

Published in final edited form as:

Microbes Infect. 2012 October ; 14(12): 1009–1016. doi:10.1016/j.micinf.2012.06.003.

Animal models to study the pathogenesis of enterotoxigenic *Clostridium perfringens* infections

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Abstract

Rabbits, mice, rats, non-human primates, sheep and cattle have been used to study the effect of *Clostridium perfringens* enterotoxin (CPE). CPE produces mostly necrosis of the small intestinal epithelium along with fluid accumulation in rabbits and mice. In the latter, CPE can bind to internal organs such as the liver, which induces lethal potassium levels in blood.

Keywords

animal models; calf; *Clostridium perfringens*; enterotoxin; lamb; mouse; non-human primate; rabbit; rat

1. Introduction

Clostridium perfringens, a Gram-positive spore forming anaerobic bacterium, is a prolific toxin producer [1]. At least 17 different *C. perfringens* toxins are reported in the literature, but individual isolates only produce some of these toxins [1]. This variability in toxin production provides the basis for a commonly-used toxin typing classification system where individual strains are assigned to one of five types based upon their production of four typing toxins, i.e., alpha beta, epsilon and iota toxins. All isolates produce alpha toxin, but type B strains also produce beta and epsilon toxins, type C isolates also produce beta toxin, type D strains also produce epsilon toxin and type E isolates also produce iota toxin.

Besides producing one or more of the typing toxins, some *C. perfringens* strains produce additional toxins. One of those non-typing toxins is *C. perfringens* enterotoxin (CPE), which can be produced by all types, except type B. Although only ~5% of type A strains produce this enterotoxin [2], those CPE-positive type A strains have exceptional biomedical importance. They cause *C. perfringens* type A food poisoning, which is the second most common bacterial illness in the USA, where about 1 million cases/year occur [3]. In addition

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these strains cause ~3-15% of all cases of human non-food borne human gastrointestinal diseases, including antibiotic-associated diarrhea and sporadic diarrhea [4]. Molecular Koch's postulate analyses strongly support CPE production as the essential factor necessary for CPE-positive type A isolates to cause intestinal pathology in animal models [5].

CPE is encoded by a gene (*cpe*) that can be located on the chromosome or on large plasmids [6]. When located on large plasmids it is associated with a putative transposon, named Tn5565, which is flanked by IS 1470 insertion sequences [7]. In type A isolates, these are two major families of *cpe* plasmids [8]. The 75.3 kb pCPF5603 *cpe* plasmid family has an IS 1151 insertion sequence near *cpe* and also carries the gene (*cpb2*) encoding beta2 toxin. In contrast, the 70.5 kb pCPF4969 *cpe* plasmid family has an IS 1470-like sequence near *cpe*; it lacks the *cpb2* gene but encodes a bacteriocin gene cluster. Both the pCPF5603 and pCPF4969 *cpe* plasmids carry the *tcp* locus [8], which has been shown to mediate conjugative transfer for some other *C. perfringens* plasmids [9]. The *tcp* region likely also mediates conjugative transfer of the *cpe* plasmids, since mixed mating studies have shown conjugative transfer of pCPF4969 [10].

CPE is a single 319 amino acid polypeptide of ~35 kDa with a unique sequence [11], except for some limited homology (of still unknown significance) with a nonneurotoxic protein made by *C. botulinum* [6]. The structure of CPE was recently solved by two groups [12,13], which revealed this toxin belongs to the aerolysin family of pore-forming toxins. Notably, this family also includes two other clostridial toxins, i.e., *C. perfringens* epsilon toxin and *C. septicum* alpha toxin. Structurally, the CPE protein consists of two domains, including a C-terminal domain that mediates receptor binding [14] and an N-terminal domain that is responsible for post binding cytotoxic functions [15,16,17], including oligomerization and membrane insertion/pore formation as described below. The D48 and I51 residues of CPE are particularly important for formation of the CPE oligomers described below [16,17]. The transmembrane domain of CPE responsible for mediating insertion of the toxin during pore formation (see below) appears to involve an alpha helical region named TM1, which spans from CPE amino acids 81 to 106 [17].

The first step in CPE action is binding of the toxin to certain members of the claudin family of tight junction proteins [18,19,20]. Claudins are ~22 kDa proteins whose structure consists of four transmembrane domains, two extracellular loops (ECLs) and a cytoplasmic tail [21]. Binding of CPE to claudin receptors involves interactions between tyrosine residues in the 30 C-terminal amino acids of CPE and ECL-2 of receptor claudins [14,22,23,21]. Interestingly, not all claudins can serve as CPE receptors [18,22,21]; recent studies have identified an Asn located in the middle of ECL-2 as being critical for CPE binding [22,23,21]. CPE receptor claudins are present on many epithelial cells, including those found in the intestines [24].

