

# Animal models of necrotizing enterocolitis

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**To cite:** Mendez YS, Khan FA, Perrier GV, *et al.* Animal models of necrotizing enterocolitis. *World Jnl Ped Surg* 2020;3:e000109. doi:10.1136/wjps-2020-000109

Received 16 January 2020

Revised 5 March 2020

Accepted 6 March 2020

## ABSTRACT

**Background** Necrotizing enterocolitis (NEC) is one of the leading causes of death in premature infants. To determine the factors present in the disease that lead to increased morbidity and mortality, manipulation of variables that are shown to have a positive response has been tested using various animal models. Testing and manipulation of these variables are unwarranted in humans due to regulatory health standards.

**Methods** The purpose of this review is to provide an update to previous summaries that determine the significance of animal models in studying the mechanisms of NEC. A large variety of animal models including rats, mice, rabbits, piglets, nonhuman primates, and quails have been described in literature. We reviewed the reported animal models of NEC and examined the pros and cons of the various models as well as the scientific question addressed.

**Results** The animals used in these experiments were subject to gavage feeding, hypoxia, hypothermia, oxygen perfusion, and other methods to induce the disease state. Each of these models has been utilized to show the effects of NEC on the premature, undeveloped gut in animals to find a correlation to the disease state present in humans. We found specific advantages and disadvantages for each model.

**Conclusions** Recent advances in our understanding of NEC and the ongoing therapeutic strategy developments underscore the importance of animal models for this disease.

premature newborns to intestinal injury leading to NEC.<sup>3,4</sup>

Naturally, development of robust animal models to aid in the study of this disease process is of critical importance. Traditionally, gavage formula fed/hypoxia/hypothermia models have been used for induction of NEC in mice, rats, and piglets. Asphyxia has been shown to hold a crucial role in the development of NEC because formula and bacterial exposure without asphyxia typically do not result in the phenotypical injury pattern seen in NEC.<sup>2</sup> Another significant variable that has been manipulated in various models is the removal of breast milk. It is well known that breast milk contains large amounts of immunoglobulin A (IgA), smaller amounts of Immunoglobulin G (IgG), active lymphocytes and macrophages, and specific antibodies against many types of microorganisms.<sup>3</sup> Barlow *et al*<sup>4</sup> theorized that without the passive immunity and intestinal flora control of breast milk, the enteric mucous barrier will be destroyed, leading to a pathologic cascade characterizing NEC.<sup>4</sup>

To induce intestinal damage and microbial dysbiosis, pathogenic bacteria can be administered to disrupt the intestinal epithelial barrier leading to an inflammatory response and translocation of pathogens.<sup>5</sup> Although this explains the presence of intestinal epithelial disruption that characterizes NEC, it does not explain the presence of intestinal necrosis that is seen in patients with this disease, nor can this explanation readily reconcile the observation that most patients who develop NEC do not actually have an antecedent ischemic event.<sup>6</sup>

The clinical NEC seen in different animal models resembles NEC presented in human patients. Robust animal models closely resembling human disease are critical to test biochemical pathways, cell receptors, enteral nourishment, mechanisms of direct bowel injury, and studies that cannot ethically be conducted in humans. Animal models of NEC utilizing rats, mice, nonhuman

## INTRODUCTION

Necrotizing enterocolitis (NEC) is a devastating disease affecting premature infants. Approximately 10% of infants born weighing less than 1500 g will develop the disease, with mortality rates for affected infants being as high as 20%–30%.<sup>1</sup> Mechanisms leading to the development of NEC are not completely understood, which makes the study of this disease difficult.<sup>2</sup> Although the exact pathophysiology of NEC is not fully understood, most experts agree that its etiology is multifactorial. It is felt that impaired gastrointestinal (GI) motility, intestinal barrier dysfunction, decreased digestive ability, poor circulatory regulation, intestinal microbial overgrowth, and immature immune defenses predispose



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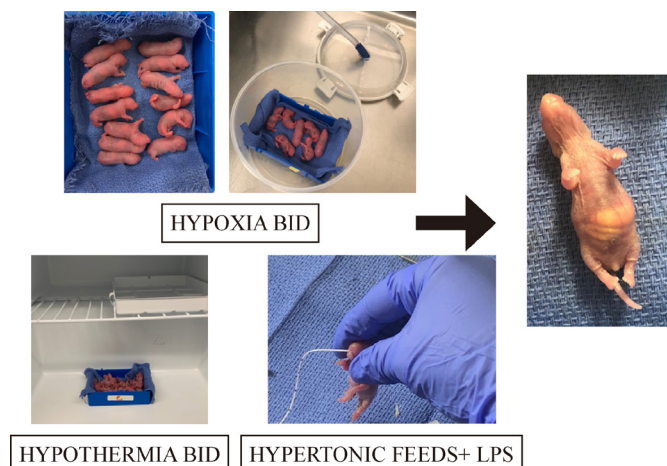
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**Figure 1** Pregnant time-dated Sprague-Dawley rats were delivered by C-section under CO<sub>2</sub> anesthesia on day 21.5 of gestation. Newborn rats were placed in an incubator and fed via gavage with formula containing 15 g Similac 60/40 in 75 mL Esbilac, providing 836.8 kJ/kg per day. Feeds were started at 0.1 mL every 4 hours beginning 2 hours after birth and advanced as tolerated up to a maximum of 0.4 mL per feeding by the fourth day of life. Animals were exposed to a single dose of intragastric LPS (2 mg/kg) 8 hours after birth, and were stressed by exposure to hypoxia (100% nitrogen for 1 min) followed by hypothermia (4°C for 10 min) two times per day beginning immediately after birth until the end of the experiment on day 5 (IRB protocol # 8180041, Loma Linda university health). LPS, lipopolysaccharide.

primates, and piglets have been used to study the pathogenesis of NEC.

