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Development of a large animal model of lethal polytrauma and intra-abdominal sepsis with bacteremia

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ABSTRACT

Background Trauma and sepsis are individually two of the leading causes of death worldwide. When combined, the mortality is greater than 50%. Thus, it is imperative to have a reproducible and reliable animal model to study the effects of polytrauma and sepsis and test novel treatment options. Porcine models are more translatable to humans than rodent models due to the similarities in anatomy and physiological response. We embarked on a study to develop a reproducible model of lethal polytrauma and intra-abdominal sepsis, which was lethal, though potentially salvageable with treatment.

Methods Our laboratory has a well-established porcine model that was used as the foundation. Animals were subjected to a rectus crush injury, long bone fracture, liver and spleen laceration, traumatic brain injury and hemorrhage that was used as a foundation. We tested various colon injuries to create intra-abdominal sepsis. All animals underwent injuries followed by a period of shock, then subsequent resuscitation.

Results All animals had blood culture-proven sepsis. Attempts at long-term survival of animals after injury were ceased because of poor appetite and energy. We shifted to an 8-hour endpoint. The polytrauma injury pattern remained constant and the colon injury pattern changed with the intention of creating a model that was ultimately lethal but potentially salvageable with a therapeutic drug. An uncontrolled cecal injury (n=4) group resulted in very early deaths. A controlled cecal injury (CCI; n=4) group had prolonged time prior to mortality with one surviving to the endpoint. The sigmoid injury (n=5) produced a similar survival curve to CCI but no animals surviving to the endpoint.

Conclusion We have described a porcine model of polytrauma and sepsis that is reproducible and may be used to investigate novel treatments for trauma and sepsis.

Level of evidence Not applicable. Animal study.

INTRODUCTION

Trauma and sepsis are both common and significant sources of death worldwide. Severe polytrauma injury patterns are particularly common in the military setting with blast injuries. In a recent review of injuries treated in Afghanistan from 2005 to 2018, over 10% of patients had multiple injuries.[1] Of these polytrauma patients, 42.9% had an injury to their head or neck and 39.9% had an injury to their abdomen and pelvic contents. The same review showed that of patients with whole body injuries, 37.9% had penetrating injuries.[1] The abdomen is one area of the body which is particularly susceptible to penetrating injuries.[2] In a review of patients in Afghanistan with penetrating abdominal wounds, the majority of injuries were to the gastrointestinal tract with the most common injury being to the small bowel.[3] While this severe constellation of injuries is uncommon in the civilian setting, concomitant hollow viscous injury with other severe injuries is prevalent among injured soldiers. As such, it is imperative to have a reproducible and dependable animal model with which to study the effects of polytrauma and hollow viscous injury on survival outcomes.

Current treatment for trauma and sepsis patients is largely limited to supportive care, resuscitation, and antibiotics, with a need for novel strategies. Currently, the majority of animal models used to study new treatments for trauma and sepsis are murine or other small animal models. Many treatments which have showed promising results in small animal models have not translated to humans.[4–6] While the advantages of using the murine model include their widespread availability and low maintenance, their history with human translation is discouraging. Porcine models are a more suitable species for trauma and sepsis models due to the similarities in anatomy and physiological response when compared with humans.[7–9]

The development of a porcine model with polytrauma and concomitant colon injury resulting in bacteremia would offer a novel approach for studying innovative therapies. The model includes a femur fracture, rectus crush, liver and spleen lacerations, traumatic brain injury (TBI), colon injury, and hemorrhagic shock with subsequent resuscitation. This is a severe, but clinically relevant injury pattern in the military setting where concomitant blunt and penetrating injury patterns are common from blast injuries. We have previously used similar polytrauma models without a septic insult that are well validated and reproducible.[10] We included a colon injury to inflict intra-abdominal sepsis. The addition of intra-abdominal sepsis is unique and merits investigation given high mortality rates.[11 12] The aim of this study was to create a valid and reproducible porcine model which can be used to study treatment methods for translational trauma research.

METHODS

Animals

Female Yorkshire swine between 40 and 44 kg were selected (Michigan State University, East Lansing, Michigan).

Anesthesia and medications

Animals were injected with 0.5 mg/kg Telazol (Pfizer, New York, New York) and then maintained under general anesthesia (GA) with inhaled isoflurane (1% to 3%) during the injury protocol and resuscitation portions of the experiment. For animals with planned attempt at extubation and survival after surgery and injuries, a 75 μg fentanyl patch was placed over a shaved area on the shoulder and covered with a piece of durable tape which was sewn into place.

