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Canadian Intellectual Property Office

CA 2976976 C 2023/02/28

(11)(21) 2 976 976

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(86) Date de dépôt PCT/PCT Filing Date: 2016/02/19
(87) Date publication PCT/PCT Publication Date: 2016/08/25
(45) Date de délivrance/Issue Date: 2023/02/28
(85) Entrée phase nationale/National Entry: 2017/08/17
(86) N° demande PCT/PCT Application No.: CA 2016/050170
(87) N° publication PCT/PCT Publication No.: 2016/131157
(30) Priorité/Priority: 2015/02/19 (US62/118,450)

(51) Cl.Int./Int.Cl. *C07K 14/33* (2006.01), *C07K 16/12* (2006.01), *C07K 16/46* (2006.01), *C12N 15/31* (2006.01), *C12N 15/85* (2006.01)

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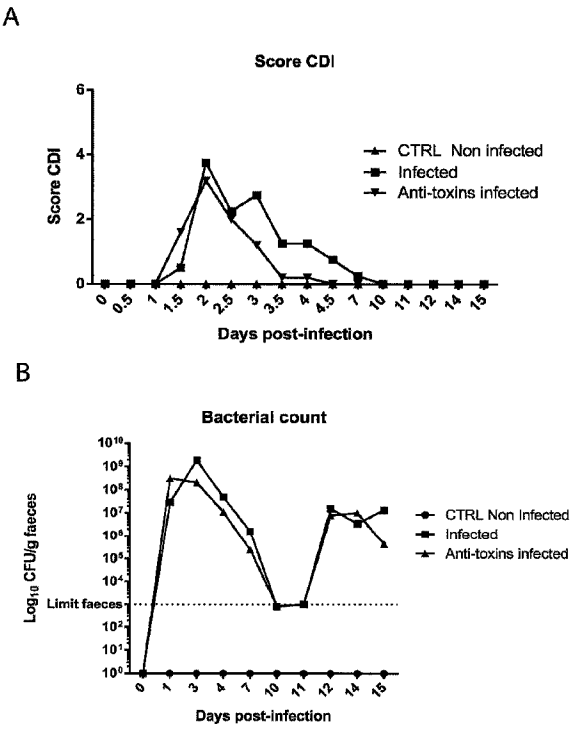
(54) Titre : ANTIGENE ET ANTICORPS EPITOPIQUE CONTRE LES TOXINES A ET/OU B DE CLOSTRIDIUM DIFFICILE, ET UTILISATIONS PHARMACEUTIQUES

(54) Title: CLOSTRIDIUM DIFFICILE TOXINS A AND/OR B ANTIGEN AND EPITOPE ANTIBODY, AND PHARMACEUTICAL USES THEREOF



(57) Abrégé/Abstract:
It is described a Clostridium difficile (C-difficile) toxins A and/or B as a target for therapy, including passive immunotherapy, and particularly prevention of C-difficile intoxication in human or other animals. It is also described a polypeptide comprising a portion of

(11)(21) 2 976 976

(13) C

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(57) Abrégé(suite)/Abstract(continued):
C-difficile toxins A and/or B sequence being an epitope for anti-toxins A and/or B antibody. It is also 5 disclosed a method for generating a neutralizing antibody directed against C-difficile toxins A and/or B. It is also provided a novel formulation that combines key toxins A and/or B epitope antibodies, located in three key domains of toxins A and/or B, for neutralisation of the toxins A and/or B, at any stage of toxins A and/or B intoxication related to C-difficile infection. The novel formulation of toxins A and/or B epitope antibodies are useful in immunotherapy, for 10 therapeutic and/or prophylactic mediation of C-difficile intoxication.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
25 August 2016 (25.08.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/131157 A1

(51) International Patent Classification:
C07K 14/33 (2006.01) *C12N 15/31* (2006.01)
C07K 16/12 (2006.01) *C12N 15/85* (2006.01)
C07K 16/46 (2006.01)

(21) International Application Number:
PCT/CA2016/050170

(22) International Filing Date:
19 February 2016 (19.02.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/118,450 19 February 2015 (19.02.2015) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH,

[Continued on next page]