The advantages and disadvantages to use animal models are to be discussed in further detail. Because of the differences in size, genetic manipulation, cost of use, and preterm viability, each animal model can be examined to highlight the beneficial aspects of how NEC can be modeled.

## ANIMAL MODELS

### Rat model

The rat model has been used most commonly for NEC analysis due to the similarity to human preterm infants. NEC mainly affects premature and low birthweight neonates and is characterized by ischemic necrosis of the bowel wall frequently leading to perforation and death.<sup>3</sup> As such, neonatal rats can be useful models for comparison due to their level of bowel immaturity at day 21 of gestation. One of the earliest studies to use rats concluded that the gut flora and absence of breast milk were suggested to play important roles in NEC pathogenesis.<sup>7</sup> In Barlow's model, a formula was developed which simulated rat breast milk and could be mixed with a bacterial contaminant. In this model, the pups are removed immediately after Cesarean section and are placed in an incubator, with avoidance of breast feeding.<sup>8</sup> The stressors used to induce a NEC like state in the animal models includes hypoxia, hypothermia, addition of a *lipopolysaccharide*

(LPS) and hyperosmolar formula during the course of feeding throughout a week long experiment (figure 1). In this model, a formula is given four times per day with an increasing caloric intake each day of the week.<sup>8</sup> At the end of the protocol, or when rat pups have clinical signs of NEC, intestines are harvested and histologically graded for intestinal injury 0–4 with lesions greater than 2 being considered as NEC (figure 2).

Several laboratories have modified this formula feeding/hypoxia/hypothermia rat NEC model using additional elements to help induce NEC. One model has administered commensal bacteria found in stool, whereas others have tried different types of bacteria.<sup>7</sup> Some researchers introduced *Cronobacter sakazakii* (CS), formerly known as *Enterobacter sakazakii*, a bacteria that was reported to be associated with NEC, to the formula-feeding/hypoxia rat NEC model.<sup>7</sup>

Rat models are an attractive option when studying NEC due to their low cost, preterm viability post-Cesarean section, and resilience to common stressors used to induce the disease.<sup>9</sup> One disadvantage seen is the lack of commercial antibodies produced to target selected receptors involved in NEC. Another disadvantage is that rats are highly tolerant to different types of bacteria and it can be difficult to achieve success when attempting to induce disease. Last but not least, another major drawback is the lack of genetic diversity found in rats compared with other species of rodents.<sup>7</sup>

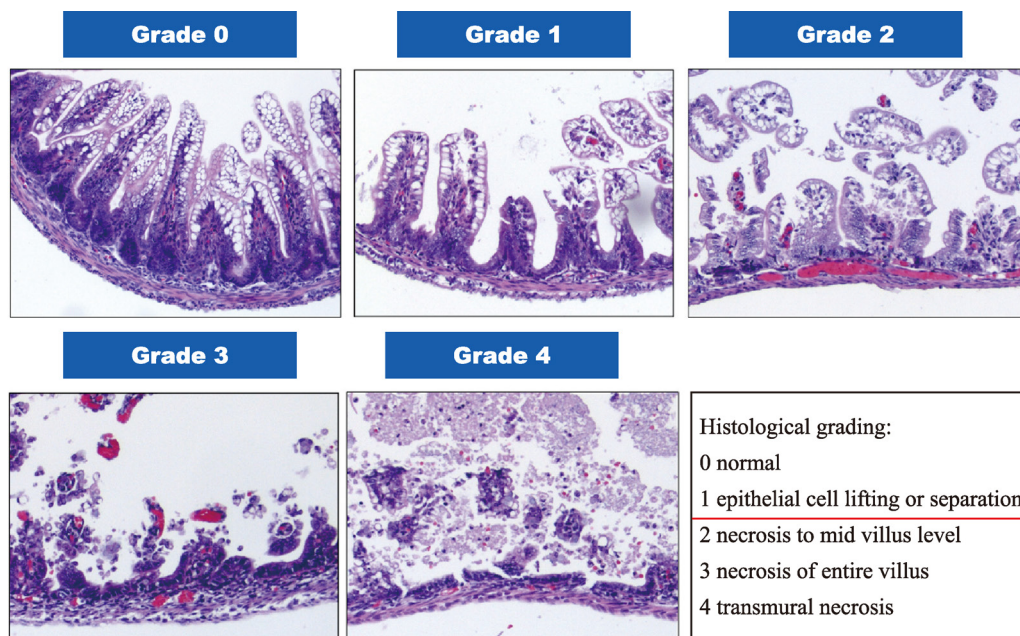
Using this model, many promising preventative and therapeutic interventions have been tested such as administration of probiotics, growth factors, stem cells, human milk oligosaccharides, tumor necrosis factor (TNF) blockers, and various antioxidants.<sup>9</sup> To date, we found more than 400 papers that have used the rat model with or without modifications from the original description.

