



### Short Communication

## Surgical procedures to an experimental polymicrobial sepsis: Cecal Ligation and Puncture

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#### Abstract

Sepsis is still a leading cause of death worldwide and the mechanism of shock remains to be completely understood. Several studies have aimed to evaluate the effects of several drugs and procedures in sepsis, and the most common models of this study are to challenge mice with LPS or to simulate a polymicrobial infection using a surgical procedure. Such procedure consists in exposure of the cecum by a midline laparotomy, ligation of ileocecal junction and perforation with a needle, squeezing cecum contents to the peritoneum cavity. Beyond the variations allowed by this model, the thickness of the needle used and the number of perforations seem to be an important factor, displaying different levels of sepsis severity. In this study, we used two mice strains (C57BL/6 and BALB/c) to describe the procedures of cecal ligation and puncture (CLP), comparing the survival rates of mice subjected to three different thicknesses of perforation.

**Key words:** sepsis, shock, polymicrobial infection, CLP, C57BL/6, BALB/c

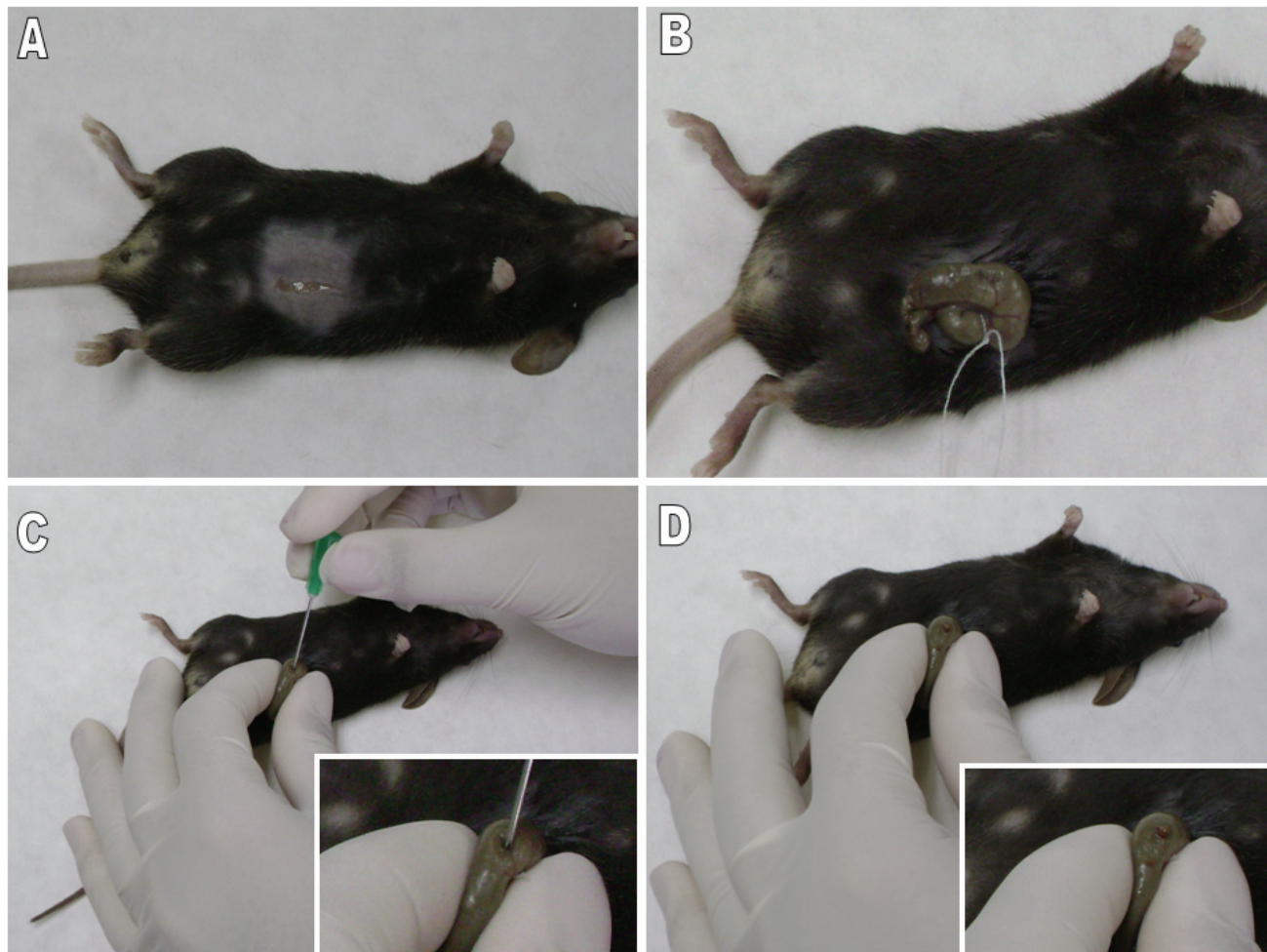
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#### Introduction

Despite intense efforts, sepsis remains a serious clinical problem, accounting for thousands of deaths every year. Several studies have shown that the incidence of sepsis is increasing worldwide, and is projecting to grow as the population ages (4). Shock by bacterial invasion of body tissues is a common complication of different traumatic injuries, and the acute events of sepsis may trigger long-term consequences, such as inhibition of leukocyte migration, immunosuppression and pulmonary complications (2).