(54) Title: CLOSTRIDIUM DIFFICILE TOXINS A AND/OR B ANTIGEN AND EPITOPE ANTIBODY, AND PHARMACEUTICAL USES THEREOF

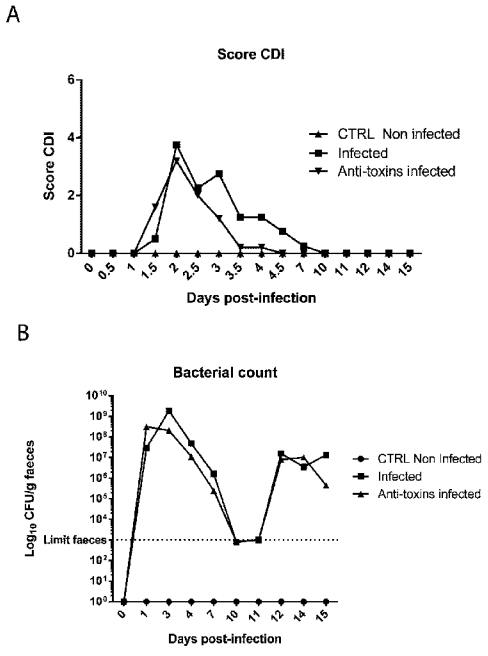

FIGURE 11

(57) Abstract: It is described a Clostridium difficile (C-difficile) toxins A and/or B as a target for therapy, including passive immunotherapy, and particularly prevention of C-difficile intoxication in human or other animals. It is also described a polypeptide comprising a portion of C-difficile toxins A and/or B sequence being an epitope for anti-toxins A and/or B antibody. It is also 5 disclosed a method for generating a neutralizing antibody directed against C-difficile toxins A and/or B. It is also provided a novel formulation that combines key toxins A and/or B epitope antibodies, located in three key domains of toxins A and/or B, for neutralisation of the toxins A and/or B, at any stage of toxins A and/or B intoxication related to C-difficile infection. The novel formulation of toxins A and/or B epitope antibodies are useful in immunotherapy, for 10 therapeutic and/or prophylactic mediation of C-difficile intoxication.

WO 2016/131157 A1

WO 2016/131157 A1

GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

CLOSTRIDIUM DIFFICILE TOXINS A AND/OR B ANTIGEN AND EPITOPE ANTIBODY, AND PHARMACEUTICAL USES THEREOF

Cross-Reference to Related Applications

[0001]

Field of the Invention

[0002] The present invention generally relates to epitopes of toxins A and/or B produced by Clostridium difficile and antibodies that specifically binding to these epitopes. The invention is also related to pharmaceutical compositions and vaccines for the prevention or treatment of Clostridium difficile infection comprising any of the epitopes or antibodies thereof.

Background of the Invention

[0003] Clostridium difficile is a Gram-positive, anaerobic, endospore-forming gastrointestinal pathogen responsible for C-difficile-associated disease (CDAD) in humans and animals with symptoms ranging in severity from mild cases of antibiotic-associated diarrhea to fatal pseudomembranous colitis (Rupnik et al, 2009; Leffler and Lamont, 2009; Songer, 2004; Kelly et al, 1994). Each year in North America, 1-3 % of hospitalized patients receiving antibiotics become infected with C-difficile, leading to thousands of deaths and over $1 billion in associated costs to the health-care system (Wilkins and Lyerly, 2003; Kyne et al, 2002; Kelly et al, 1994). C-difficile produces two primary virulence factors, toxin A (TcdA) and toxin B (TcdB), which are large (308 kDa and 269 kDa, respectively), single-subunit exotoxins composed of a catalytic, a translocation and a cell-receptor binding domain (RBD) (Jank and Aktories, 2008; Jank et al, 2007). Recently it was suggested TcdB is solely responsible for C-difficile virulence (Lyras et al, 2009), although earlier studies have shown both anti-TcdA and anti-TcdB monoclonal antibodies (mAbs) were required for full

Date Reçue/Date Received 2021-03-12

protection of hamsters from CDAD (Babcock et al, 2006; Kink and Williams, 1998) and anti-TcdA mAbs were required for protection in mice (Corthier et al, 1991).