Once bound to a claudin receptor, the toxin becomes localized in a still poorly characterized small complex [25]. This SDS-sensitive complex is ~90 kDa in size and apparently contains both claudin receptors and nonreceptor claudins [26,25]. Small complex formation will occur at 4°C but CPE action proceeds no further at low temperature [27].

At 37°C, CPE in small complexes apparently oligomerizes into an SDS-resistant complex named CH-1 [26]. CH-1 is ~450 kDa in size and contains, besides 6 copies of CPE, both receptor and nonreceptor claudins. Initial formation of CH-1 occurs on the membrane surface; however at 37°C, the CH-1 prepore rapidly inserts into membranes to form an active pore [17].

CPE pore formation results initially in a rapid breakdown of membrane permeability properties for small molecules of <200 Daltons [28]. Of particular importance, formation of

the CPE pore causes a Ca^{2+} influx into CPE-treated cells that activates cell death pathways [29,30]. At low CPE doses there is a modest Ca^{2+} influx that triggers a classical caspase-3 cell death pathway. In contrast, high CPE doses induce a massive Ca^{2+} influx that causes cell death by oncosis.

Formation of the CPE pore also results in morphologic damage that exposes the basolateral surface of sensitive host cells [31]. This effect results in formation of CH2, a second SDS-resistant CPE complex of ~600 kDa [26,32]. CH-2 contains, at minimum, 6 copies of CPE, receptor and nonreceptor claudins and a second tight junction protein named occludin. The presence of occludin in CH-2 is most likely due to occludin:claudin interactions since CPE does not bind directly to occludin [32].

CPE-induced cell death fosters the development of microscopic damage in the intestines. CPE-induced microscopic damage includes villus blunting, epithelial desquamation and epithelial necrosis [24]. This damage appears to trigger the fluid and electrolyte fluxes responsible for diarrhea based upon the fact that only CPE doses causing microscopic damage are capable of causing fluid/electrolyte changes in rabbit ileal loops [33]. In addition, the development of fluid and electrolyte alterations in CPE-challenged rabbit ileal loops correlates closely with the onset of microscopic damage [34].

Several animal models have been used extensively to study the intestinal and systemic activity of CPE [35,36,37,38,39,34]. We review here the information published on animal models to study the pathogenesis of CPE-positive *C. perfringens* type A infections.

2. Rabbit models

Rabbit intestinal loops have been used for many years to study the effects of CPE *in vivo* [40,36,41,24]. The effect of CPE has been studied mostly in the small intestine (jejunum and ileum) of rabbits. Purified CPE caused net secretion of fluid and electrolytes, together with microscopic damage in the rabbit ileum and jejunum, but not in the duodenum [38,37,42]. Thus it appears that, within the rabbit small intestine, the sensitivity to the action of CPE increases moving aborally from the proximal duodenum, with the terminal ileum being the most responsive [38].

Microscopic changes observed in the small intestinal loops of rabbits treated with CPE are dose-dependent and they consist of necrosis of superficial epithelium and lamina propria, villus blunting and fusion, mucosal and submucosal edema and hemorrhage [36,41,24] (Fig. 1). Initial CPE-induced intestinal damage in rabbits begins at villus tips in small intestinal loops [36,34]. This was also confirmed by electron microscopy studies, in which CPE enterocyte damage and heavy cellular blebbing were found predominantly at villus tips of CPE-treated rabbit ileum [42]. Immunolocalization results on rabbit intestinal loops indicated that more CPE binds to villus tips than elsewhere on intestinal villi, which coincides with greater CPE receptor (Claudin-4) density in villus tips [24]. The correlation between claudin-4 density and CPE binding at villus tips also suggests this claudin, which is a known CPE receptor [19,20,26], plays an important role in intestinal CPE binding. However, since certain other claudins can also bind CPE *in vitro* [18], this correlation does not preclude the possibility that other claudins also contribute to CPE binding *in vivo*.