Considering the lack of a complete understanding of the sepsis mechanism, that can justify the relative failure of effective treatments, different experimental models of sepsis have been described. Bacterial inoculation, injection

of endotoxin/LPS (lipopolysaccharide) and cecal ligation and puncture (CLP) are very frequent in literature, the latter two models representing the most commonly used. LPS can trigger signals and symptoms of infection, and its infusion in animals can simulate a Gram-negative sepsis, whereas CLP simulates the clinical situation of polymicrobial sepsis (6). It is assumed that LPS-mediated events contribute to the pathogenesis of sepsis, but among the obvious differences of these models, LPS-independent apoptotic pathways in thymus, spleen, lung and gut have been described in CLP model (3). Indeed, LPS-insensitive mice or LPS-receptor deficient mice (TLR-4 and TLR-2) are not protected from endotoxemia via CLP (6). As these findings do not support a major role for LPS in sepsis disturbances, it can be assumed that the CLP model can more adequately simulate the clinical condition of septic



**Figure 1 – Cecal Ligation and Puncture procedures:** Mice were anesthetized and the cecum was exposed to subsequent ligation and puncture. A – laparotomy; B – cecum ligation; C – cecum perforation, D- squeeze for cecum contents extrusion.

shock.

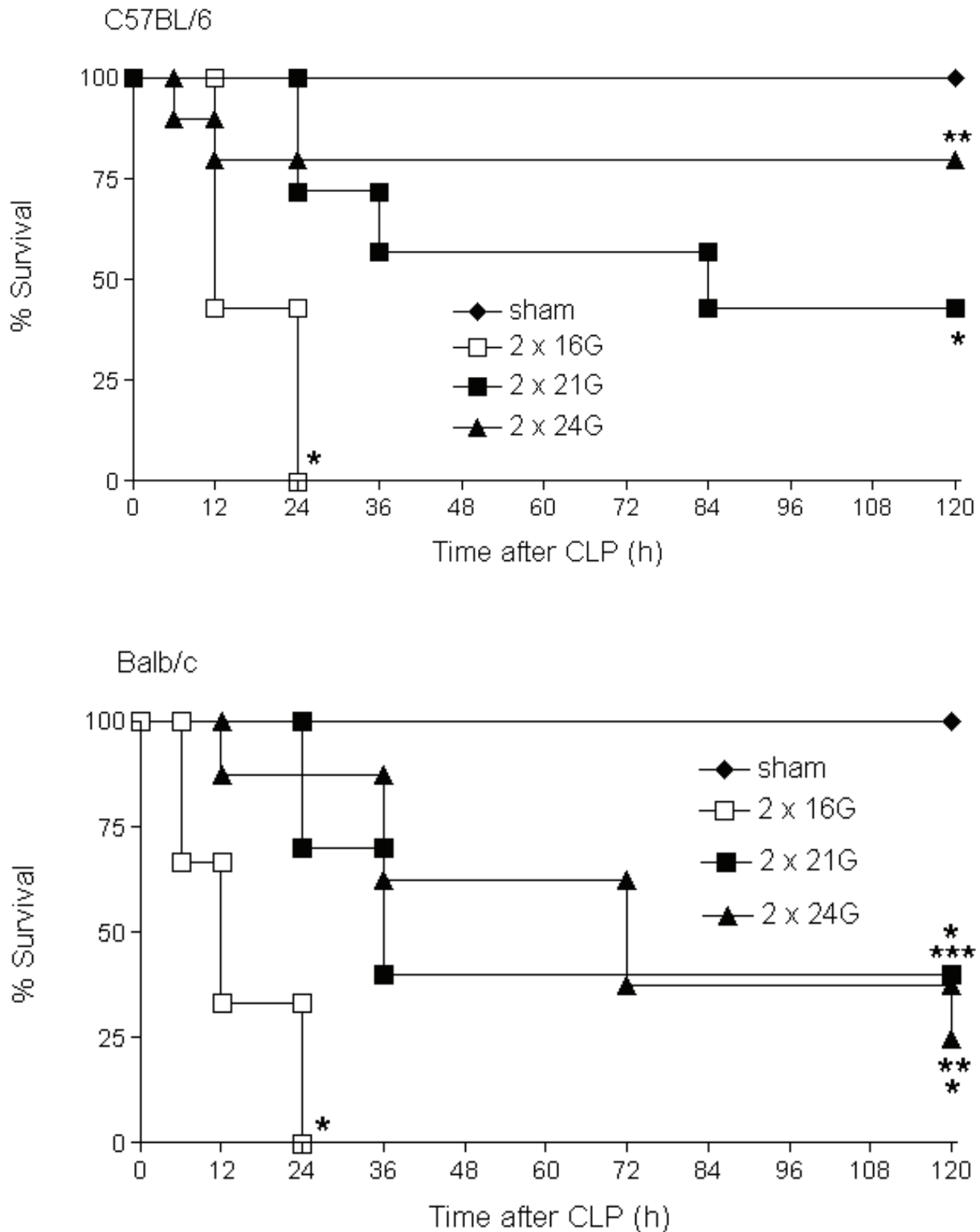
Considering the utility of the CLP model for the study of sepsis, several groups have described different protocols that vary in thickness of needle, the number of perforations, and the amount of cecum contents that is squeezed through the punctures. Indeed, it is very difficult to reproduce accurately such findings, as often there is no detailed description of the method in most part of the studies. Also, there is no obvious evidence in literature concerning differential sensitiveness among mice strains. These variations can imply in different mortality rates and inflammatory findings. In this study, we aimed to compare two mice strains that were submitted to three different protocols of CLP, detailing the whole surgical procedure.