Line placement and monitoring

In bilateral groins, vascular cutdowns were performed to expose the femoral vessels. The femoral artery was cannulated with a 5 Fr 11 cm catheter and the femoral vein was cannulated with an 8 Fr 11 cm catheter. The arterial line was used for blood pressure monitoring, controlled hemorrhage and blood draws for laboratory measurements. The venous line was used for fluid resuscitation. A laparotomy was done for placement of an open cystostomy tube. Under ultrasound guidance, the right external jugular vein was accessed percutaneously for sheath placement. A pulmonary artery catheter was floated for cardiac output (CO) and pulmonary artery pressure monitoring.

After placement of all lines, we began the injury protocol which consisted of the following: rectus crush, bone fracture (rib fracture if extubating the animal or femur fracture if remaining under GA), liver laceration, spleen laceration, colon injury, hemorrhage and TBI (figure 1). The rectus muscle on the right side was dissected free from the skin and subcutaneous tissue over a span of 5 cm. This was then crushed using a Kelly clamp to create a soft tissue injury. A laparotomy was then performed. In the initial survival experiments a rib fracture was created to avoid postoperative mobility issues. Rib fracture was performed by scoring the peritoneum overlying the inferior most complete rib in the mid-clavicular line. The neurovascular bundle under the rib was cleared with a ribbon retractor, and the rib was completely fractured using a rongeur. In models designed to be lethal hours prior to extubation, a femur fracture was performed consistent with our prior polytrauma models. The right femur was cut down onto, and a captive bolt gun with 22 G shell (Ramset Mastershot, Glendale Heights, Illinois) was fired directly onto the femur to create a long bone fracture. The