[0004] The current approach for treating most CDAD infections involves administration of antibiotics, most commonly metronidazole or vancomycin (Leffler and Lamont, 2009). Antibiotic treatment places selection pressure on the organism, can lead to antibiotic resistance, and suppresses or eliminates beneficial commensal microbes. However, there are several other emerging challenges warranting the development of novel therapeutics. First, there is no acute CDAD treatment targeting TcdA and/or B. These toxins are responsible for loss of epithelial barrier function in the colon by disrupting tight junctions and increasing membrane permeability, causing diarrhea and promoting severe inflammation (Rupnik et al, 2009; Jank and Aktories, 2008). Second, hypervirulent strains of C-difficile, such as the NAP1/027 isolate, over-express TcdA and TcdB (Warny et al, 2005) and have been associated with increased mortality rates and disease severity (O'Connor et al, 2009; Pepin et al, 2005). Third, an estimated 20-25% of patients suffering from CDAD experience symptomatic relapse after the initial infection is cleared, with 45% of these patients prone to subsequent relapses (Johnson, 2009). Taken together, there is a need for non-antibiotic based reagents targeting and inhibiting TcdA and TcdB for CDAD therapy. Individuals who are asymptomatic C-difficile carriers and patients who experience mild cases of CDAD tend to possess high anti-toxin A titers (Kyne et al, 2001; Kyne et al, 2000; Warny et al, 1994; Viscidi et al, 1983). Conversely, patients susceptible to relapsing C-difficile infection have low anti-TcdA immunoglobulin titers, specifically IgM, IgG2 and IgG3 isotypes (Katchar et al, 2007; Kyne et al, 2001). TcdA-neutralizing secretory IgA antibodies are also thought to play a role in regulating CDAD severity (Johal et al 2004; Kelly et al 1992). Therefore, the introduction of anti-toxin antibodies to patients suffering from severe C-difficile infection may be a therapeutically useful approach.

[0005] A limited number of animal and human studies have illustrated the effectiveness of anti-toxin Abs for treatment of CDAD. Babcock et al (2006) intravenously administered anti-TcdA and anti-TcdB mAbs to hamsters and found a significant reduction in hamster mortality in prophylactic, primary disease and relapse models when both anti-toxin mAbs were administered. A recently completed clinical trial involving these two humanized mAbs appears promising (Lowy et al, 2010). In another study, intravenous administration of anti-TcdA mAbs raised against the RBD followed by oral challenge with C-difficile resulted in

protection of mice (Corthier et al, 1991). Elsewhere, a toxoid vaccine given by the intraperitoneal route to hamsters conferred protection against oral C-difficile challenge (Giannasca et al, 1999) and mice vaccinated with DNA encoding the TcdA RBD resulted in full protection from oral TcdA challenge (Gardiner et al, 2009). In humans, a number of uncontrolled studies have reported intravenous immunoglobulin (IVIG) therapy to be successful for the treatment of severe CDAD (Juang et al, 2007; Hassoun and Ibrahim, 2007; McPherson et al, 2006; Wilcox, 2004; Salcedo et al, 1997; Leung et al, 1991). IVIG involves administration of high concentrations (150 - 400 mg/kg) of human immunoglobulins from healthy donors which are thought to contain neutralizing anti-toxin antibodies as an estimated 60% of healthy adults have detectable TcdA- and TcdB-specific serum IgG antibodies (Viscidi et al, 983). Given that C-difficile toxins rely on attachment to epithelial cells for entry (Jank and Aktories, 2008; Jank et al, 2007), neutralizing the toxins within the lower gastrointestinal tract with antibodies may block the first step in CDAD pathogenesis. In animals, orally administered bovine immunoglobulin concentrate (BIC) containing TcdA and TcdB neutralizing IgGs were able to prevent hamster mortality when used as a propholyactic (Lyerly et al, 1991) and protected rats from the enterotoxic effects of TcdA in vivo (Kelly et al, 1996). Chicken IgY antibodies specific for toxin RBDs were shown to reduce hamster mortality when administered orally to infected animals (Kink and Williams, 1998). In humans, there have been limited reports on CDAD therapy with orally delivered Abs. Tjellstrom et al (1993) reported the successful treatment of a 3 ½ year old boy suffering from severe CDAD with IgA antibody orally. Warny et al (1999) and Kelly et al (1997) examined the passage of anti-toxin bovine IgG through the human gastrointestinal tract and found a significant reduction in IgG activity, likely due to proteolytic degradation within the upper gastrointestinal tract. The limited success of both oral and systemic anti-toxin immunotherapy in clinical settings has likely been hampered by the high immunoglobulin dose requirements (150 - 400 mg/kg), the associated costs of these doses, and a lack of published clinical data showing the effectiveness of these treatments.