### Mouse model

One of the major challenges and limitations of various animal models is that humans have an inherently different pattern of gene expression and therefore immune response to the development of NEC. In addition to this limitation, mice are also different due to their size compared with newborn rats or piglets. However, despite these limitation, mouse models have produced greater insights into the significance of prematurity in animals who develop NEC.<sup>7</sup>

Humanized mouse models have initiated an area of interest for modeling NEC through genetic manipulation. Humanized mice can be defined as animals that are genetically manipulated to express human genes.<sup>10</sup> This model also relies on the use of hyperosmolar formula gavage feeds, hypoxia, and hypothermia. The addition of LPS to increase the incidence of NEC is variable across the published literature.

The most useful advantage of the mouse model comes from the ability to monitor and manipulate genetic variability and the use of transgenic animals to compare knockout (KO) mice to wild-type. The advantage of a



**Figure 2** Histologic injury score in rat pups subjected to experimental NEC. Shown are representative H&E-stained sections showing Grade 0, normal intestine, Grade 1, epithelial cell lifting or separation, Grade 2, sloughing of epithelial cells to the mid villous level, Grade 3, necrosis of the entire villous, and Grade 4, transmural necrosis. Magnification  $\times 40$ . H&E, hemotoxylin and eosin; NEC, necrotizing enterocolitis.

humanized mouse model is the interrogation of human tissue while examining the progression of the disease.<sup>7</sup> With this ability, the complex biological processes can be appreciated better in an attempt to model human neonates. For example, KO and transgenic (TG) models have been used to test the role of different growth factors in the prevention and treatment of NEC such as epidermal growth factor (EGF) and heparin-binding EGF-like growth factor (HB-EGF).<sup>11 12</sup>

HB-EGF TG mice were designed to specifically overexpress the human HB-EGF precursor (proHB-EGF) in the intestine.<sup>11</sup> Using this model, the investigators were able to demonstrate that decreased endogenous HB-EGF expression predisposed the intestines to the development of NEC and the opposite being true for HB-EGF TG mice that overexpressed HB-EGF.<sup>11</sup>

The advantages of this model lie in the ability to easily breed mice, the ability to genetically alter lines, and the cost-benefit of using a smaller animal model compared with the other animal models. On the other hand, the smaller size of the pups at birth predisposes them to a increased rate of complications. The mouse pups are more difficult to handle and gavage feed with formula through an orogastric catheter compared with rats.

### Piglet model

Compared with rodents, piglets share a high degree of anatomical, developmental, nutritional, and physiological similarity to the GI tract of humans.<sup>7</sup> The piglet is therefore a good model for studies looking at pathogenesis of NEC, evaluation of specific feeding regimens, preclinical drug studies for prevention and potential

therapy, as well as for the development of radiological diagnostic methods.<sup>7</sup> During the neonatal period, protein deposition is very rapid, and owing to similarities of post-natal nutrition and intestinal development to humans, the piglet can be viewed as an accelerated model of post-natal growth and development.<sup>13</sup> In addition, the piglet model is also appropriate for modeling other biological systems that may have an impact on the development of NEC such as liver function and metabolism due to its similarity with human systems.<sup>13</sup>

Generally, with small variations, the model involves piglets that are delivered by C-section at 90% term. Following birth, preterm piglets go through a period of natural hypoxia/hypothermia, and then are formula fed to induce injury.<sup>14</sup> A variation uses total parental nutrition at birth followed by a transition to enteral formula feeds on the second day of life.<sup>9 15 16</sup> Many investigators have used variations of this model as outlined in table 1.

Cohen *et al*<sup>17</sup> described a model in which newborn piglets were subjected to a hypoxic insult for 30 min and hypothermic stress (core temperature reduced to 35°C for 30 min). After 3–4 days, approximately half of the animals developed histological changes that resembled NEC. Other investigators induced NEC through a combination of cow-based formula and ischemia/reperfusion such as the model described by Crissinger *et al*<sup>18</sup> or by means of administered isosmolar acidified casein solution into intestinal segments of 1-day-old piglets.<sup>19</sup>

All these reported variations of the piglet model serve to underline the importance of this model to study the pathogenesis of NEC.