Although several studies [43,34] have supported a linkage between CPE-induced cytotoxicity, intestinal microscopic damage and the onset of intestinal fluid transport alterations, more recent studies [19,20,44,45] reported that 4-6 h treatment with noncytotoxic, but binding-capable, C-terminal CPE fragments can induce claudin internalization and alter transepithelial resistance in cultured cells. In addition, those C-terminal CPE fragments were unable to induce microscopic damage in the ileum and alter

transepithelial resistance and dextran absorption in ileal loops [44]. Those observations could have indicated that C-terminal CPE fragments can, independently of cytotoxicity or microscopic changes, induce paracellular permeability changes that contribute to intestinal fluid transport changes. However, no fluid accumulation was measured in the intestine in the study referred to [44]. To address this, the *in vivo* effects of CPE versus two rCPE variants that are binding-capable, yet non-cytotoxic *in vitro*; i.e. rCPE₁₆₈₋₃₁₉ and rCPE D48A [46,16] were compared [24]. In that study, CPE (which is cytotoxic) caused both intestinal fluid accumulation and microscopic damage in rabbit ileal loops [24]. However, neither of those effects was observed when small intestinal loops were treated with similar pathophysiologically-relevant (up to 100 µg/ml) doses of the rCPE₁₆₈₋₃₁₉ or rCPE D48A non-cytotoxic variants of CPE, even though both those rCPE variants were demonstrated to bind to the small intestinal epithelium (Figs. 2 and 3). These findings support cytotoxic activity as being important for the development of both the microscopic and intestinal fluid transport effects of CPE in this model.

Results of the study referred to above [24] suggested the following model for CPE enterotoxicity. Once produced in the small intestine during sporulation, CPE first binds to certain claudin receptors at the villus tips because cells in that region have more abundant and probably more exposed (due to normal cellular extrusion processes) enterotoxin receptors. This CPE binding results in small complex formation, followed by formation of hexameric CH-1 pores on the enterocyte surface. These prepore CH-1 complexes then penetrate the plasma membrane to form a pore that permits a Ca²⁺ influx to drive death of villus tip cells, which are already primed for apoptosis. The resultant death of villus tip enterocytes exposes the basolateral membranes of adjacent enterocytes, allowing the process to repeat such that epithelial desquamation developing at the villus tips impairs intestinal absorption. As the process continues, intestinal villi become increasingly blunted and denuded, causing net fluid secretion in the small intestine. These effects then manifest clinically as diarrhea and abdominal cramps.

Information about the action of CPE in the colon is scant and sometimes contradictory. Because the colon is involved primarily in fluid reabsorption, it is thought that the diarrhea characteristic of the human disease could be caused in part by inhibition of fluid reabsorption or by enhancement of fluid secretion by the colon [38]. In a study of the effect of CPE on small and large human intestine *ex-vivo*, the toxin bound to ileal epithelium and induces microscopical damage concurrently with reduced short-circuit current, transepithelial resistance and net water resorption [47]. In the same study, it was also found that CPE binds to the human colon but causes only slight morphological and transport changes. Rabbit colonic loops inoculated with CPE had no apparent response to the CPE in the transport of fluid and electrolytes, or microscopic damage [38]. This seems to be in disagreement with human cases of fatal necrotizing colitis that occurred in people consuming turkey contaminated with enterotoxigenic *C. perfringens* [48]. However, colonic epithelial cells bound ¹²⁵I-labeled CPE at levels even greater than those observed in the ileum. Based on these results, Mc Donel and Demers [38] concluded that although binding of CPE is necessary for its biological activity, biological activity does not necessarily follow binding to specific receptors.

Rabbits have also been used to study the binding of CPE to tissues other than the intestine [35]. ¹²⁵I-labeled CPE was incubated with homogenates of rabbit liver, kidney and brain. CPE bound in a specific manner to tissue homogenates from liver and kidney but not brain. The amount of CPE molecules that bound was similar in intestine, liver and kidney. This binding was demonstrated to be heat-labile, as the binding ability was lost after CPE was heated for 10 minutes at 60°C [35]. These results, coupled with similar additional studies in mice [49], suggest that CPE absorbed from the intestine can be responsible for systemic

alterations, which may help explain the lethality observed in some cases of experimental animals and human patients [48].

3-Mouse models

Mice have been used, although less extensively than rabbits, to study the pathogenesis of CPE intoxication [50,49]. In mouse models, both the intestinal and systemic effects of CPE were studied.

Sakaguchi et al [51] found that the lethal CPE dose to a mouse by i.v. injection is about 2 µg. Death of animals usually occurred within 30 minutes after i.v. injection of CPE and animals that survived for 30 minutes rarely died later [51,52]. When anesthetized mice received an i.v. inoculation with purified CPE, a 50 µg/kg dose was lethal [53]. Death in these mice was preceded by changes of the ECG, rapid fall of blood pressure and transient hyperpnea followed by respiratory depression. Elevated levels of plasma potassium were detected in these mice, suggesting that the ECG alterations and death were produced by hyperkalemia. This was supported by the fact that even large doses of CPE (up to 100 µg) failed to produce significant cardiotoxicity on the isolated heart.