[0006] Despite such advances, there remains a need in the art for a safe and effective therapeutic for treating C-difficile-associated disease as well as for sensitive and effective reagents for the detection of toxins A and B, the factors responsible for C-difficile-associated disease.

Summary of the Invention

[0007] The aforesaid and other objectives of the present invention are realized by generally providing specific Clostridium difficile (C-difficile) toxins A and/or B as a target for therapy, including passive immunotherapy, and particularly prevention of toxins A and/or B intoxication in human or other animals.

[0008] One aspect of the present invention is to provide an isolated polypeptide comprising a portion of Clostridium difficile toxins A and/or B sequence, the portion of toxins A and/or B sequence being an epitope for anti-toxins A and/or B antibody, the portion comprising a sequence being SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or any combination thereof.

[0009] Preferably, the portion of toxins A and/or B sequence may comprise a combination of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4.

[0010] The aforesaid isolated peptides may be used for immunizing an animal against Clostridium difficile infection.

[0011] Another aspect of the present invention is to provide a pharmaceutical composition for generating a neutralizing antibody directed against Clostridium difficile toxins A and/or B and comprising at least two different polypeptides, each polypeptide comprising a portion of Clostridium difficile toxins A and/or B sequence, the portion of toxins A and/or B sequence being an epitope for anti-toxins A and/or B antibody, and the portion comprising a sequence being SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or any combination thereof.

[0012] Preferably, the pharmaceutical composition may comprise four different polypeptides, wherein the portion of toxins A and/or B sequence comprises a combination of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4.

[0013] The aforesaid pharmaceutical compositions may further comprise a pharmaceutically acceptable carrier.

[0014] The pharmaceutical compositions may be used for immunizing an animal against Clostridium difficile infection.

[0015] Another aspect of the present invention is to provide a vaccine composition for prevention or treatment of Clostridium difficile infection comprising at least one polypeptide

which comprises a portion of Clostridium difficile toxins A and/or B sequence,the portion of toxins A and/or B sequence being an epitope for anti-toxins A and/or B antibody, and the portion comprising a sequence being SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or any combination thereof.

[0016] Preferably, the aforesaid vaccine composition may comprise the portion of toxins A and/or B sequence comprising a combination of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4.

[0017] The aforesaid vaccine compositions may further comprise a pharmaceutically acceptable adjuvant.

[0018] Another aspect of the present invention is to provide a nucleic acid vaccine or DNA vaccine composition for prevention or treatment of Clostridium difficile infection comprising nucleic acids encoding at least one polypeptide which comprises a portion of Clostridium difficile toxins A and/or B sequence, the portion of toxins A and/or B sequence being an epitope for anti-toxins A and/or B antibody; the portion comprising a sequence being SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or any combination thereof.

[0019] Preferably, the aforesaid nucleic acid vaccine composition may comprise the portion of toxins A and/or B sequence comprising a combination of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4.

[0020] The aforesaid nucleic acid vaccine compositions may further comprise a pharmaceutically acceptable adjuvant.

[0021] Another aspect of the present invention is to provide a method of generating a neutralizing antibody directed against Clostridium difficile toxins A and/or B. The method comprises a first step of administrating to a host an isolated polypeptide comprising a portion of the Clostridium difficile toxins A and/or B sequence, the portion comprising a sequence being SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or any combination thereof, for generating antibodies in the host. The method also comprises a second step of obtaining the antibodies from the host. The host may be a mammal or a bird, including bird eggs, such as but not limited to chicken eggs.

[0022] Preferably, in the first step of the method, the portion of the Clostridium difficile toxins A and/or B sequence comprises a combination of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4.

[0023] Another aspect of the present invention is to provide a purified antibody adapted for binding to a toxins A and/or B peptide, the peptide comprising a sequence being SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or any combination thereof.

[0024] The aforesaid antibody may also be an active fragment thereof, a chimeric antibody, a veneered antibody, a humanized antibody or a single chain recombinant antibody based thereon as well as a bird polyclonal antibody or a bird humanized recombinant antibody.

