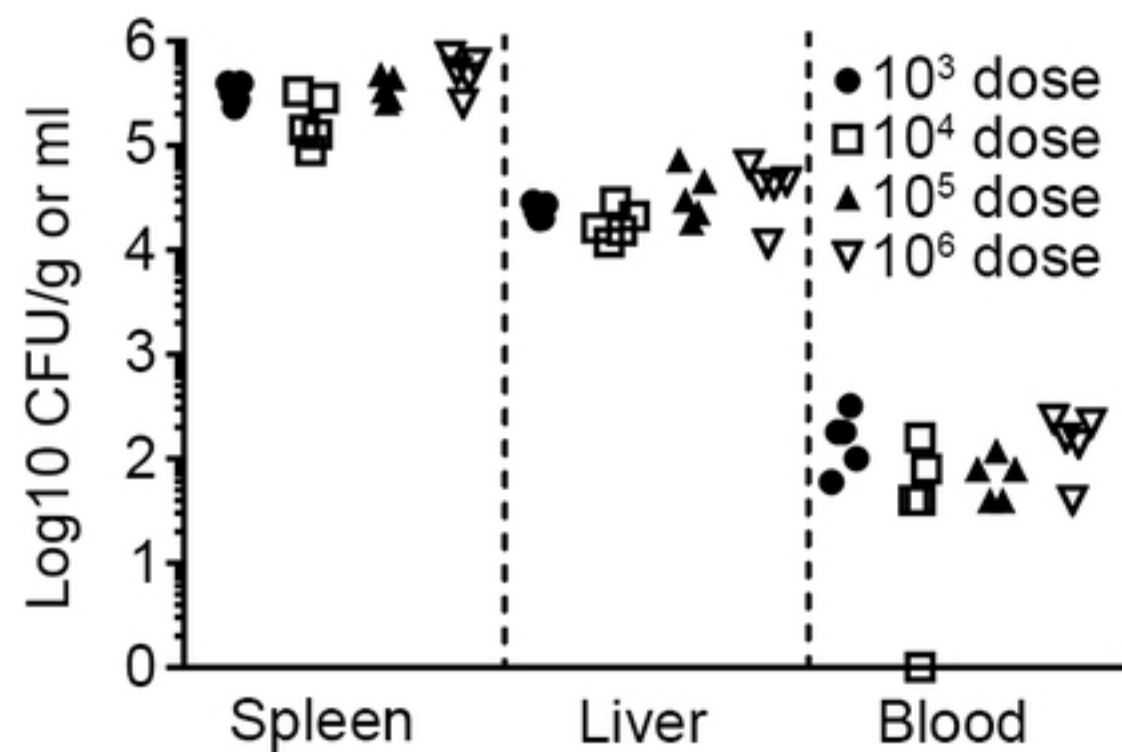
A C57Bl/6J, strep+

B Nramp1$^{+/+}$, strep+