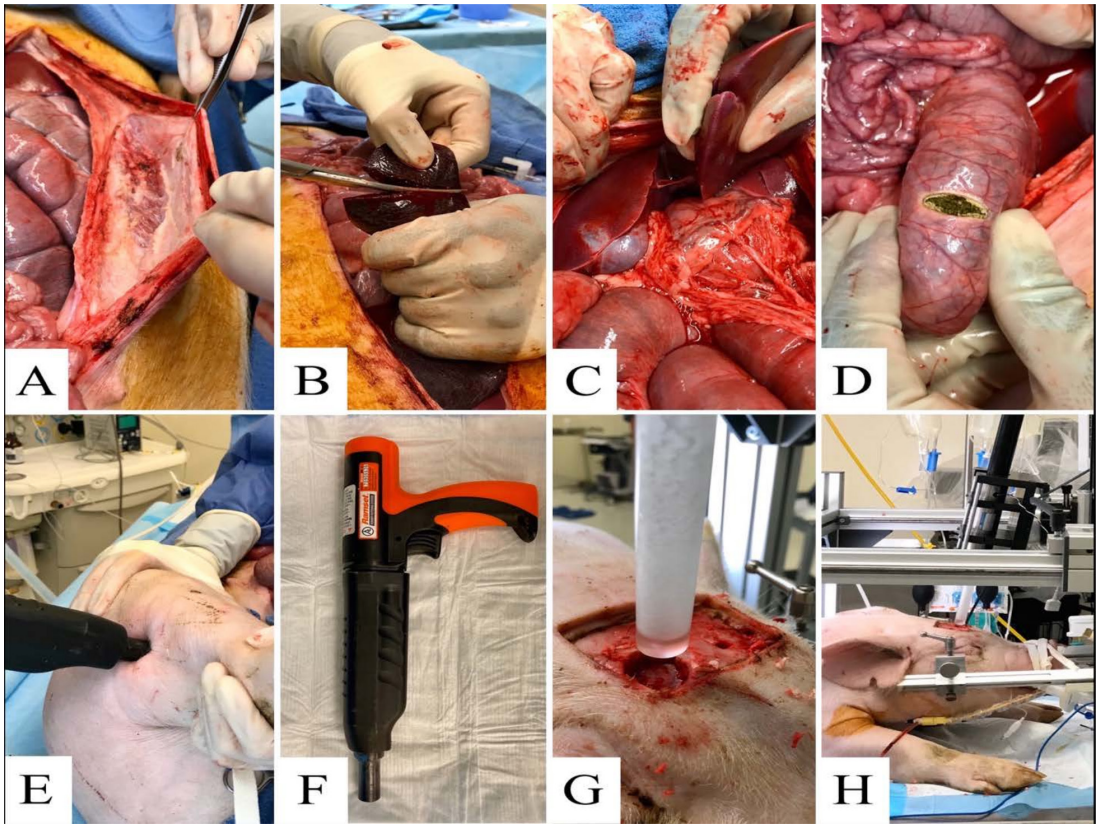

Figure 1 Rectus crush, abdominal injuries, femur fracture and traumatic brain injury. (A) Rectus abdominis separated from skin and subcutaneous tissue, exposing 5 cm of the muscle and crushed with a Kelly clamp to create a soft tissue injury. (B) Section of spleen 5 cm from its distal tip is transected sharply. Remaining spleen is oversewn to prevent excess blood loss. (C) Left median lobe of liver transected sharply 5 cm from its anterior tip. Cut edge of liver is allowed to hemorrhage freely for 30 seconds before being closed by oversewing the cut edge, cauterizing the surface, and packing with laparotomy pads. (D) 3 cm injury created in the sigmoid colon 10 cm proximal to the anterior peritoneal reflection, exposing fecal matter. The colotomy in left open. (E) Small cruciate incision is used to cut down onto the lateral femur. Femur fractured with .22 caliber bullet using a captive bolt gun, creating a long bone fracture. 4×4 gauze pads are placed into wound after fracture to prevent excess bleeding. (F) Close-up of the bolt gun used. (G) A 21 mm burr hole was made just anterior and to the right of the bregma to expose the dura for traumatic brain injury. A 5 mm burr hole was made anterior and left of the bregma for placement of an intracranial pressure monitor. (H) Controlled cortical impact device set up to deliver a 12 mm impact.

left median lobe of the liver was exposed and transected sharply 5 cm from its anterior tip. The liver was allowed to hemorrhage for 30 seconds, after which the hemorrhage was controlled by oversewing the cut edge, cauterizing the raw liver surface and packing the liver with laparotomy pads. Estimated blood loss was approximately 50 mL from the uncontrolled liver hemorrhage, which allowed us to maintain reproducibility between animals. The spleen was transected sharply 5 cm from its distal tip and immediately oversewn; this resulted in scant blood loss.

Several variations of colon injury were attempted with significant trial and error. We initially attempted extubating and allowing the animals to survive after the injury and resuscitation periods (survival group; n=7). This group received a rectus crush, rib fracture, liver laceration, spleen laceration, colon injury, hemorrhage and TBI. The survival group was followed by three groups that were non-survival which included an uncontrolled cecal injury (UCI; n=4), controlled cecal injury (CCI; n=4) and sigmoid injury (SI; n=5). These non-survival groups received the same injuries as the survival group except a femur fracture was performed in place of a rib fracture. The severity of the colon injury was the only variation between the non-survival groups. UCI was created with an approximately 4 cm cecal injury between taeniae; this resulted in a large volume fecal contamination. CCI was subsequently performed in an attempt to control the peritoneal contamination by making a cecal injury, collecting 25 mL of stool and repairing the injury immediately. The collected stool was then placed in the right paracolic gutter for a very controlled amount of fecal contamination. The SI was the last colon injury used in the model and consisted of a 3 cm full thickness injury in the sigmoid colon 10 cm proximal to the anterior peritoneal reflection. The colon injury was left open; the hardened stool consistency at the sigmoid colon resulted in minimal intraperitoneal fecal burden. The abdomen was then closed, and the animal flipped prone.

A U-shaped incision was made on the top of the skull and skin flap raised to expose the bregma. A 21 mm burr hole was made just anterior and to the right of the bregma to expose the dura for TBI. A 5 mm burr hole was made anterior and to the left of the bregma for placement of an intracranial pressure monitor. Hemorrhage and TBI were inflicted concurrently. The TBI was made using a controlled cortical impact device (University of Michigan Innovation Center, Ann Arbor, Michigan). The impact was made with a 20 mm diameter impactor, 4 m/s velocity, 100-millisecond dwell time and depth of 12 mm. Via the femoral artery cannula, the animal was hemorrhaged 45% of its estimated blood volume (estimated total volume (mL)=-weight (g)×0.06+0.77) over 20 minutes using a Masterflex pump (Cole Palmer, Vernon Hills, Illinois). If the mean arterial pressure (MAP) dropped below 30 mm Hg, hemorrhage was paused and a 50–100 mL bolus of normal saline (NS) was given to maintain the MAP.

At the completion of hemorrhage, animals were left in shock and not resuscitated for 1 hour to simulate military medic response time. During the 1-hour shock phase MAP was maintained between 30 and 35 mm Hg by titrating isoflurane to minimize animal-to-animal variability in response to the injury protocol.

Whole blood was centrifuged and separated into packed red blood cells (pRBCs) and pRBCs were stored for transfusion later in the experiment.

To reflect a resource scarce field care environment and delayed transfer to definitive care that may occur in combat settings, animals were initially resuscitated with NS which was administered via the central venous catheter.