**Table 1** A summary of the studies using the piglet model

Author	Title	NEC protocol	Gestational age (days)	Area of study
Robinson <i>et al</i> <sup>30</sup>	Prematurity reduces citrulline-arginine-nitric oxide production and precedes the onset of necrotizing enterocolitis in piglets	Piglets gavaged with formula, and tracer induced TPN for 42 hours, starting day 3 of life (DOL)	103 110 114	Effects of tracers including citrulline, arginine, and nitric oxide in lowering the incidence of necrotizing enterocolitis
Zamora <i>et al</i> <sup>15</sup>	Low abdominal near-infrared spectroscopy values and elevated plasma intestinal fatty acid-binding protein in a premature piglet model of necrotizing enterocolitis	Piglets gavaged with 3–5 mL/kg/h of infant formula every 3 hours for 48 hours starting on day of life 3	103	Detecting necrotizing enterocolitis prior to the onset of clinical symptoms through continuous abdominal near-infrared spectroscopy measurements
Burrin <i>et al</i> <sup>31</sup>	Delayed initiation but not gradual advancement of enteral formula feeding reduces the incidence of necrotizing enterocolitis in preterm pigs	Early abrupt group: gavaged with formula every 3 hours starting day of life 2, late abrupt: gavaged with formula every 3 hours starting day of life 5	103	Early versus late initiation and abrupt versus gradual advancement of enteral feeding of an intact versus hydrolyzed protein formula on incidence of necrotizing enterocolitis
Burrin <i>et al</i> <sup>32</sup>	Near-infrared spectroscopy measurement of abdominal tissue oxygenation is a useful indicator of intestinal blood flow and necrotizing enterocolitis in premature piglets	Piglets gavaged with cow's milk formula every 3 hours after 72 hours of life. Piglets attached to abdominal near-infrared spectroscopy probe for continuous monitoring	105–107	Developing a non-invasive method for early detection for necrotizing enterocolitis using near-infrared spectroscopy probes
Sun <i>et al</i> <sup>33</sup>	Necrotizing enterocolitis is associated with acute brain responses in preterm pigs	Gavaged with increasing doses of human donor milk for 8 days, starting day of life 0	106	The effects of necrotizing enterocolitis lesions on the neuro development of the hippocampus
Jensen <i>et al</i> <sup>16</sup>	Similar efficacy of human banked milk and bovine colostrum to decrease incidence of necrotizing enterocolitis in preterm piglets	Piglets gavaged with either human donor milk, bovine colostrum, and infant formula every 2 hours, starting day of life 3	105	Developing supplemental replacements for formula
Bjornvad <i>et al</i> <sup>34</sup>	Enteral feeding induces diet-dependent mucosal dysfunction, bacterial proliferation, and necrotizing enterocolitis in preterm pigs	Piglets gavaged with either porcine colostrum, bovine colostrum, or infant formula every 3 hours, starting day of life 0 for 20–40 hours	105–108	Effects of formula on the incidence of necrotizing enterocolitis
Buddington <i>et al</i> <sup>35</sup>	The risk of necrotizing enterocolitis differs among preterm pigs fed formulas with either lactose or maltodextrin	Piglets gavaged with colostrum, lactose, and maltodextrin	105	Removing maltodextrin in formula to lower incidence of necrotizing enterocolitis
Good <i>et al</i> <sup>36</sup>	<i>Lactobacillus rhamnoses</i> HN001 decreases the severity of necrotizing enterocolitis in neonatal mice and preterm piglets: evidence in mice for a role of TLR9	Piglets were gavaged with 15 mL/kg of formula every 3 hours for 4 days containing <i>Lactobacillus rhamnoses</i> DNA or live <i>Lactobacillus rhamnoses</i>	105–108	Potential of <i>Lactobacillus rhamnoses</i> in lowering the incidence of necrotizing enterocolitis

### Rabbit model

Gurien *et al*<sup>20</sup> evaluated the protective role of *Lactococcus lactis* against NEC using a rabbit model of NEC. In their description, 2-day-old preterm New Zealand white rabbit pups were gavage fed and given CS in addition to oral ranitidine and indomethacin after performing an anal blockage using adhesive tape. The anal blockage

was intended to simulate the poor intestinal function and dysmotility of preterm neonates. Bozeman *et al*<sup>21</sup> provided evidence that in this model the incidence of NEC increased with the duration and completeness of the anal blockage. Their model is similar to that in research of Gurien *et al*<sup>20</sup> and utilized premature C-section delivered rabbits.

To examine the clinical applicability of Doppler sonography in a rabbit model of NEC, Choi *et al.*<sup>22</sup> induced experimental NEC using a combination of endotoxin, hypoxia, and cold stress.

Reduced oxygen to the small intestine induces mucosal injury and may contribute to neonatal NEC. Since little is known about the relationship between fetal hypoxia and GI motility, Sase *et al.*<sup>23</sup> assessed the potential effects using a rabbit model. These experiments gave insight into the GI motility, proving that it was significantly decreased by maternal hypoxia during the last third of gestation. It also highlighted that hypoxia-induced reduction in GI motility might contribute to neonatal NEC.

Miller *et al.*<sup>24</sup> reproduced NEC in rabbits by means of transmural injection of intestinal loops with an acidified solution of casein and calcium gluconate, mimicking the luminal milieu of afflicted neonates. Using this model, they demonstrated that NEC could be established by a luminal insult that caused local generation of free

radicals and exaggerated release of prostaglandins and leukotrienes.<sup>24</sup>

Predominant enterobacteria from infants with NEC was examined for detecting unusual ability of fermenting lactose by Carbonaro *et al.*<sup>25</sup> One such isolate, a *Klebsiella pneumoniae* strain, partially induced lactose operon expression in tryptone containing media, and was pathogenic in a rabbit ileal loop model for NEC. The results of these studies on the intraluminal biochemistry of infants with NEC support the hypothesis that an increased ability for lactose fermentation may be a bacterial pathogenic trait with respect to NEC.<sup>25</sup> Many investigators have used variations of this model as outlined in table 2.