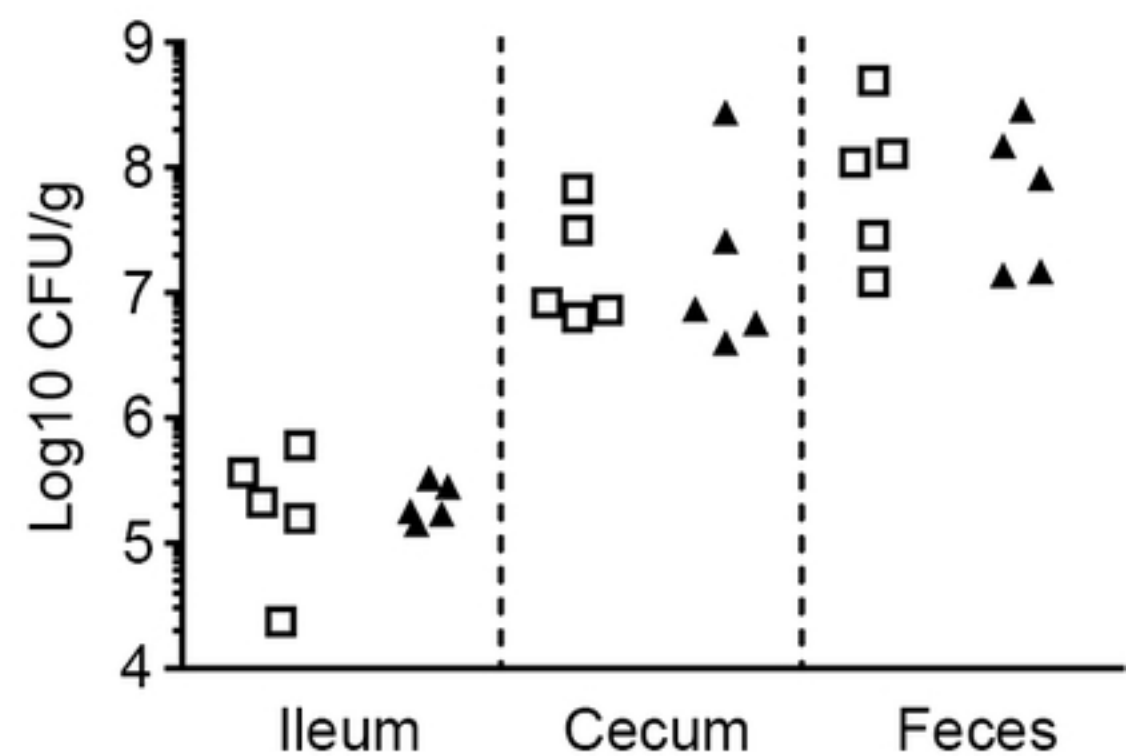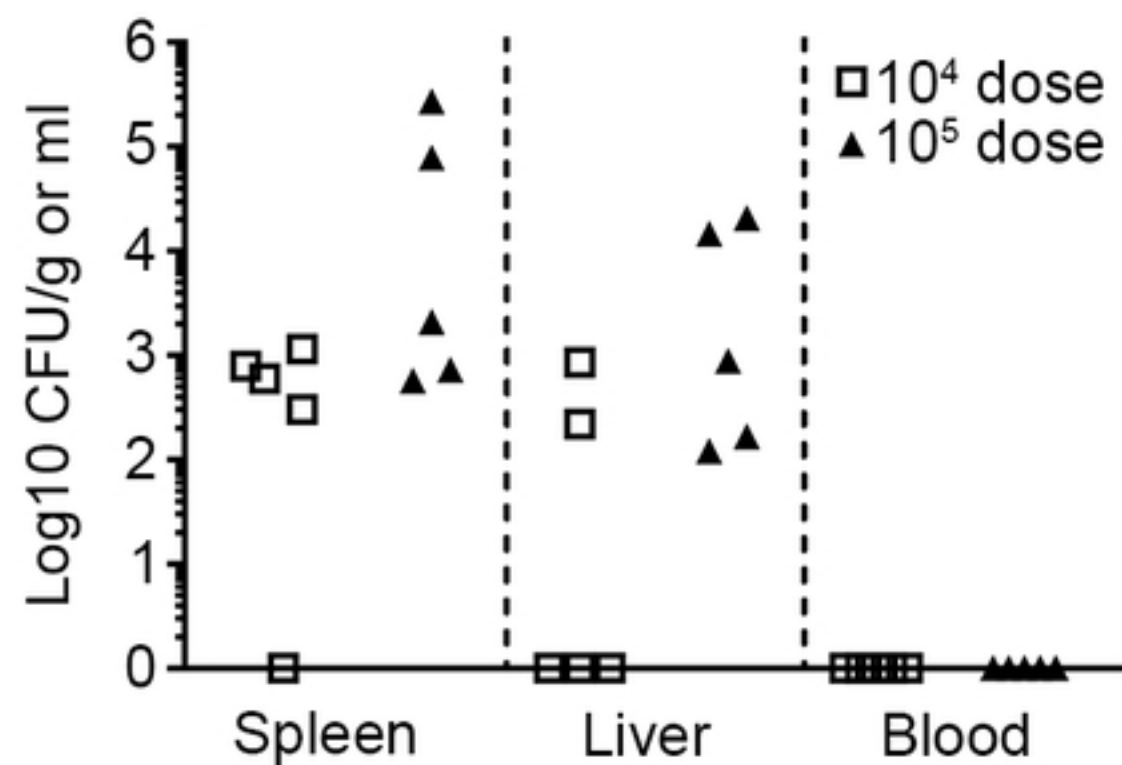
Fig 3