[0025] The aforesaid antibody may be used for detecting pure toxins A and/or B or a presence of toxins A and/or B produced by Clostridium difficile in cell culture.

[0026] Another aspect of the present invention is to provide an antibody composition for prevention or treatment of Clostridium difficile infection, comprising different antibodies adapted for binding to at least two epitopes of toxins A and/or B, the at least two epitopes comprising a sequence being SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or any combination thereof.

[0027] Preferably, the aforesaid antibody composition may comprises different antibodies adapted for binding to four different epitopes, the epitopes comprising a combination of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4.

[0028] Another aspect of the present invention is to provide a pharmaceutical composition for the prevention or the treatment of Clostridium difficile toxins A and/or B intoxication, the composition comprising at least one antibody adapted for binding to at least one epitope of toxins A and/or B, the at least one epitope comprising a sequence being SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or any combination thereof.

[0029] Preferably, the aforesaid pharmaceutical composition may comprises at least one epitope comprising a combination of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4.

[0030] The aforesaid pharmaceutical compositions may further comprise a pharmaceutically acceptable carrier.

[0031] The aforesaid pharmaceutical compositions may be used for making a medicament for preventing or treating Clostridium difficile infection.

[0032] The aforesaid pharmaceutical compositions may also be used for the capture and neutralisation of Clostridium difficile toxins A and/or B, allowing a passive immunotherapy of mammals, such as humans.

[0033] The features of the present invention which are believed to be novel are set forth with particularity in the appended claims.

Brief Description of the Drawings

[0034] The above and other objects, features and advantages of the invention will become more readily apparent from the following description, reference being made to the accompanying drawings in which:

[0035] Figure 1 is a schematic representation of the targeted portion of toxins A and/or B epitope antibodies.

[0036] Figures 2A and 2B illustrate the amino acid sequences of Clostrodium Difficile toxins A and B, respectively, with the sequences of the targeted epitopes underlined.

[0037] Figure 3 is a photograph of a western blot showing recognition of toxins A and/or B by toxins A and/or B epitope antibody IBSCD1, according to a preferred embodiment of the present invention.

[0038] Figure 4 is a photograph of a western blot showing recognition of toxins A and/or B by toxins A and/or B epitope antibody IBSCD2, according to a preferred embodiment of the present invention.

[0039] Figure 5 is a photograph of a western blot showing recognition of toxins A and/or B by toxins A and/or B epitope antibody IBSCD3, according to a preferred embodiment of the present invention.

[0040] Figure 6 is a photograph of a western blot showing recognition of toxins A and/or B by toxins A and/or B epitope antibody IBSCD4, according to a preferred embodiment of the present invention.

[0041] Figure 7 is a set of microscope photographs showing the neutralisation effect on the cell rounding and dead of Caco-2 intestinal cell line by toxins A and/or B epitope antibodies formulation in presence of the supernatant of C-difficile NAP1/027 hypervirulent strain, according to a preferred embodiment of the present invention.

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[0042] Figure 8 is a set of microscope photographs showing the neutralisation effect on the cell rounding and dead of Caco-2 intestinal cell line by toxins A and/or B epitope antibodies formulation in presence of the supernatant of C-difficile NAP1/027 hypervirulent strain and purified toxin A and toxin B, according to a preferred embodiment of the present invention.

[0043] Figure 9 is a set of epifluorescence microscope photographs showing improvement of Caco-2 cell viability by the blocking antibodies against C-difficile toxin A and/or B, according to a preferred embodiment of the present invention.

[0044] Figure 10 is a set of graphs showing the protection of the Caco-2 monolayer integrity by the blocking antibodies against C-difficile toxin A and/or B, according to a preferred embodiment of the present invention.

[0045] Figure 11 is a set of graphs showing the reduction of Clostridium difficile infection in vivo by the blocking antibodies against C-difficile toxin A and/or B, according to a preferred embodiment of the present invention.

[0046] Figure 12 is a set of microscope photographs showing the reduction of mucosal damage in murine colon by the blocking antibodies against C-difficile toxin A and/or B, according to a preferred embodiment of the present invention.