Experimental inoculation of CPE i.v. [53] provided valuable information on the pathogenesis of the systemic effects of CPE. However, i.v. administration does not closely mimic natural CPE action, where CPE is produced in the intestines. To overcome this problem, intestinal loop models were developed in mice [50,49]. When CPE was inoculated into ligated small intestinal loops of mice, similar intestinal effects to those seen in rabbits were observed [50,49]. CPE caused marked fluid accumulation; the effect being proportional to the applied CPE dose and occurring as early as 10 minutes after toxin inoculation [50]. This effect, however, was arrested by washing the loop with saline or by injection of anti-CPE antibodies into the loop as late as 30 minutes after inoculation of CPE. Based on these results, the authors suggested that CPE is neither bound firmly to the mucosal membrane nor does it permeate into the cells of the intestinal wall [50]. These results are in contrast with the irreversible nature of CPE binding to intestinal cells observed in several other studies [35]. This discrepancy might suggest that the rapid onset of fluid accumulation observed in one mouse intestinal loop study [50] involved the presence of another factor in addition to CPE.

Mouse intestinal loop models have also been used to study the systemic effects of CPE *in vivo* [49], something which cannot be evaluated in rabbit intestinal loops mainly because, in rabbit studies, multiple intestinal loops are typically constructed in a single animal to test different inocula. In addition, use of multiple loops in one rabbit is not conducive to time course studies since the rabbit is euthanized when the first time-point is reached. In the mentioned study [49], significant lethality occurred when mouse intestinal loops were treated with 100 or 200 µg CPE doses for several hours (Fig. 4). Those CPE doses rarely cause lethality in rabbit small intestinal loop models, even after 6 h of incubation. It was then suggested that CPE absorbed from the intestine into the circulation could have been responsible for the lethality observed with the mouse intestinal loop model. This suggestion was based on the fact that seroprotection against CPE-induced lethality was observed in the mouse intestinal loop model after i.v. injection of CPE antibody, and the presence of CPE was detected in sera of these mice. The difference in the size of mice and rabbits (and therefore, a difference in doses per Kg of body weight) was also suggested as a possible explanation for the difference in response between rabbits and mice since similar doses were used in both species [49].

CPE treated mouse intestinal loops showed dose- and time-dependent intestinal histopathologic damage mimicking that observed in rabbit ileal loops, thus validating the

usefulness of mouse intestinal loops for studying CPE action. However, no luminal fluid accumulation was observed in the intestinal loops of CPE-treated mice, probably due to the short (up to 4 h) experimental challenge of mice used in the model [49], compared to the 6 or more h incubation period usually used in rabbits. Experiments using longer treatment times are required to clarify this issue.

When potassium levels in sera were measured in mice whose intestinal loops were treated for 2 h with 200 μg of CPE, a lethal (~ 14 mM) concentration of potassium was detected (Fig. 5). The mouse lethality results in the intestinal loop model described by Caserta et al [49], appears to reproduce results by Sakaguchi et al [51] who detected hyperkalemia in mice inoculated i.v. with CPE. These results may also help to explain the unusually high lethality associated with the recent *C. perfringens* type A food poisoning outbreaks in mental institutions [48]. Specifically, the study by Caserta et al [49] has found that CPE can be absorbed from the intestines into the circulation and this absorbed CPE can have lethal consequences. The atypical lethality associated with CPE in psychiatric patients referred to before in Oklahoma was thought to involve drugs that cause constipation as a side effect [48]. Constipation would prevent diarrhea from flushing CPE out of the intestines and thus prolong intestinal exposure to CPE (and perhaps other *C. perfringens* toxins). The mouse ligated intestinal loop model developed by Caserta et al [49] mimics somewhat the situation of constipated human patients. The mechanism by which CPE gains access to the bloodstream is currently unknown. CPE might gain access to the blood simply due to the overwhelming tissue destruction in the CPE-treated intestine, which could expose the underlying microvasculature. However, in the study by Caserta et al [49], small amounts of CPE were still detected in the blood of mice inoculated with 50 μg of CPE that had minimal intestinal damage. This observation suggests that substantial gross or microscopic damage may not be essential for CPE to gain access to the circulation.

Early studies determined that, when i.v.-injected into mice, most CPE distributes to the liver and, to a lesser extent, the kidney [54]. More recently liver and kidney homogenates were prepared from mice whose intestinal loops had been challenged with 200 μg of CPE. Analysis of those homogenates by Western blotting with an anti-CPE antibody showed that CPE had bound and formed a CH-1-like complex in both the liver and kidney. However, no CPE binding or complex formation was observed in homogenates prepared from the livers and kidneys of mice whose loops had received 50 μg of CPE. In addition, no CH-2-like complex was detected in homogenates prepared from liver or kidney of mice intestinally challenged with either the 50 or 200 μg CPE dose. The study referred to [49] also showed that CPE can bind and form complexes resembling CH-1 in internal organs, including the liver and kidney. The ability of CPE to bind to liver and kidney is consistent with the strong expression of claudins, including some known claudin CPE receptors, in these two organs [55]. As the portal vein system connects the GI tract directly to the liver, this might explain the abundance of CPE binding to this organ.