C57Bl/6J, Nramp1$^{+/+}$, strep-, 10^8 dose

Fig 4

A C57Bl/6J

B Nramp1^{+/+}

Survival

C

D Strep+

E

F Strep-
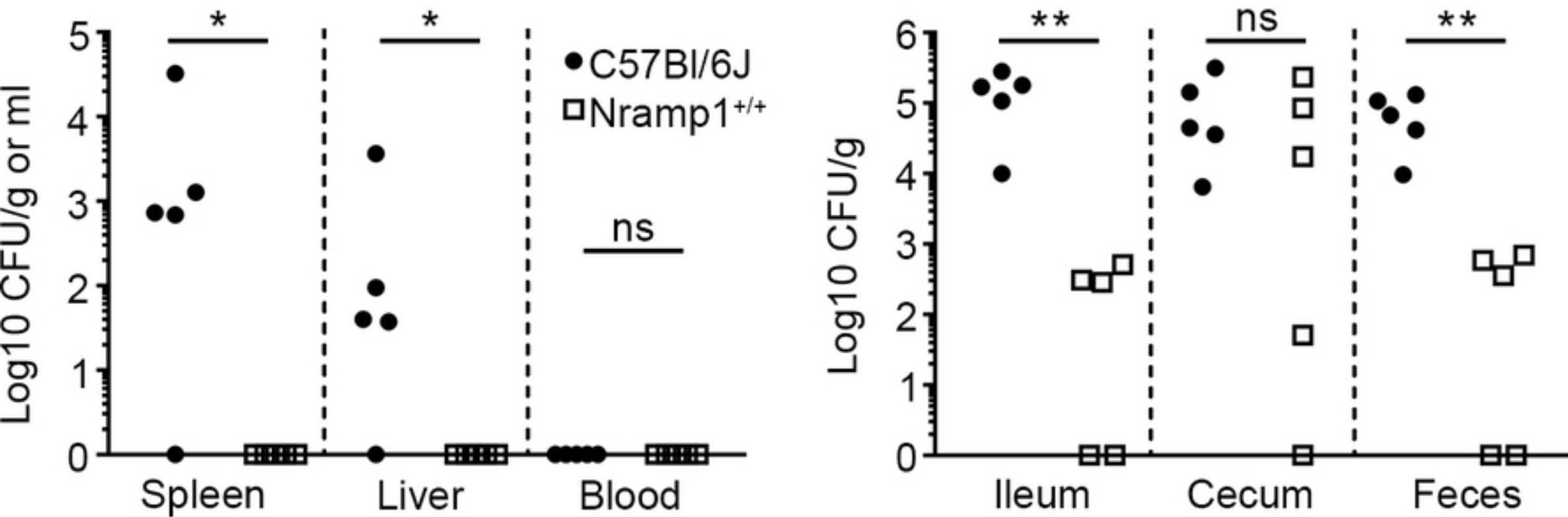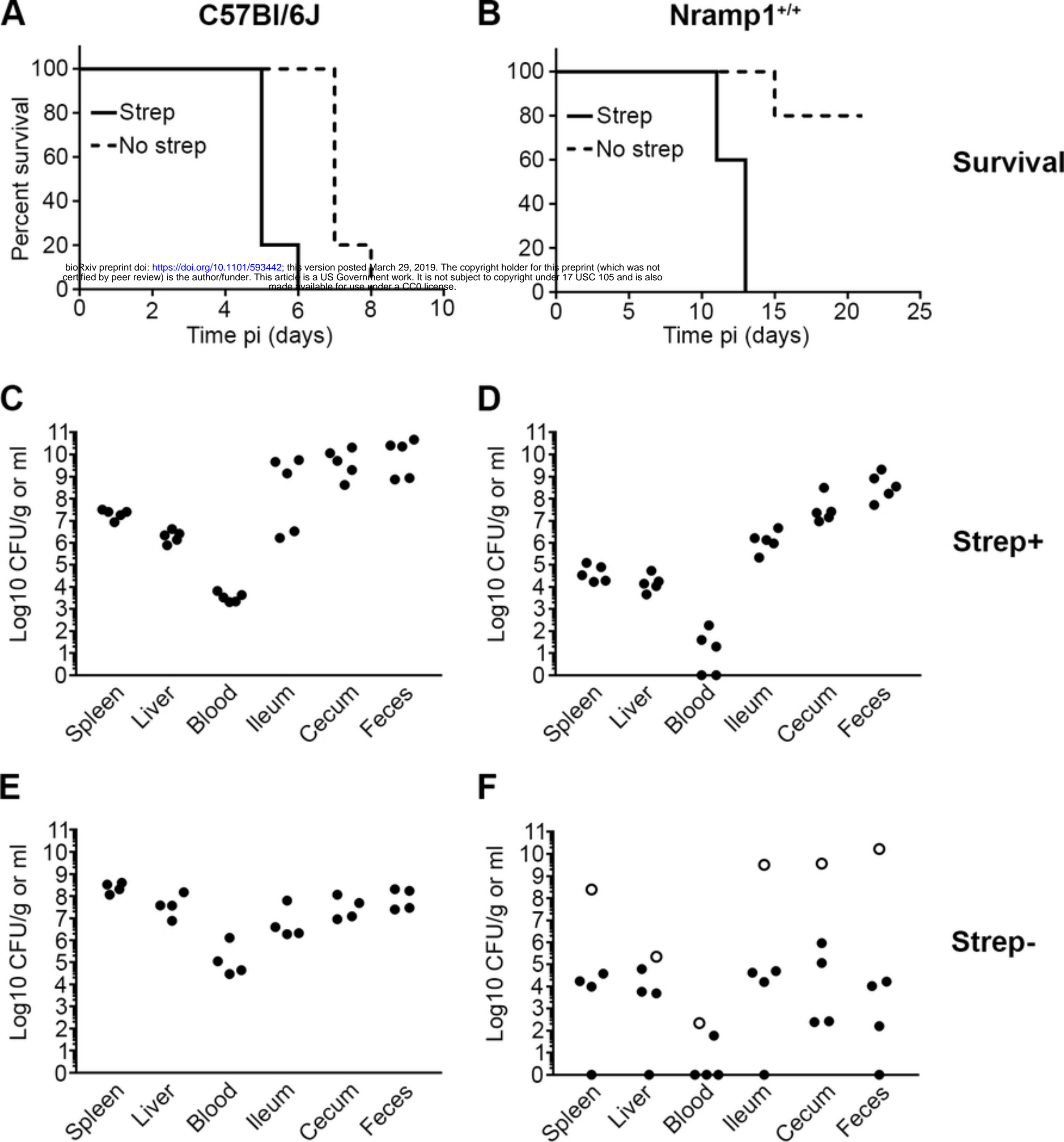
Fig 5