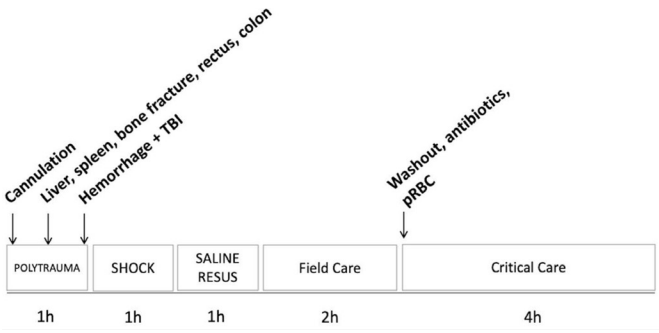

Figure 2 Experimental timeline. Invasive lines were placed (cannulation), followed by rectus muscle, bone, liver, spleen and colon injuries, then hemorrhage and traumatic brain injury (TBI). Animals were left in shock for 1–2 hours, depending on the model (survival animals had 2-hour shock, all other animals had 1-hour shock). Then treatment began with normal saline resuscitation given over 1 hour. Autologous packed red blood cells (pRBC) were given 2 hours after resuscitation. Concurrent with pRBC transfusion, the laparotomy was reopened; the colon injury was repaired and abdomen irrigated with normal saline; and antibiotics were administered. The animal was then monitored for an additional 4 hours at which point the experiment was ended for non-survival models.

Transfer to definitive care

Three hours after the start of NS resuscitation, animals were transfused autologous pRBCs, the colon injury was repaired and cefoxitin administered to reflect transitioning an injured patient from the field to a place of definitive care. The entire volume of pRBCs hemorrhaged earlier in the experiment was returned to the animal via the central venous line at a rate of 25 mL/h. The previous laparotomy was reopened, and the colon injury closed in two layers. The abdomen was irrigated with 1 L of NS. The abdomen was then closed again. Cefoxitin 1 g was administered intravenously.

Survival: For animals that we planned to emerge from anesthesia, extubate and survive after surgery, isoflurane was weaned. The animals were then extubated after demonstrating the ability to maintain adequate tidal volume and exhibit appropriate neurologic alertness (moving all four limbs, blinking, chewing on endotracheal tube). Animals were then individually housed in their cage and serially monitored.

Non-survival: In non-survival models, animals were monitored for 4 hours after the start of the pRBC transfusion to the predetermined endpoint of the study. An experimental timeline is shown in figure 2.

Laboratory monitoring

Throughout the experiment, arterial blood gases drawn from a femoral arterial line were serially monitored every 30 minutes. Hypoglycemia and hyperkalemia were common for both groups. Hypoglycemia was treated with 25 g dextrose when blood glucose reached <60 mg/dL. Hyperkalemia was treated with 1 U insulin and 25 g dextrose for potassium >6 mmol/L.

Blood cultures

Immediately prior to the transfer to definitive care and antibiotic administration, blood was obtained sterilely from an anterior abdominal wall vein after cleaning the area with chlorhexidine twice. Blood was transferred to aerobic and anaerobic blood culture containers, and blood cultures were performed

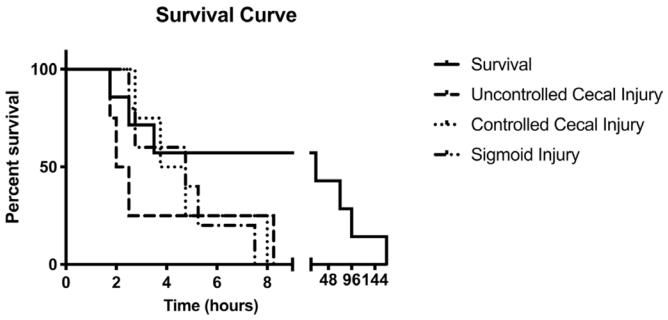

Figure 3 Kaplan-Meier survival curves. X-axis shows hours since start of traumatic injuries.

by Michigan State University Veterinary Diagnostic Laboratory (East Lansing, Michigan).

Statistics

GraphPad Prism V.8 was used for statistical analyses. Values are presented as mean±SD. Survival was analyzed using Kaplan-Meier curves and log-rank testing. Analysis of variance was used when comparing groups for all other variables. Statistical significance was defined as p value <0.05.

RESULTS

Survival

Survival is depicted in figure 3. In the survival group (n=7), the length of survival was highly variable. Several animals died early after injury whereas others survived up to a week. Late deaths were all based on decisions for euthanasia based on consensus from a group of veterinarians and physicians. These decisions were based primarily on lack of appetite and very poor activity level. UCI (n=4) had three extremely early deaths and another single animal that lived 8 hours to the predetermined endpoint. When controlling the cecal injury (CCI group, n=4), mortality was more stepwise over 8 hours with a single animal that lived 8 hours to the predetermined endpoint. Moving the colon injury to the left side (SI group, n=5) produced a similar survival curve but none of the animals lived to the end of the experiment.