Administration of CPE by the i.v. route also caused elevated levels of blood potassium, which resulted in cardiac dysfunction and, eventually, respiratory depression and death [54,53]. This is consistent with results of CPE treatment of liver *ex vivo* shown to cause a massive potassium loss, suggesting the liver as a major source of the lethal blood potassium levels measured in mice receiving an i.v. CPE injection [54]. In mice receiving a CPE injection into their intestinal loops, both a CPE dose- and time-dependent increase in serum potassium were found and this increase exceeded lethal levels. Furthermore, these increased serum potassium levels slightly preceded the onset of lethality in mice that were intestinally-challenged with CPE, consistent with a cause and effect relationship. These results suggest that formation of a CH-1-like pore in liver may be responsible, at least in part, for the

observed potassium release and subsequent lethality observed in some intestinally-challenged mice.

Interestingly, formation of a CH-2-like complex was not observed in mouse intestinal tissue that had been CPE-treated *in vivo* or in mouse intestinal homogenates treated with CPE *in vitro*. Since Western blot analysis showed that virtually all of the CPE bound to intestinal tissue had localized in CH-1 and no CH-2 formation was detected *in vivo*, formation of a CH-1-like CPE complex appears to be sufficient for CPE intestinal action *in vivo*. Similarly, no CH-2-like complex was detected in kidney or liver tissue from mice whose intestinal loops had been CPE-treated or in homogenates of those organs treated *in vitro* with CPE.

4-Rat Models

Rats have also been used, albeit less extensively than mice and rabbits, to study the *in vivo* effects of CPE [53]. Similarly to what was observed in mice (see above), when anesthetized rats were inoculated i.v. with purified CPE, 50 µg/kg of this toxin were enough to produce lethality. Death in these rats was preceded by changes of the ECG, rapid fall of blood pressure and transient hyperpnea followed by respiratory depression. Elevated levels of plasma potassium were also detected in these rats, suggesting that the ECG alterations and death were produced by hyperkalemia. This was supported by the fact that even large doses of CPE (up to 100 µg) failed to produce significant cardiotoxicity on the isolated heart of rats. By perfusing isolated rat organs with CPE, the same authors [53] demonstrated that there was a marked increase of potassium and cytoplasmic enzymes (GPT, GOT and LDH) in the eluent of the liver (but not lungs or lower extremities), indicating that the elevated potassium found in plasma originated, at least partly, in the liver. Based on these results, Sugimoto et al [53] concluded that hyperkalemia elicited by the cytotoxic action of CPE on hepatocytes caused cardiac failure leading to death. Binding to liver cells was demonstrated to be specific on hepatocytes rather than on stromal cells and on these specific for the plasma membrane [56].

5. Other animal models

To the best of our knowledge, only one report has been published describing the use of non-human primates to study the effects of CPE. Uemura et al [57] fed cynomolgus monkeys with 5 mg of purified CPE, causing vomiting and diarrhea only when the normally low gastric pH had been neutralized by giving the animals sodium bicarbonate. Only diarrhea was consistently observed in all the monkeys dosed orally with CPE positive *C. perfringens* type A and sodium bicarbonate. No lethality was observed and no CPE antibodies were detected in any of the monkeys up to 21 days after the challenge. Based on these results, the authors concluded that no significant amount of CPE is absorbed from the intestine (it should be noted, however, that the development of serum antibodies against CPE has been detected in human food poisoning victims [58]). Unfortunately no post mortem examinations were performed on these animals and no information on gross or histopathological changes of non-human primates intoxicated with CPE is therefore available.

Niilo [59,60] reported that i.v. administration of crude cell extracts from sporulating cells of enterotoxigenic strains of *C. perfringens* type A into calves caused partial loss of villus epithelium associated with diarrhea and other systemic reactions. He also reported variable damage in ligated loops of lambs when CPE was placed in the intestinal lumen. Hauschild et al [61] reported no significant lesions in lambs challenged with cells or culture filtrates of enterotoxigenic *C. perfringens* strains.