Detailed Description of the Preferred Embodiment

[0047] A novel composition of anti-toxins A and/or B antibodies and toxins A and/or B epitopes will be described hereinafter. Although the invention is described in terms of specific illustrative embodiment(s), it is to be understood that the embodiment(s) described herein are by way of example only and that the scope of the invention is not intended to be limited thereby.

[0048] A broad aspect, the present invention provides a method that uses a combination of toxins A and/or B epitope antibodies, including but not limited to bird's epitope antibodies, for the capture and neutralisation of C-difficile toxins A and/or B, which permit a passive immunotherapy of humans or others mammals. The method of neutralisation aims three different key portions of toxins A and/or B among the four domains of C-difficile toxins, which are the combined repetitive oligopeptides c-terminal domain (CROPs) **10**, the delivery pore forming domain **20**, the auto-protease domain **30**, and the N-terminal glucosyltransferase domain **40**, as illustrated in **Figures 1** and **2**. These different portions of toxins A and/or B includes four different epitopes adapted for generating toxins A and/or B epitope antibodies in

mammals or birds. Furthermore, **Figure 2** shows the location of the four epitopes within the toxins A and B amino acid sequences as part of the present invention.

[0049] According to a first embodiment of the present invention, it is provided an isolated peptide having the amino acid sequence: DSKKYYFNTNTAEAA (SEQ ID NO: 1). This particular peptide is a toxins A and/or B epitope peptide encompassing amino acids 2084-2098 of toxins A and/or B, which has been identified as a shared toxins A and/or B epitope using the chicken polyclonal antibodies IBSCD1 (see **Figure 3**).

[0050] According to a second embodiment of the present invention, it is provided an isolated peptide having the amino acid sequence: ANQYEVRINSEGR (SEQ ID NO: 2). This particular peptide is a toxins A and/or B epitope peptide encompassing amino acids 739-751 of toxins A and/or B, which has been identified as a shared toxins A and/or B epitope using the chicken polyclonal antibodies IBSCD2 (see **Figure 4**).

[0051] According to a third embodiment of the present invention, it is provided an isolated peptide having the amino acid sequence GHGKDEFNTDIFAG (SEQ ID NO: 3). This particular peptide is a toxins A and/or B epitope peptide encompassing amino acids 652-665 of toxins A and/or B, which has been identified as a shared toxins A and/or B epitope using the chicken polyclonal antibodies IBSCD3 (see **Figure 5**).

[0052] According to a forth embodiment of the present invention, it is provided an isolated peptide having the amino acid sequence DEYNKLTTNNNENKYL (SEQ ID NO: 4). This particular peptide is a toxins A and/or B epitope peptide encompassing amino acids 31-46 of toxins A and/or B, which has been identified as a shared toxins A and/or B epitope using the chicken polyclonal antibodies IBSCD4 (see **Figure 6**).

[0053] In accordance with another embodiment of the present invention, the isolated peptides, including combinations of one or more thereof, are adapted for generating antibodies which recognize toxins A and/or B and have a neutralising therapeutic or prophylactic activity in immunizing animals, particularly mammals, most particularly humans, who have a C-difficile intoxication.

[0054] In accordance with a preferred embodiment of the present invention, it is provided a combination of polypeptides, which comprises all the afore-mentioned amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4, or any combination thereof.

[0055] The afore-mentioned polypeptides may include an immunogenic peptide, particularly comprising amino acid sequence of any of SEQ ID NOS: 1-4, or an immunogenic fragment thereof. These polypeptides may also include immunogenic receptor of peptides, wherein such polypeptides comprise a combination of at least one immunogenic receptor peptide comprising amino acid sequence of any of SEQ ID NOS: 1-4, or immunogenic peptide fragment thereof.

[0056] In accordance with another embodiment of the present invention, it is provided a method for immunizing an animals, particularly mammals or birds comprising administering a toxins A and/or B epitope peptide or an immunogenic fragment thereof, whereby the animal produces antibodies that are immunoreactive with the epitope peptide exposed on partial or full length toxins A and/or B produced by C-difficile bacteria. The method for immunizing mammals or birds may comprise administering a toxins A and/or B peptide comprising amino acid sequence of any of SEQ ID NOS: 1-4 or an immunogenic fragment thereof, whereby the animal produces antibodies that are immunoreactive to full length toxins A and/or B produced by C-difficile bacteria. According to a preferred embodiment, the aforesaid method comprises the use of the four different toxins A and/or B peptides with the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4.