Hemodynamic parameters

Hemodynamic parameters are outlined in figure 4. The X-axis on the graphs shows the amount of time that has passed since the beginning of the traumatic injuries. The heart rate (HR) starts around a baseline value of 110–115 beats per minute which sharply increases after the beginning of hemorrhage. As the experiment continues, the HR shows a slow downward trend before ending just above the baseline value. The MAP starts around a baseline value of just above 60 mm Hg, but sharply drops when hemorrhage takes place. After the 1-hour shock period (MAP controlled to be between 30 and 35 mm Hg), the MAP increases with NS resuscitation, though never returns to baseline. The CO started around a baseline value of 4 L/min. While there is some variability between the survival group and the rest of the groups, the general trend is that the CO decreases after hemorrhage and then rises again when resuscitation with

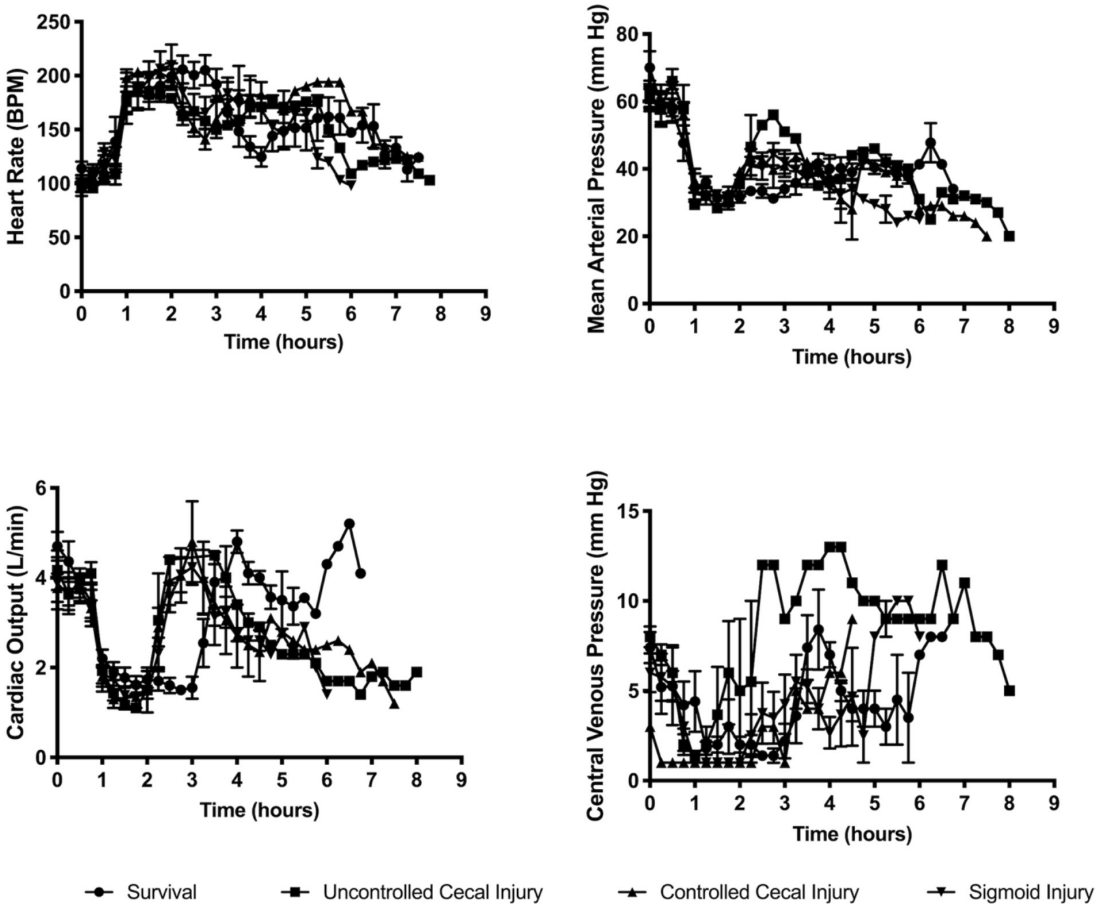

Figure 4 Physiologic parameters. Heart rate (HR) in beats per minute (BPM), mean arterial pressure (MAP) in mm Hg, cardiac output (CO) in liters per minute (L/min) and central venous pressure (CVP) in mm Hg. X-axis shows hours since start of traumatic injuries.

fluids occurs, before tapering off at later timepoints. The central venous pressure (CVP) shows great variability between the groups but in general hemorrhage results in decreased CVP and it then increases with resuscitation.

Laboratory values

Over the course of the experimental timeline, animals exhibited metabolic acidosis (online supplemental table 1). At baseline and the beginning of shock, the pH for the animals was similar; however, as the study progressed animals became acidotic reflected by their pH, lactate and bicarbonate. Animals also demonstrated an anemia as would be expected with large volume hemorrhage followed by NS resuscitation. Blood cultures from all animals demonstrated bacteremia, regardless of the intestinal injury or time of death (online supplemental table 2).