### Quail model

The quail model has been used in studies evaluating the role of bacteria in NEC. Development of cecal NEC-like lesions in quails requires the combination of two major factors that are present in preterm human infants:

**Table 2** A summary of the studies using the rabbit model

Author	Title	NEC protocol	Gestational age (days)	Area of study
Gupta <i>et al.</i> <sup>37</sup>	Occurrence of necrotizing enterocolitis may be dependent on patterns of bacterial adherence and intestinal colonization: studies in caco-2 tissue culture and weanling rabbit model	Adherent <i>E. coli</i> isolates, from necrotizing enterocolitis cases, were able to cause pathologic changes typical of necrotizing enterocolitis in a weanling rabbit ileal loop model	No age specification	The role of intestinal microbial ecology in the pathophysiology of necrotizing enterocolitis
Garston <i>et al.</i> <sup>38</sup>	The role of intraluminal tension and pH in the development of necrotizing enterocolitis: an animal model	A closed intestinal loop, 20 cm distal to the cecum was created. A solution containing fatty acids at pH 3 or 5 were infused into the loop at a pressure of 10 or 40 mm Hg	6- to 8-weeks old	Relative effects of pH and intraluminal tension on colonic mucosa
Bozeman <i>et al.</i> <sup>21</sup>	An animal model of necrotizing enterocolitis in preterm rabbits	Pups were intestinally blocked to stimulate abdominal distention. Pups were then bolus fed 120 kcal/kg/day with kitten milk replacer mixed with <i>Enterobacter cloacae</i>	2 days premature	Developing a non-invasive, premature animal model that closely mimics clinical conditions
Mark <i>et al.</i> <sup>24</sup>	Super oxide dismutase prevents damage and attenuates eicosanoid release in a rabbit model of necrotizing enterocolitis	Transmural injection of intestinal loops with an acidified solution of casein and calcium gluconate, mimicking the luminal milieu of afflicted neonates	No age specification	Localizing eicosanoids released from necrotic and healthy intestine in response to proinflammatory agents
Hwa-Young <i>et al.</i> <sup>39</sup>	Bowel sonography in sepsis with pathological correlation: an experimental study	Animals received 1 mg/kg of <i>E. coli</i> O55-B5 lipopolysaccharide to induce sepsis. Bowel wall thickness and injury were evaluated.	1-week old	Facilitating early detection of intestinal injury in septic infants with necrotizing enterocolitis
Erdener <i>et al.</i> <sup>40</sup>	Pentoxifylline does not prevent hypoxia/reoxygenation-induced necrotizing enterocolitis	Pups were pretreated with pentoxifylline 15 min prior to being exposed to 5 min hypoxic/reoxygenation episodes 3 times a day for 3 days	1-day old	Investigating the protective effects of pentoxifylline in necrotizing enterocolitis

lactose in diet and colonization by lactose-fermenting bacteria. Experimental infection of germ-free quails with either *Clostridium butyricum* (CB) strains (monobiotic quails) or fecal specimens (polybiotic quails), both originating from preterm infants with NEC, reproduced many aspects of the pathology of NEC, such as thickening of the cecal wall with gas cysts, hemorrhagic ulcerations, necrotic areas, and intestinal pneumatosis.<sup>26</sup> This model allowed the study of the role of bacterial strains involved in NEC in gnotobiotic quails as the experimental model. This model looked at Clostridia species, and demonstrated that this species were strongly implicated in NEC through excessive production of butyric acid as a result of colonic lactose fermentation.<sup>26</sup> This model also served to understand the protective role of bifidobacteria through a decrease in clostridial populations and in butyric acid. Oligofructose dietary supplementation was shown to

enhance the effect with an increase in the bifidobacterial level and consequently a greater decrease in clostridia, as described in table 3.<sup>27</sup>

In addition, inoculation of germ-free quails with bacteria associated with NEC have improved our understanding of the inducible nitric oxide synthase (iNOS) pathway activation prior to the development of macroscopic lesions in NEC.<sup>28</sup>

### Nonhuman primate model

Though not as common, nonhuman primates have been utilized as models to examine the disease progression of NEC. Nonhuman primate models most closely resemble human disease states because of their homology in several organ systems, specifically seen in regard to their gastrointestinal development.<sup>13</sup>