[0057] In accordance with another embodiment of the present invention, it is provided a pharmaceutical composition for generating a neutralizing antibody directed against Clostridium difficile toxins A and/or B, the pharmaceutical composition comprising a toxins A and/or B peptide, particularly with amino acid sequence of any of SEQ ID NOS: 1-4, and a pharmaceutically acceptable carrier. According to a preferred embodiment, the pharmaceutical composition comprises the four different toxins A and/or B peptides with the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4, and a pharmaceutically acceptable carrier.

[0058] In accordance with another embodiment of the present invention, it is provided a vaccines or immunogenic compositions that comprise one or more toxins A and/or B peptide, particularly with amino acid sequence of any of SEQ ID NOS: 1-4, and a pharmaceutically acceptable adjuvant. According to a preferred embodiment, the vaccine composition comprises the four different toxins A and/or B peptides with the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4, and a pharmaceutically acceptable adjuvant.

[0059] The vaccine may also be used for treatment of a subject, such as a mammal, particularly a human subject, suffering from a C-difficile intoxication. Such vaccine may comprise an immunogenic amount of one or more toxins A and/or B peptides, particularly with amino acid sequence of any of SEQ ID NOS: 1-4 or immunogenic fragment thereof, and a pharmaceutically acceptable adjuvant. The aforesaid toxins A and/or B peptides may be conjugated to a carrier.

[0060] In accordance with another embodiment of the present invention, it is provided a nucleic acid vaccines or DNA vaccines comprising nucleic acids encoding immunogenic toxins A and/or B peptides, particularly with amino acid sequence of any of SEQ ID NOS: 1-4, and a pharmaceutically acceptable adjuvant. According to a preferred embodiment, the nuclei acid vaccine composition comprises the nucleic acids encoding the four different toxins A and/or B peptides with the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4, and a pharmaceutically acceptable adjuvant. The afore-mentioned nucleic acid vaccine may further comprises at least one other polypeptide, particularly an immunomodulatory molecule peptide derived from C-difficile.

[0061] In accordance with another embodiment of the present invention, it is provided a method for diagnosis of Clostridium difficile infection comprising the steps of contacting a biological sample of a subject with at least one peptide fragment of toxins A and/or B and detecting antigen-antibody complex formation. Such at least one peptide fragment may comprises an epitope for anti-toxins A and/or B antibody with an amino acid sequence of any of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or any combination thereof.

[0062] In accordance with another embodiment of the present invention, it is provided a kit for the diagnosis or detection of Clostridium difficile infection, the kit comprising at least one peptide fragment of toxins A and/or B and directions for diagnosing or detecting anti-toxin A and/or B antibody. Such at least one peptide fragment may comprise an epitope for anti-toxin A and/or B antibody with an amino acid sequence of any of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or any combination thereof.

[0063] As previously mentioned, toxins A and/or B polypeptides with the amino acid sequence of any of SEQ ID NOS: 1-4 are adapted for generating toxins A and/or B epitope antibodies in mammals or birds. Such antibodies recognize toxins A and/or B and have

neutralising effect on the activity of these toxins, so that the antibodies may be used for a therapeutic or prophylactic treatment against C-difficile intoxication in humans and animals.

[0064] In accordance with another embodiment of the present invention, it is provided a method of generating a neutralizing antibody directed against Clostridium difficile toxins A and/or B. The method comprises a first step of administrating to a host an isolated polypeptide comprising a portion of the Clostridium difficile toxins A and/or B sequence. The portion comprising a sequence being SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or any combination thereof, for generating antibodies in the host. The method comprises a second step of obtaining the antibodies from the host. The host may be a mammal or a bird, including the bird eggs, such as but not limited to chicken eggs.

[0065] The aforesaid method may also comprise a first step, wherein the portion of the Clostridium difficile toxins A and/or B sequence comprises a sequence being SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4.

[0066] Referring to **Figure 3**, an antibody, called IBSCD1, is adapted for binding to a key toxins A and/or B epitope, encompassing amino acids 2084-2098 of toxin A and/or B located in combined repetitive oligopeptides c-terminal domain (CROPs) **10** of toxins A and/or B. This domain plays a role in the binding to human or animal cells, as illustrated in **Figure 1**, step 1.