6. Conclusions

Although significant progress has been made over the last few decades towards the understanding of CPE-positive *C. perfringens* type A infections, new insights into the pathogenesis of these infections, including the mechanism of action of CPE, have come to light using animal models. Several examples showed that CPE has different effects depending on the physiologic condition of the patient. An example of this is the necrotizing colitis presented by psychiatric patients medicated with drugs that produced constipation; an effect not usually observed in other patients. It is also important to point out that different animal species provide tools to study different aspects of CPE effects. For instance, although the rabbit intestinal loop model has been very useful to study the intestinal effect of CPE, that model could not be used to study the systemic effects of this toxin. The latter could only be at least partly understood when the new mouse model was used.

It is also worth mentioning that most *in vivo* research performed to date was done using CPE. To the best of our knowledge no animal model has been developed based on CPE-positive *C. perfringens* infections because it is difficult to obtain good levels of *in vivo* sporulation, as needed for CPE production, in small animals. Such a model would be very useful to fully understand the pathogenesis of these infections. This aspect of CPE disease might also benefit from the development of non-human primates model/s.

Acknowledgments

This work was supported by Public Health Service grant R37 AI019844-29 (BAM) from the National Institute of Allergy and Infectious Diseases. We thank Ms. S. Fitsemanu and N. Harrigan for excellent secretarial help.

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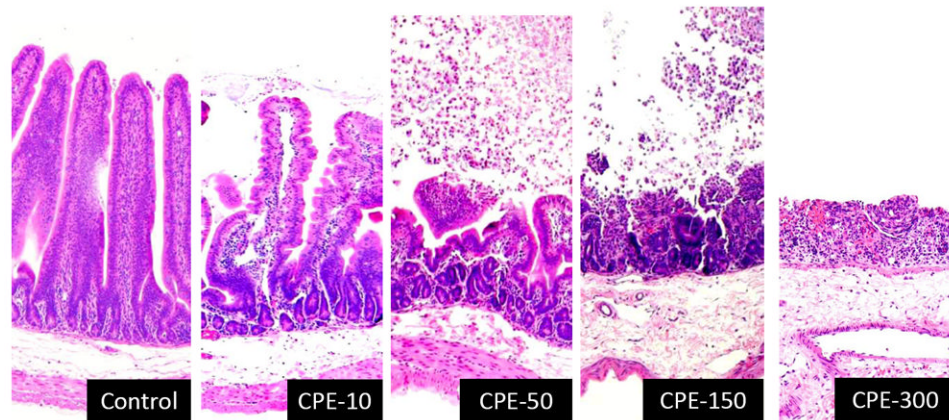


Figure 1.

Histology of rabbit small intestinal loops treated with buffer (control), 10 μg of CPE (CPE-10), 50 μg of CPE (CPE-50), 150 μg of CPE (CPE-150) or 300 μg of CPE (CPE-300). After 6 h treatment, loop tissues were formalin fixed, embedded in paraffin and processed for the production of 4 μm thick, hematoxylin and eosin stained sections. Final magnification: X200.

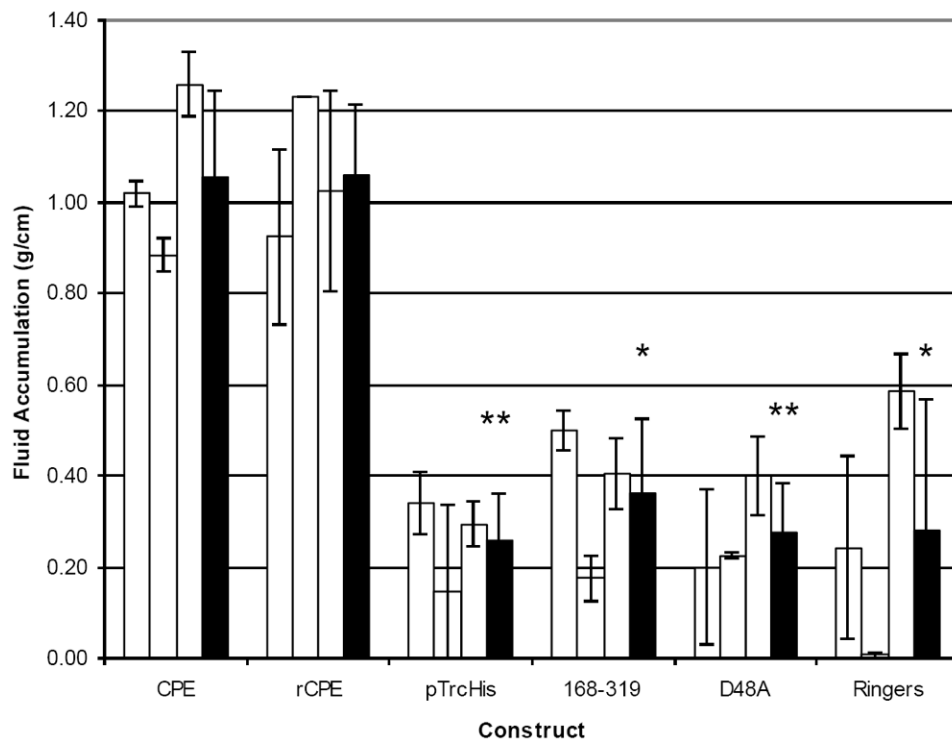


Figure 2.