DISCUSSION

Polytrauma leading to intra-abdominal sepsis is a common problem among trauma patients with hollow viscous injuries. Patients with intra-abdominal sepsis have high rates of mortality and morbidity. Current treatment options are limited to the administration of antibiotics, source control, and supportive care. The cause of that mortality is certainly multifactorial but we hypothesize some portion of lethality can be attributed to initial cellular damage in patients that is unrecoverable despite current surgical and medical therapy. Our laboratory has used several prosurvival cellular therapies on similar large animal models which have shown survival improvement.[10 13] To advance treatment options and reduce mortality rates, it is important to have a translational model with which to study potential new therapies. To our knowledge, this is the first large animal model that combines polytrauma with concomitant colon injury resulting in bacteremia that is reproducible in the hands of experienced operators.

A widely used model to study sepsis is cecal ligation and puncture (CLP), which is performed in rodents. The model involves perforating the cecum which allows fecal matter to spill into the peritoneal cavity. While the model is consistent and reproducible, it is not necessarily translatable to humans. In fact, investigational new drug applications are required to have preclinical data from non-rodent animals prior to approval by the Food and Drug Administration.[14] This mandates appropriate non-rodent models for new drug testing. Porcine models are appropriate for translational investigations due to the similarities to human physiological response, size, and anatomy.[8] Our described experience demonstrates how challenging large animal model development can be, highlighting the importance of establishing reproducible models for future work. The porcine model we describe allows for translatable research which is also consistent and reproducible. Using controlled hemorrhage for the majority of the blood loss maintains the reproducibility of the model. Following the injury pattern described allows for minimal variability between animals. The similarities in hemodynamic parameters and the fact that all animals demonstrated bacteremia also support the consistent and reproducible nature of the model. The porcine model allows for a more clinically realistic setting due to the similarities in size and anatomy to humans. As we have done in this model, using a large animal allows the same level of intensive care monitoring and resuscitation that a human would receive. The porcine model also allows for simulating a more realistic timeline of care starting with colon injury, prehospital care and finally definitive care with laparotomy, repair of injury and irrigation of the abdomen. This is starkly different from uncontrolled intra-abdominal sepsis with CLP murine models.

Others have used models of isolated hollow viscous injury,[15 16] or attempted to replicate Gram-negative sepsis with endotoxin infusion.[17] While these models do create uncontrolled intra-abdominal sepsis, there is no transition to a period of source control that would be clinically realistic. Additionally, the isolated colon injury is clearly a different insult than the model we describe that is akin to the polytrauma patient that is common after combat-related injury. We chose to use a colon injury in this sepsis model due to the large bacterial burden. Since the porcine colon injury is not well investigated, it took significant effort to find an injury pattern that would allow development of a model that is both lethal but potentially salvageable with treatment. The pig has a much larger cecum than a human, and the stool is very liquid at this location. Even with a small injury to the cecum there was a very large amount of initial and ongoing stool spillage. This led us to change the model from survival to non-survival and to limit the amount of gross spillage due to the severity of the injury. Our laboratory has extensive experience surviving animals after porcine models of TBI and hemorrhagic shock and polytrauma. Compared with this previous experience, the addition of the hollow viscous injury does not translate well to attempts at long-term survival for ethical reasons because of markedly worsened appetite, energy and mobility. Still the experiment timeline in the non-survival models allows adequate time to investigate early response to injury with simulated transitions in care. Despite the lethality of the model, our laboratory has found that it is salvageable by using histone deacetylase inhibitors as a treatment.[13] This indicates that other treatments can be tested on this model to observe their effectiveness.

The applicability of this model with prolonged time from injury to definitive treatment may be difficult to imagine in a civilian setting in the USA. However, in the most recent military conflicts there has been increasing use of ‘far forward’ military units in truly austere environments. The scenario of a patient suffering a major injury and being multiple hours from definitive care is a reality faced by those delivering care in combat settings. In fact, some of our collaborative efforts with the Department of Defense (DoD) have tested simulated prolonged field care up to 72 hours based on current military needs and DoD forecasts of future military conflicts.[13]

While this model is novel and has many benefits, we recognize that there are limitations. First, the model is complex and involves many traumatic injuries. While we intentionally developed the model with a complex injury pattern because it is relevant in the combat setting, its reproducibility requires an experienced practitioner in a laboratory equipped to perform large animal studies. There are certainly pros to clinically realistic large animal models; however, they are clearly labor intensive and costly to perform. This places practical limits on the sample size attainable. However, the sample size used here is similar to previous studies performed in models of trauma using swine. Another limitation is that the complexity of the model and large number of injuries makes it hard to specify the exact cause of death, though it was likely multifactorial. Without further studies, it is impossible to know how the injuries interact or compound on one another. An additional limitation is that the bacteremia resulted from a colon injury rather than the small bowel injury that is more common in human trauma. We chose this due to the higher bacterial burden brought on by this injury. This model, however, does not account for what would happen in cases of stool spillage from foregut or midgut injuries. Presumably, the bacterial insult would be less from the foregut or

the midgut, but we anticipate that those would likely also result in a severe inflammatory insult from large volume liquid succus contamination. Another limit to the model is that it is a model of trauma with concomitant colon injury which resulted in bacteremia. It does not model late development of sepsis after trauma, which has a different clinical relevance. The last major limitation of the study is that it would be difficult to use this model to study long-term effects of bacteremia. Early on in our model development, we attempted to perform long-term survival of the animals; however, they were subjectively too sick to continue these efforts as poor appetite, dehydration, and clinical deterioration were observed. In the future, experiments intended to study long-term survival could be done by maintaining these animals in an intensive care unit environment which could also further enhance the clinical relevance.