**Table 3** A summary of the studies using the quail model

Author	Title	NEC protocol	Gestational age (days)	Area of study
Dupriet <i>et al</i> <sup>26</sup>	Evidence for clostridial implication in necrotizing enterocolitis through bacterial fermentation in a gnotobiotic quail model	2-week old, germ-free quail were orally inoculated with bacterial strains: <i>K. pneumoniae</i> , <i>C. perfringens</i> , <i>C. difficile</i> , <i>C. paraputrificum</i> , <i>C. butyricum</i> . Quails were sacrificed after 3 weeks	2-week old	To analyze the role of <i>K. pneumoniae</i> , <i>C. perfringens</i> , <i>C. difficile</i> , <i>C. paraputrificum</i> in the development of necrotizing enterocolitis
Dupriet <i>et al</i> <sup>28</sup>	Short-chain fatty acids (SCFAs) and polyamines in the pathogenesis of necrotizing enterocolitis: Kinetics aspects in gnotobiotic Quails	3-day old quail were orally inoculated with <i>C. butyricum</i> . Quails were sacrificed at day 7, 12, 18, and 24	3-day old	Investigating if short-chain fatty acids and polyamines are primary elements in the pathogenesis of necrotizing enterocolitis like lesions
Butel <i>et al</i> <sup>41</sup>	Oligofructose and experimental model of neonatal necrotizing enterocolitis	Quails were fed a 6% lactose diet that included lactose-fermenting bacteria such as <i>C. butyricum</i> or fecal flora specimens	No age specification	Assessing how low endogenous lactase activity, lactose in diet and lactose-fermenting bacteria onset intestinal lesions
Butel <i>et al</i> <sup>42</sup>	Clostridial pathogenicity in experimental necrotizing enterocolitis in gnotobiotic quail and protect role of bifidobacteria	Quails were fed a lactose diet and orally inoculated either <i>C. butyricum</i> or whole necrotizing enterocolitis flora (including three clostridial species <i>C. butyricum</i> , <i>C. perfringens</i> , <i>C. difficile</i> —each from premature infants suffering from necrotizing enterocolitis) or Bifidobacterium strain	2-week old	Determining the involvement of clostridia in the development of necrotizing enterocolitis. As well as determining the protective role of bifidobacteria in necrotizing enterocolitis
Bouseboua <i>et al</i> <sup>43</sup>	Experimental cecitis in gnotobiotic quails monoassociated with clostridium butyricum strains isolated from patients with neonatal necrotizing enterocolitis and from healthy newborns	Animals were initially fed a commercial diet ad libitum then transitioned into a lactose-based diet after day 3 of life. At day 13, quails were split and given <i>C. butyricum</i> bacterial strains were obtained from sick premature babies	13-day old	Determining the involvement of clostridia strains in the development of necrotizing enterocolitis

**Table 4** Challenges and limitations of the necrotizing enterocolitis animal models

Models	Rat	Mouse	Piglet	Rabbit	Quail	Nonhuman primate
Advantages	Low cost Preterm viability Larger size High resilience to stressors	Transgenic lines available Low cost High reproductive rate Short lifecycle	Larger size Repeated tissue sampling Physiology and anatomy closely resembles humans Ability for invasive monitoring	Larger size	Low cost Germ-free quail availability	Larger size Physiology and anatomy closely resembles humans Ability for invasive monitoring High degree of homology at DNA level
Disadvantages	Lack of transgenic lines Lack of biomolecular reagents Resilience to stress disadvantageous to NEC High endotoxin and bacterial contamination tolerance	Difficulty gavage feeding—small size Low preterm viability	Costly model Limited analytic tools—antibodies The model creates global intestinal injury including the stomach	Costly model Lack of transgenic lines	Lack of transgenic lines Model not commonly used	Extremely expensive Longer life span Long breeding period Model rarely used

NEC, necrotizing enterocolitis.

Namachivayam *et al.*<sup>29</sup> described a NEC model using premature baboons, delivered via C-section at 125 days gestation, which was equivalent to a 27-week gestation human. The baboons were treated with mechanical ventilation, antibiotics, enteral feeds, and other necessary treatments similar to those provided to a neonate with sepsis. In this model, intravenous fluids were initiated at birth through a central line and parenteral nutrition was started at 24 hours. Enteral feeds were initiated after day 5 using a primate or human infant formula. Outcomes were compared that showed a difference in the transforming growth factor-beta expression in premature intestines during NEC.<sup>29</sup>

However, the main drawback with the use of nonhuman primates is the prohibitive cost in some instances. The expensive costs for housing and a longer lifespan and breeding period make these models less commonly observed, as explained in [table 4](#).

## CONCLUSION

Animal models of NEC have been around for a long time and they are of significant value to help us better understand the pathogenesis of NEC. Without them, our current understanding of pathways that lead to NEC would be very limited. These models have helped identify a number of promising and novel strategies such as probiotics, growth factors, stem cells, and breast milk to reduce the burden of NEC. There are significant variabilities to each and every model whether it is related to the animal size, genetic variability, or the protocol used to generate the model. However, they each have distinct

advantages and drawbacks. As there is no “gold standard” animal model for the study of NEC, our paper highlights the importance of using animal models that are appropriate to answer the specific scientific question. We found that the most commonly used model remained the rat pup one, mainly due to its low cost, larger size of the pups and ease to learn and reproduce. As many authors have emphasized, there is a significant need for research and further development and funding in the field of NEC.

**Contributors** RA is responsible for the concept, design, and critical editing of the manuscript. MYS and PGV are responsible drafting of the manuscript and literature review. KF is responsible for the revision of the manuscript and literature review.