Fluid accumulation in rabbit small intestinal loops inoculated with 300 μ g of CPE, rCPE, two non-cytotoxic CPE variants (168-319 and D48A) or two different buffers (negative controls) and then incubated for 6 h while rabbits were under anesthesia. At the conclusion of the experiment, loop fluid content was measured and the amount of accumulated fluid was related inversely to the length of each loop (g/cm). White bars for each inoculum preparation represent the average of duplicate ileal loop samples in a single rabbit, while black bars represent the combined average results for three rabbits. Error bars represent the standard deviation from the mean. Statistical significance of <0.005 between a construct and rCPE is indicated with a ‘***’, whereas ‘*’ similarly indicates statistical significance of <0.05 . Copyright © American Society for Microbiology, Infection and Immunity, 76, 2008, 3793-3800, DOI: 10.1128/IAI.00460-08. Used with permission.

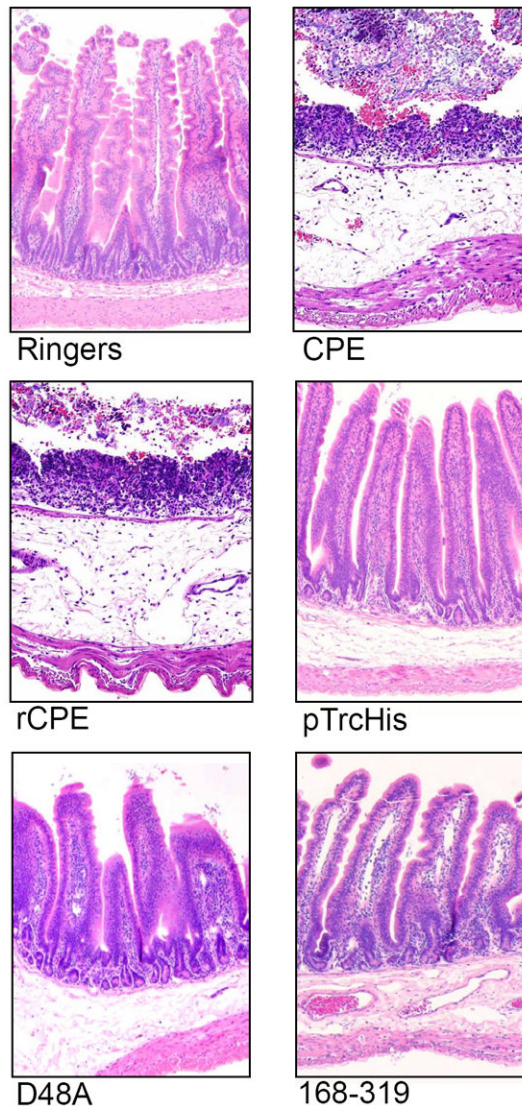


Figure 3. Histopathological changes induced by the toxin preparations described in Figure 2, in rabbit small intestinal loops. After the 6 h fluid accumulation experiments, rabbit small intestinal loops selected for histological examination were formalin-fixed and embedded in paraffin. Hematoxylin and eosin was used to stain 4 μ m thick sections of intestinal tissue treated with the indicated construct. The preparation used to treat each loop is noted at the bottom of each panel. Final magnification: 200 \times . Copyright © American Society for Microbiology, Infection and Immunity, 76, 2008, 3793-3800, DOI: 10.1128/IAI.00460-08. Used with permission.