Despite these limitations, this porcine model of polytrauma and bacteremia is reproducible and dependable. The porcine model described here presents an excellent opportunity to validate findings in rodents in a translatable and clinically realistic way. It is an innovative tool for our laboratory and can be for others to develop novel treatments for the clinical problem of concurrent trauma and bacteremia.

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Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The University of Michigan Institutional Animal Care and Use Committee approved the animal protocol. The investigators adhered to the Animal Welfare Act Regulations and other federal statutes relating to animals and experiments involving animals and the principles set forth in the current version of the Guide for Care and Use of Laboratory Animals, National Research Council.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information.

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Supplemental Table 1

		Baseline	Shock	Resus	pRBC + Source Control
pH	Survival	7.44 (0.02)	7.49 (0.03)	7.28 (0.07)	7.37 (0.07)
	UCI	7.50 (0.05)	7.46 (0.08)	7.36 (0.01)	7.40
	CCI	7.42 (0.05)	7.47 (0.13)	7.36 (0.14)	7.44
	SI	7.46 (0.05)	7.48 (0.04)	7.41 (0.09)	7.23 (0.05)
Hgb (g/dL)	Survival	9.4 (1.3)	10.6 (1.0)	10.0 (1.6)	7.0 (1.0)
	UCI	9.5 (1.6)	9.1 (0.4)	7.9 (0.6)	5.9
	CCI	11.0 (0.8)	10.3 (0.7)	9.3 (0.5)	7.1
	SI	9.6 (0.6)	10.5 (2.3)	10.2 (1.7)	8.1 (0.4)
K (mM)	Survival	3.8 (0.2)	4.5 (0.2)	5.1 (0.5)	4.6 (0.9)
	UCI	4.0 (0.1)	4.0 (0.1)	4.9 (0.8)	4.9
	CCI	3.7 (0.2)	3.9 (0.3)	5.1 (1.3)	5.8
	SI	4.0 (0.4)	4.5 (0.5)	5.0 (0.4)	4.1 (0.1)
Na (mM)	Survival	137 (0.5)	135 (0.8)	136 (3.6)	139 (2.2)
	UCI	140 (2.5)	140 (1.7)	138 (0.7)	144
	CCI	136 (3.6)	135 (4.6)	143 (4.5)	141
	SI	140 (1.8)	139 (2.6)	145 (2.6)	144 (2.1)
Ca	Survival	1.32 (0.05)	1.27 (0.04)	1.29 (0.11)	1.32 (0.08)

2

(mM)	UCI	1.34 (0.04)	1.27 (0.07)	1.29 (0.11)	1.34
	CCI	1.35 (0.06)	1.31 (0.06)	1.29 (0.11)	1.34
	SI	1.34 (0.05)	1.31 (0.05)	1.26 (0.07)	1.24 (0.10)
Cl (mM)	Survival	102 (2.6)	102 (2.8)	103 (3.7)	111 (2.6)
	UCI	104 (3.8)	110 (5.5)	107 (2.1)	114
	CCI	104 (4.4)	103 (5.5)	105 (2.9)	113
	SI	104 (2.1)	105 (2.2)	105 (2.9)	112 (2.1)
Glucose (mg/dL)	Survival	95 (7)	167 (44)	136 (65)	110 (31)
	UCI	83 (19)	140 (42)	140 (30)	89
	CCI	100 (12)	156 (36)	179 (88)	103
	SI	87 (14)	197 (60)	170 (88)	157 (26)
Lactate (mmol/L)	Survival	1.5 (0.6)	3.1 (1.5)	9.2 (6.3)	4.9 (3.8)
	UCI	1.3 (0.5)	4.5 (2.0)	11.7 (3.0)	5.0
	CCI	1.1 (0.4)	2.6 (1.5)	9.1 (4.7)	6.1
	SI	1.6 (0.6)	3.5 (1.6)	8.1 (3.0)	8.5 (3.8)
HCO_3	Survival	28.3 (1.9)	27.5 (2.1)	18.3 (7.2)	21.0 (4.1)
	UCI	30.2 (2.1)	22.8 (4.4)	17.0 (2.6)	17.5
	CCI	28.9 (0.8)	28.0 (2.4)	21.7 (5.8)	20.3
	SI	28.0 (1.1)	28.7 (4.2)	21.6 (4.0)	15.2 (3.2)

Select arterial blood gas data: Data presented as group means with standard deviation shown in parentheses. Hgb = hemoglobin; K = potassium; Na = sodium; Ca = calcium; Cl = chloride;

3

HCO_3 = bicarbonate; Shock = end of hemorrhage/start of shock; Resus = end of shock/start of normal saline resuscitation; pRBC + Source Control = start of blood transfusion, colon injury closure, abdominal washout and antibiotic administration; Survival = survival group; UCI = uncontrolled cecal injury group; CCI = controlled cecal injury group; SI = sigmoid injury group.