### Cecal Ligation and Puncture procedures

C57BL/6 and BALB/c (18–20 g) mice were used in this study. Animals were housed and used in accordance to the guidelines of the Committee on Care and Use of Laboratory Animal Source of Federal University of Minas Gerais, Brazil. The procedures of CLP were approved by

this committee in another study. These guidelines are similar to those of the National Research Council, USA. The mice were housed in cages in temperature-controlled rooms and received water and food *ad libitum*.

Animals were anesthetized with an i.p. injection of a mixture of 10 mg/kg xylazine and 100 mg/kg ketamine hydrochloride and a tricotomy of abdomen was gently done with a hair machine. A 1 cm midline laparotomy (Fig. 1A) was made on the anterior abdomen, and the cecum was exposed and ligated distal of the ileocecal valve, without causing intestinal obstruction (Fig. 1B). The cecum was punctured twice in the midline, with a 0.5 cm distance between the punctures (Fig. 1C). As shown in the inset, it was preferred the region of the cecum with absence of larger vessels in order to avoid hemorrhage. Three different thicknesses of needles were used in different protocols: 16G (1.65 mm), 21G (0.80 mm) and 24G (0.55 mm). Then, the cecum was squeezed to extrude contents (Fig. 1D) in a 2 mm of fecal amount (inset). The incision was closed with a 4-0 suture wire and each mouse received 1 ml of sterile saline for fluid resuscitation. The animals were allowed to free access to food and water after



**Figure 2 – Survival curve after CLP in C57BL/6 and BALB/c mice.** Mice were subjected to two perforations of the cecum using three different thicknesses of needle (16G, 21G and 24G). The survival rate was assessed for 5 days. Differences in survival were analyzed by a Kaplan-Meier survival plot and the log-rank statistic. \* - indicates statistical significance in comparison to sham group, \*\* - between 2 x 16G and 2 x 24G, and \*\*\* - between 2 x 16G and 2 x 21G (p<0.05)

induction of sepsis. In sham operated animals, the cecum was exteriorized without ligation and puncture. The survival rate was observed for seven days and at least 6 animals were used in all groups. Differences in survival were analyzed by a Kaplan-Meier survival plot and the log-rank statistic.

Survival rate after CLP – Variations of the method

All mice that were subjected to CLP procedures presented clinical signs of bacterial infection, as piloerection, lethargy and tachypnea. As shown in Figure 2 a differential survival rate that varies mainly due to the thickness of the needle used to cecum perforation was observed. The perforation with a 16G needle displays a severe sepsis, leading to a 100% death rate in both strains in the initial 24 hours. The reduction of the thickness of the needle (to 21 and to 24G) leads to a progressive decrease of lethality in both strains, displaying a 50% and 75% of survival rate, respectively in C57BL/6 strain. BALB/c mice were more sensitive to CLP, as only 25% and 50% of mice resisted to perforations using 21G and 24G needle, respectively. It can be suggested that the diameter of the perforation is directly related to the amount of bacteria that extrude from the cecum, implying in the severity of the infection. Indeed, differential survival rates can be achieved using different mice strains. The incapacity of mice subjected to CLP procedures to combat infection in the peritoneal cavity may be due to the inhibition of leukocyte migration, mainly neutrophil, to the site of infection (1). Indeed, the excessive NO production during the polymicrobial infection can down-regulate the expression of chemokine receptors on the surface of neutrophils (5), leading to an impairment of emigration, increasing the severity of the infection.

Once established the survival rate of this model, it is possible to study at different intervals several patterns of inflammatory response, such as bacteremia and the number of bacteria in the peritoneal fluid, leukocyte migration to peritoneal cavity, cytokine levels and other inflammatory mediators in fluids, besides the effect of different drugs in this process.

The CLP model is one of the most widely used models of sepsis and septic shock. It can trigger clinical

signs that are not achieved in other models, as LPS infusion and single bacterial infection. It can also produce a different spectrum of sepsis severity, which varies with the surgical procedure. It allows the investigator to choose from an acute (lethal) to a chronic approach. Furthermore, this model of CLP in rodents is extremely simple, widely used and relatively low cost.

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