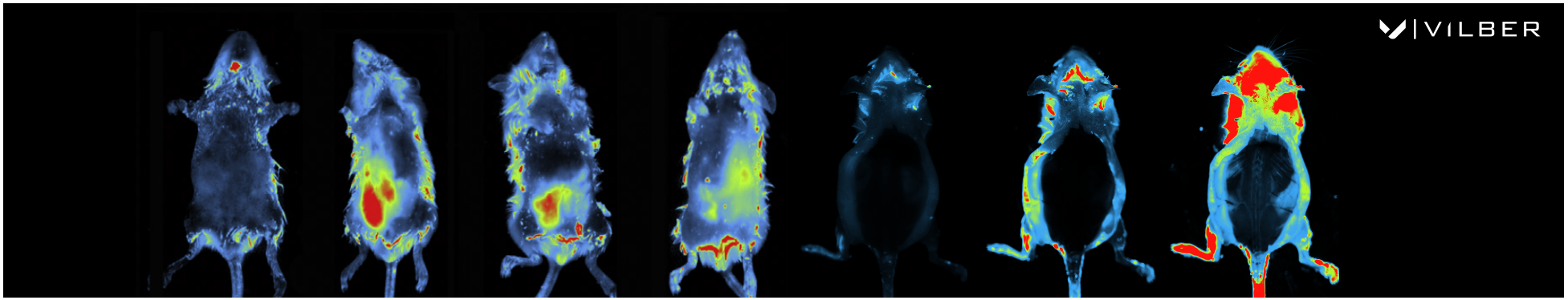

IN VIVO BIOLUMINESCENT TRACKING OF ENTEROTOXIGENIC ESCHERICHIA COLI (ETEC) IN MICE WITH NEWTON FX7

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INTRODUCTION

Enterotoxigenic *Escherichia coli* (ETEC) is a leading etiologic agent of diarrhea worldwide, causing millions of diarrheic episodes and approximately 120,000 deaths every year. Adhesion to the human intestinal tract is one of the most important features of ETEC representing a crucial step toward colonization. Unfortunately, the colonization dynamics and colonic receptor interactions of this human pathogen remain largely uncharacterized. Bioluminescence imaging (BLI) has rapidly progressed in the field of bacterial pathogenesis to facilitate the visualization and quantitation of host-pathogen interactions in live animals. *In vivo* tracking of bacteria allows us to understand the role of ETEC during bacterial infection. BLI permit the extent of pathogenic infection to be determined in real-time in living animals, providing temporal and spatial information regarding labeled bacteria and their metabolic activities. Bioluminescence is an enzymatic process by which the enzyme luciferase produces visible light in the presence of a specific substrate, oxygen and an energy source. The application of bioluminescence technology to study ETEC under *In vivo* conditions may elucidate the behavior of this bacterium in the gastrointestinal tract in further detail.

OBJECTIVE

The aim of this study was to evaluate ETEC colonization by performing bioluminescent tracking during *In vivo* mouse infection. We generated a vector harboring the *luc* gene under the regulation of the *dnaK* gene promoter. Light emission and duration of light-emitting bacteria were determined *in vitro*, and bioluminescent ETEC colonization was studied during *In vivo and ex vivo* mouse infection.

MATERIAL & METHODS

***In vitro, In vivo,* and *Ex vivo* Bioluminescence Assays**

For *in vitro* agar media assays, 200 µL of luciferin (15 ng/µL) were spread on LB agar plates before the bacteria were cultured. Strains harboring the luciferase reporter vector were cultured overnight (12 h) at 37°C on LB agar media with luciferin. The light emission of the samples was captured for 2–10 min using a Fusion FX imaging system (Vilber Lourmat; SU, Germany).

Three sets of 2- to 4-week-old BALB/c mice were intraperitoneally administered 200 µL (15 ng/µL) of luciferin prior to orogastric or intraperitoneal inoculation at different times. Animals were anesthetized with ketamine/acepromazine at a dosage of 0.3 IU (100-2.5mg/kg ratio) per g of weight. Mouse gastrointestinal tracts were dissected following euthanization. Complete gastrointestinal tract tissue was placed in 1x PBS and washed, and tissues were immediately placed into the Fusion FX for ex vivo imaging. Images of mice and petri dishes, are presented as pseudocolor images indicating light intensity (red being the most intense and blue the least intense). The colors are superimposed over grayscale reference images. The signal is expressed as the total number of photons emitted per second (photons/s). Images were captured and analyzed using a Fusion FX imaging system (Vilber Lourmat; SU, Germany).

RESULTS

Figure 1. *E. coli* K-12 harboring pRM*kluc* generates bioluminescence.
(A–C) E. coli K-12 harboring pRMkluc. (D–F) E. coli K-12 harboring pBR322 (negative control). Fig 1A and Fig1D: Captured under white light and fig1C: pseudocolor representation of the petri dish. (Red = intense, blue = less intense). *In vitro* bioluminescence assays were carried out to measure reporter gene activity prior to animal infection. The strains were cultured overnight in petri dishes containing LB agar with luciferin substrate. E. coli K-12 pBR322 (Figures 1D–F) did not emit bioluminescence, indicating that neither E. coli K-12 nor the pBR322 vector carried elements that confer bioluminescence. In contrast, E. coli K-12 harboring the pRMkluc vector emitted a bioluminescent signal (Figures 1A–C).

Figure 2. *In vivo* and *Ex vivo* Mouse Infections to Investigate ETEC Bioluminescence Emission
Bioluminescence tracking of *In vivo* bacterial colonization. Light capture began following gastric inoculation (Figure 2A), and the light signals in mice inoculated with ETEC FMU073332 harboring pRMkluc at this time point confirmed bacterial inoculation (0 h) (Figures 2A, 3A). After inoculation, the bioluminescent signals displaced toward the small intestine. 48H after gastric inoculation, the bioluminescent signals indicated bacterial passage through the mouse intestine. The bacterial bioluminescent signals remained in the mouse intestine after the 120 h post-inoculation (Figures 2A, 120 h and 3C), which corresponded to the bacterial shedding data. However, no signals were recovered from the distal portion of the mouse intestine. E. coli K-12 bioluminescent signals were localized in the cecum (Figures 2, 3). Bioluminescent signals were captured at 0h, 48h and 120h post inoculation. The bioluminescent signal is depicted as pseudocolor (red= intense, blue = less intense). Arrows indicate the bioluminescent signal. n=3.

Figure 3. *Ex vivo* bioluminescence imaging of bacterial passage through the mouse gastrointestinal tract.
After 120 h of *E. coli* infection, mouse gastrointestinal tracts were extracted to perform *ex vivo* imaging. Intestinal tract dissection comprised the esophagus to the rectum. The bioluminescent signals emitted by ETEC FMU073332 harboring pRM*kluc* were located in the proximal mouse ileum approximately 6 cm from the cecum.

CONCLUSION

BLI imaging suggests that passage of ETEC FMU073332 through the intestinal tract is slower than the movement of E. coli K-12(cf. to publication). The appearance of BLI signals may explain the results observed in the colonization assay where ETEC FMU073332 is likely retained in the intestine due to the presence of its adhesive structures and biochemical characteristics that facilitate intestinal colonization. *Ex vivo* imaging allowed us to locate the region of the intestine colonized by ETEC. This study investigates the use of Fusion FX imaging system (Vilber Lourmat; SU, Germany) and enlightens the fact that the system is perfectly suited for BLI imaging during *in vitro*, *In vivo*, and *ex vivo* assays.