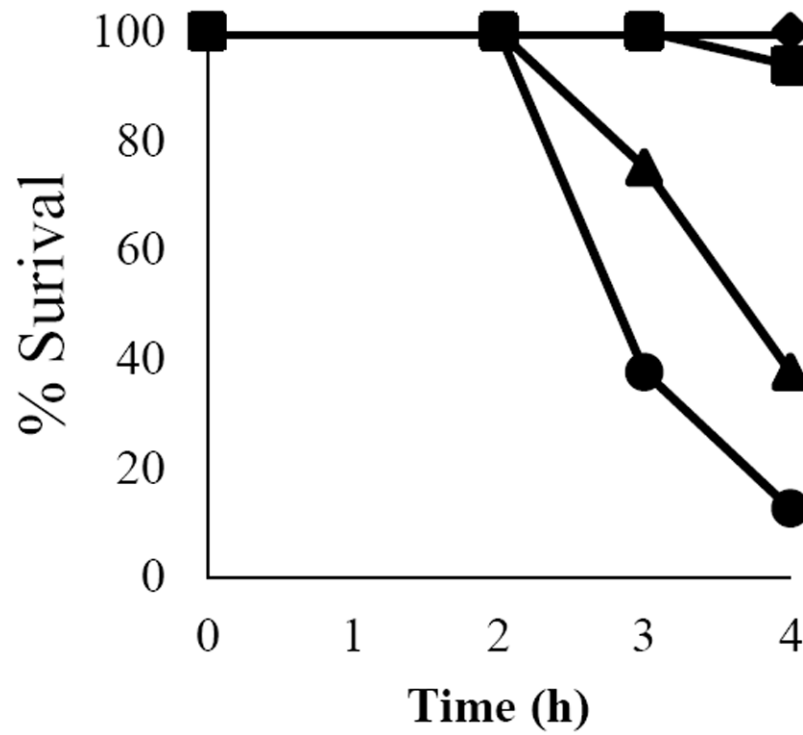


Figure 4.

Dose- and time-dependent representation of mouse survival after intraduodenal CPE challenge. The percent survival of mice was calculated and plotted against the time of treatment (in hours, x-axis) for each dose of CPE at 1, 2, 3, and 4 h. Buffer control animals (+JmY-), 50 +A7w-g treatment (-+JaA-), 100 +A7w-g treatment (-+JbI-), 200 +A7w-g treatment (-+Jc8-). Survival was calculated from 8 animals for each CPE dose. Copyright +AKk- American Society for Microbiology, Infection and Immunity, 79, 2011, 3020-3027, DOI: 10.1128/IAI.01342-10. Used with permission.

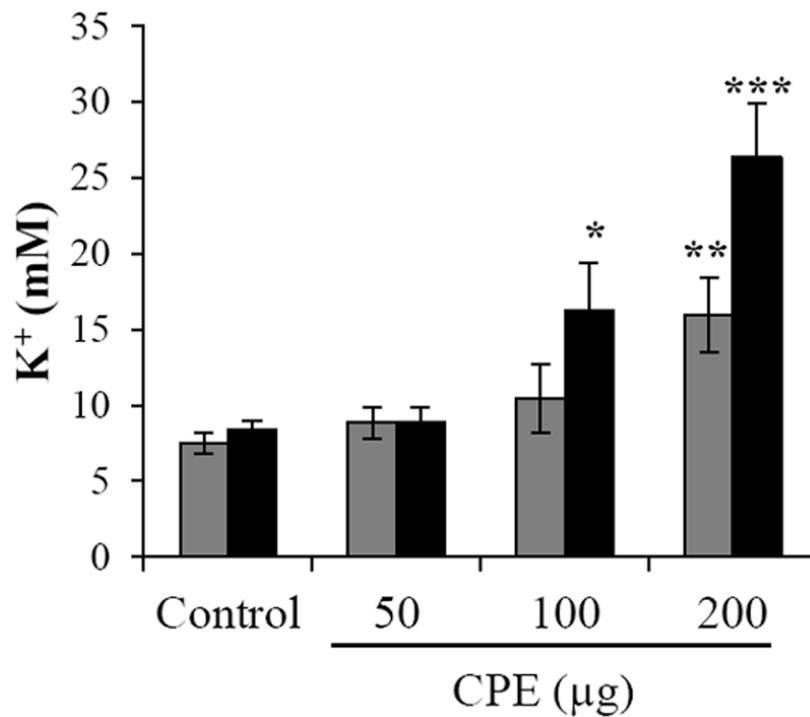


Figure 5.

Serum potassium levels after CPE intraduodenal challenge of mice. After 2 h (gray bars) or 3 h (black bars) of intestinal loop treatment with buffer control or buffer containing 50 µg, 100 µg, or 200 µg of CPE, sera were collected from mice. Those sera were analyzed by utilizing inductively coupled argon plasma emission spectrometry. After precipitation of proteins, the protein-free supernatant of each sample was analyzed for potassium levels. Accuracy of results was measured by analyzing quality assurance sera obtained from the Veterinary Laboratory Association Quality Assurance Program. Data were accepted if analyzed quality assurance serum values were within 2 standard deviations of the reference values. Data shown are mean values obtained using 8 mice. Error bars represent the standard error of the mean. * $p < 0.02$, ** $p < 0.01$, or *** $p < 0.0002$ compared to control. Copyright © American Society for Microbiology, Infection and Immunity, 79, 2011, 3020-3027, DOI: 10.1128/IAI.01342-10.