**Funding** The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

**Competing interests** None declared.

**Patient consent for publication** Not required.

**Ethics approval** It's not required for this review article.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data availability statement** No data are available. Not applicable.

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## REFERENCES

- Olson JK, Navarro JB, Allen JM, *et al.* An enhanced *Lactobacillus reuteri* biofilm formulation that increases protection against experimental necrotizing enterocolitis. *Am J Physiol Gastrointest Liver Physiol* 2018;315:G408–19.



- 2 Sodhi C, Richardson W, Gribar S, *et al.* The development of animal models for the study of necrotizing enterocolitis. *Dis Model Mech* 2008;1:94–8.
- 3 Grave GD, Nelson SA, Walker WA, *et al.* New therapies and preventive approaches for necrotizing enterocolitis: report of a research planning workshop. *Pediatr Res* 2007;62:510–4.
- 4 Barlow B, Santulli TV, Heird WC, *et al.* An experimental study of acute neonatal enterocolitis--the importance of breast milk. *J Pediatr Surg* 1974;9:587–95.
- 5 Tanner SM, Berryhill TF, Ellenburg JL, *et al.* Pathogenesis of necrotizing enterocolitis: modeling the innate immune response. *Am J Pathol* 2015;185:4–16.
- 6 Young CM, Kingma SDK, Neu J. Ischemia-Reperfusion and neonatal intestinal injury. *J Pediatr* 2011;158:e25–8.
- 7 Lu P, Sodhi CP, Jia H, *et al.* Animal models of gastrointestinal and liver diseases. animal models of necrotizing enterocolitis: pathophysiology, translational relevance, and challenges. *Am J Physiol Gastrointest Liver Physiol* 2014;306:G917–28.
- 8 Sulistyo A, Rahman A, Biouss G, *et al.* Animal models of necrotizing enterocolitis: review of the literature and state of the art. *Innov Surg Sci* 2018;3:87–92.
- 9 Ares GJ, McElroy SJ, Hunter CJ, *et al.* The science and necessity of using animal models in the study of necrotizing enterocolitis. *Semin Pediatr Surg* 2018;27:29–33.
- 10 Shultz LD, Ishikawa F, Greiner DL. Humanized mice in translational biomedical research. *Nat Rev Immunol* 2007;7:118–30.
- 11 Radulescu A, Zhang H-Y, Yu X, *et al.* Heparin-binding epidermal growth factor-like growth factor overexpression in transgenic mice increases resistance to necrotizing enterocolitis. *J Pediatr Surg* 2010;45:1933–9.
- 12 Radulescu A, Yu X, Orvets ND, *et al.* Deletion of the heparin-binding epidermal growth factor-like growth factor gene increases susceptibility to necrotizing enterocolitis. *J Pediatr Surg* 2010;45:729–34.
- 13 Puiman P, Stoll B. Animal models to study neonatal nutrition in humans. *Curr Opin Clin Nutr Metab Care* 2008;11:601–6.
- 14 Sangild PT, Siggers RH, Schmidt M, *et al.* Diet- and colonization-dependent intestinal dysfunction predisposes to necrotizing enterocolitis in preterm pigs. *Gastroenterology* 2006;130:1776–92.
- 15 Zamora IJ, Stoll B, Ethun CG, *et al.* Low abdominal NIRS values and elevated plasma intestinal fatty acid-binding protein in a premature piglet model of necrotizing enterocolitis. *PLoS One* 2015;10:e0125437–18.
- 16 Jensen ML, Sangild PT, Lykke M, *et al.* Similar efficacy of human banked milk and bovine colostrum to decrease incidence of necrotizing enterocolitis in preterm piglets. *Am J Physiol Regul Integr Comp Physiol* 2013;305:R4–12.
- 17 Cohen IT, Nelson SD, Moxley RA, *et al.* Necrotizing enterocolitis in a neonatal piglet model. *J Pediatr Surg* 1991;26:598–601.
- 18 Crissinger KD, Burney DL, Velasquez OR, *et al.* An animal model of necrotizing enterocolitis induced by infant formula and ischemia in developing piglets. *Gastroenterology* 1994;106:1215–22.
- 19 Di Lorenzo M, Bass J, Krantis A. An intraluminal model of necrotizing enterocolitis in the developing neonatal piglet. *J Pediatr Surg* 1995;30:810.
- 20 Gurien LA, Stallings-Archer K, Smith SD. Probiotic *Lactococcus lactis* decreases incidence and severity of necrotizing enterocolitis in a preterm animal model. *J Neonatal Perinatal Med* 2018;11:65–9.
- 21 Bozeman AP, Dassinger MS, Birusingh RJ, *et al.* An animal model of necrotizing enterocolitis (NEC) in preterm rabbits. *Fetal Pediatr Pathol* 2013;32:113–22.
- 22 Choi Y-H, Kim I-O, Cheon J-E, *et al.* Doppler sonographic findings in an experimental rabbit model of necrotizing enterocolitis. *J Ultrasound Med* 2010;29:379–86.