A. Time required to eat chow

Time (min) vs Fasting time (h): 4h, 8h, 14h, 4h

10^4 (open squares), 10^8 (filled circles)
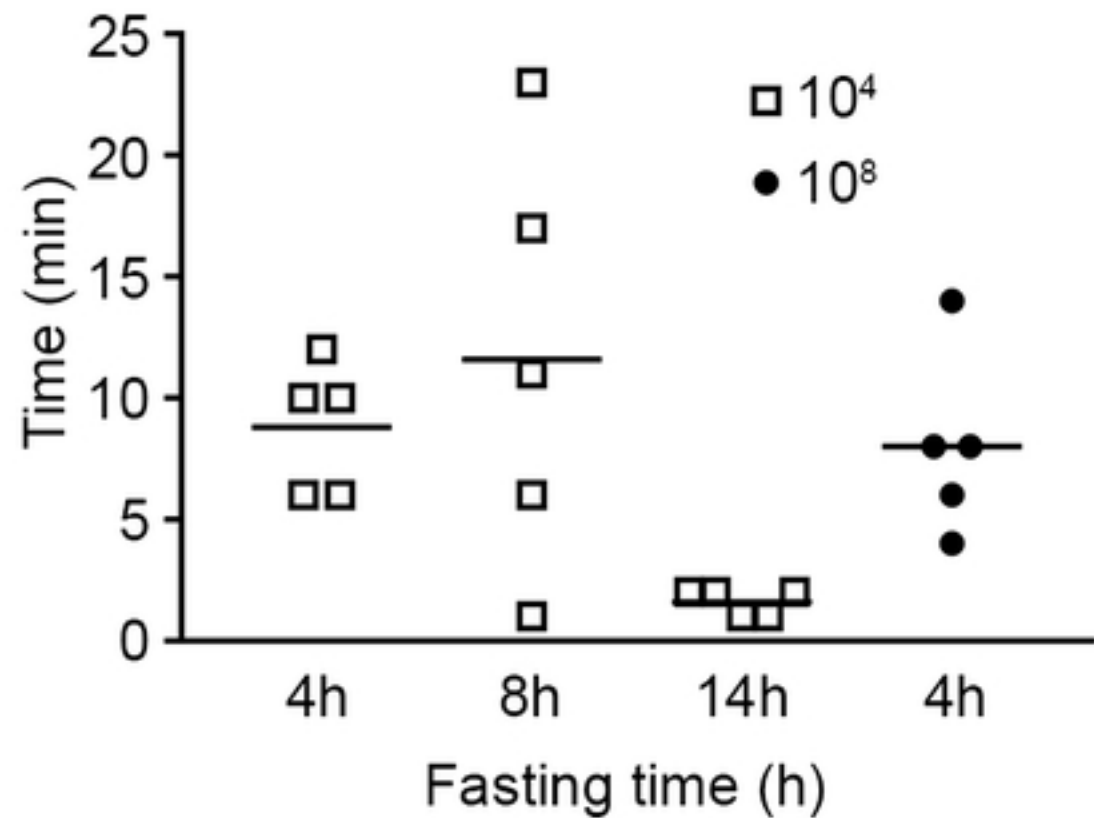
B. Bacterial loads, 4h fast (day 3 pi)

Log10 CFU/g: Spleen, Liver, Blood, Ileum, Cecum, Feces

Fig 6