Supplemental Table 2

Cecal Injury	*Pseudomonas* (2) *Streptococcus* *Sphingomonas* *Prevotella* *E. coli* (2) *Enterococcus* (2) *Chryseobacterium* (2) *Stenotrophomonas* *Klebsiella*
Sigmoid Injury	*Pseudomonas* (3) *Klebsiella* (2) *B hemolytic Streptococcus* (1) *Sphinomonas* (1) *Streptococcus pluranimalium* (1) *Enterococcus* (1) *E. coli* (1)

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	Chryseobacterium (1) *Stenotrophomonas* (1)_

Blood culture results: Peripheral blood cultures drawn immediately before antibiotics and source control, if the animal survived to that point. If the animal died prior, blood cultures were drawn at time of death. Results displayed as bacterium grown on aerobic or anaerobic blood culture.

1

Supplemental Table 1

		Baseline	Shock	Resus	pRBC + Source Control
pH	Survival	7.44 (0.02)	7.49 (0.03)	7.28 (0.07)	7.37 (0.07)
	UCI	7.50 (0.05)	7.46 (0.08)	7.36 (0.01)	7.40
	CCI	7.42 (0.05)	7.47 (0.13)	7.36 (0.14)	7.44
	SI	7.46 (0.05)	7.48 (0.04)	7.41 (0.09)	7.23 (0.05)
Hgb (g/dL)	Survival	9.4 (1.3)	10.6 (1.0)	10.0 (1.6)	7.0 (1.0)
	UCI	9.5 (1.6)	9.1 (0.4)	7.9 (0.6)	5.9
	CCI	11.0 (0.8)	10.3 (0.7)	9.3 (0.5)	7.1
	SI	9.6 (0.6)	10.5 (2.3)	10.2 (1.7)	8.1 (0.4)
K (mM)	Survival	3.8 (0.2)	4.5 (0.2)	5.1 (0.5)	4.6 (0.9)
	UCI	4.0 (0.1)	4.0 (0.1)	4.9 (0.8)	4.9
	CCI	3.7 (0.2)	3.9 (0.3)	5.1 (1.3)	5.8
	SI	4.0 (0.4)	4.5 (0.5)	5.0 (0.4)	4.1 (0.1)
Na (mM)	Survival	137 (0.5)	135 (0.8)	136 (3.6)	139 (2.2)
	UCI	140 (2.5)	140 (1.7)	138 (0.7)	144
	CCI	136 (3.6)	135 (4.6)	143 (4.5)	141
	SI	140 (1.8)	139 (2.6)	145 (2.6)	144 (2.1)
Ca	Survival	1.32 (0.05)	1.27 (0.04)	1.29 (0.11)	1.32 (0.08)

2

(mM)	UCI	1.34 (0.04)	1.27 (0.07)	1.29 (0.11)	1.34
	CCI	1.35 (0.06)	1.31 (0.06)	1.29 (0.11)	1.34
	SI	1.34 (0.05)	1.31 (0.05)	1.26 (0.07)	1.24 (0.10)
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	CCI	1.1 (0.4)	2.6 (1.5)	9.1 (4.7)	6.1
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	CCI	28.9 (0.8)	28.0 (2.4)	21.7 (5.8)	20.3
	SI	28.0 (1.1)	28.7 (4.2)	21.6 (4.0)	15.2 (3.2)

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Supplemental Table 2

Cecal Injury	*Pseudomonas* (2) *Streptococcus* *Sphingomonas* *Prevotella* *E. coli* (2) *Enterococcus* (2) *Chryseobacterium* (2) *Stenotrophomonas* *Klebsiella*
Sigmoid Injury	*Pseudomonas* (3) *Klebsiella* (2) *B hemolytic Streptococcus* (1) *Sphinomonas* (1) *Streptococcus pluranimalium* (1) *Enterococcus* (1) *E. coli* (1)

4

	Chryseobacterium (1) *Stenotrophomonas* (1)_

Blood culture results: Peripheral blood cultures drawn immediately before antibiotics and source control, if the animal survived to that point. If the animal died prior, blood cultures were drawn at time of death. Results displayed as bacterium grown on aerobic or anaerobic blood culture.