- 23 Sase M, Lee JJ, Ross MG, *et al.* Effect of hypoxia on fetal rabbit gastrointestinal motility. *J Surg Res* 2001;99:347–51.
- 24 Miller MJ, McNeill H, Mullane KM, *et al.* SOD prevents damage and attenuates eicosanoid release in a rabbit model of necrotizing enterocolitis. *Am J Physiol Gastrointest Liver Physiol* 1988;255:G556–65.
- 25 Carbonaro CA, Clark DA, Elseviers D. A bacterial pathogenicity determinant associated with necrotizing enterocolitis. *Microb Pathog* 1988;5:427–36.
- 26 Waligora-Dupriet A-J, Dugay A, Auzeil N, *et al.* Evidence for clostridial implication in necrotizing enterocolitis through bacterial fermentation in a gnotobiotic quail model. *Pediatr Res* 2005;58:629–35.
- 27 Butel M-J, Waligora-Dupriet A-J, Szyliet O. Oligofructose and experimental model of neonatal necrotising enterocolitis. *Br J Nutr* 2002;87 (Suppl 2):S213–9.
- 28 Waligora-Dupriet A-J, Dugay A, Auzeil N, *et al.* Short-Chain fatty acids and polyamines in the pathogenesis of necrotizing enterocolitis: kinetics aspects in gnotobiotic quails. *Anaerobe* 2009;15:138–44.
- 29 Namachivayam K, Blanco CL, MohanKumar K, *et al.* Smad7 inhibits autocrine expression of TGF- $\beta$ 2 in intestinal epithelial cells in baboon necrotizing enterocolitis. *Am J Physiol Gastrointest Liver Physiol* 2013;304:G167–80.
- 30 Robinson JL, Smith VA, Stoll B, *et al.* Prematurity reduces citrulline-arginine-nitric oxide production and precedes the onset of necrotizing enterocolitis in piglets. *Am J Physiol Gastrointest Liver Physiol* 2018;315:G638–49.
- 31 Ghoneim N, Bauchart-Thevret C, Oosterloo B, *et al.* Delayed initiation but not gradual advancement of enteral formula feeding reduces the incidence of necrotizing enterocolitis (NEC) in preterm pigs. *PLoS One* 2014;9:106888.
- 32 Gay AN, Lazar DA, Stoll B, *et al.* Near-Infrared spectroscopy measurement of abdominal tissue oxygenation is a useful indicator of intestinal blood flow and necrotizing enterocolitis in premature piglets. *J Pediatr Surg* 2011;46:1034–40.
- 33 Sun J, Pan X, Christiansen LI, *et al.* Necrotizing enterocolitis is associated with acute brain responses in preterm pigs. *J Neuroinflammation* 2018;15:180.
- 34 Bjornvad CR, Thymann T, Deutz NE, *et al.* Enteral feeding induces diet-dependent mucosal dysfunction, bacterial proliferation, and necrotizing enterocolitis in preterm pigs on parenteral nutrition. *Am J Physiol Gastrointest Liver Physiol* 2008;295:G1092–103.
- 35 Buddington RK, Davis SL, Buddington KK. The risk of necrotizing enterocolitis differs among preterm pigs fed formulas with either lactose or maltodextrin. *J Pediatr Gastroenterol Nutr* 2018;66:e61–6.
- 36 Good M, Sodhi CP, Ozolek JA, *et al.* *Lactobacillus rhamnosus* HN001 decreases the severity of necrotizing enterocolitis in neonatal mice and preterm piglets: evidence in mice for a role of TLR9. *Am J Physiol Gastrointest Liver Physiol* 2014;306:G1021–32.
- 37 Panigrahi P, Gupta S, Gewolb IH, *et al.* Occurrence of necrotizing enterocolitis may be dependent on patterns of bacterial adherence and intestinal colonization: studies in Caco-2 tissue culture and weanling rabbit models. *Pediatr Res* 1994;36:115–21.
- 38 Garstin WI, Kenny BD, McAneaney D, *et al.* The role of intraluminal tension and pH in the development of necrotizing enterocolitis: an animal model. *J Pediatr Surg* 1987;22:205–7.
- 39 Kim H-Y, Kim I-O, Kim WS, *et al.* Bowel sonography in sepsis with pathological correlation: an experimental study. *Pediatr Radiol* 2011;41:237–43.
- 40 Erdener D, Bakirtas F, Alkanat M, *et al.* Pentoxifylline does not prevent hypoxia/reoxygenation-induced necrotizing enterocolitis. *Neonatology* 2004;86:29–33.
- 41 Butel M-J, Waligora-Dupriet A-J, Szyliet O. Oligofructose and experimental model of neonatal necrotising enterocolitis. *Br J Nutr* 2002;87(Suppl 2):S213–9.
- 42 Butel MJ, Roland N, Hibert A, *et al.* Clostridial pathogenicity in experimental necrotising enterocolitis in gnotobiotic quails and protective role of bifidobacteria. *J Med Microbiol* 1998;47:391–9.
- 43 Cassir N, Benamar S, Khalil JB, *et al.* *Clostridium butyricum* Strains and Dysbiosis Linked to Necrotizing Enterocolitis in Preterm Neonates. *Clin Infect Dis* 2015;61:1107–15.