[0067] Referring to **Figures 4** and **5**, two antibodies, called IBSCD2 and IBSCD3, are adapted for binding to toxins A and/or B epitopes, encompassing respectively amino acids 739-751 and 652-665 of toxins A and/or B located in the delivery pore forming domain **20**. This domain plays a role in the endosome pore formation of the infected cell, as illustrated in **Figure 1**, step 3.

[0068] Referring to **Figure 6**, another antibody, called IBSCD4, bind the toxins A and/or B epitope encompassing amino acids 31-46 of toxins A and/or B located in the glucosyltransferase domain **40**. This domain plays a role in the inactivation of small GTPases in the affected cells, as illustrated in **Figure 1**, step 4.

[0069] In accordance with a preferred embodiment of the present invention, it is provided a novel formulation that combines key toxins A and/or B epitope antibodies, located in three key domains of toxins A and/or B, for neutralisation of the toxins A and/or B, at any stage of toxins A and/or B intoxication related to C-difficile infection. Therefore, the novel

formulation of toxins A and/or B epitope antibodies may be used in immunotherapy, for therapeutic and/or prophylactic mediation of C-difficile intoxication.

[0070] In accordance with another embodiment, it is provided a purified antibody to a toxin A and/or B peptide comprising the amino acid sequence of any of SEQ ID NOS: 1-4. The above-described antibodies may specifically detect pure toxins A and/or B or the presence of these toxins produced by C-difficile in culture.

[0071] The antibody may be selected from antibodies IBSCD1, IBSCD2, IBSCD3, IBSCD4 or active fragments thereof. The antibody may include both polyclonal and monoclonal antibodies prepared by known genetic techniques, as well as bi-specific antibodies, and antibodies including other functionalities suiting them for diagnostic or therapeutic use. The antibody may consist of bird polyclonal or bird humanized recombinant antibodies. Furthermore, the antibody may include, but not limited to, naturally raised and recombinant prepared antibodies or fragments thereof, including single chain variants and Fv. The antibodies may also include chimereic antibodies, veneered antibodies, humanized antibodies, chicken polyclonal antibodies, chicken recombinant humanized antibodies, domain antibodies, calemized antibodies and single chain recombinant antibodies. Such antibodies can be used for passive immunization to reduce C-difficile intoxication, particularly in humans.

[0072] In accordance with another embodiment of the present invention, it is provided a pharmaceutical composition for preventing or treating C-difficile toxins A and/or B intoxication. The pharmaceutical composition may comprise at least one antibody adapted for binding to at least one epitope of toxins A and/or B, and a pharmaceutically acceptable carrier. Such epitope may comprise a amino acid sequence of any of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or any combination thereof. According to a preferred embodiment, the pharmaceutical composition comprises the antibodies adapted for binding to the four different toxins A and/or B peptides with the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4, and a pharmaceutically acceptable carrier.

[0073] In accordance with a another embodiment of the present invention, it is provided therapeutic methods based upon the activity of an antibody, or active fragments thereof, adapted for binding to a toxins A and/or B peptide comprising amino acid sequence of any of SEQ ID NOS: 1-4. In particular, the method may comprise antibodies, or active fragments

thereof, and chimeric or synthetic antibodies derived therefrom, and can be prepared in pharmaceutical compositions, including a suitable vehicle, carrier or diluent, for administration in instances wherein therapy is appropriate, such as to treat C-difficile infection. Such methods may include oral formulations of avian of mammal anti-toxins A and/or B for prevention of toxins A and/or B intoxication. Such methods may also include modulating the half-life of the binding members, antibodies or fragments by methods known in the art such as pegylation. Such methods may further comprise additional antibodies or therapeutic agents.

[0074] In accordance with a another embodiment of the present invention, it is provided a method for diagnosis of Clostridium difficile infection comprising the steps of contacting a biological sample of a subject with at least one anti-toxins A and/or B antibody adapted for binding to at least one epitope of toxins A and/or B, and detecting antigen-antibody complex formation. Such at least one epitope may comprise an amino acid sequence of any of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or any combination thereof.

[0075] In accordance with a another embodiment of the present invention, it is provided a kit for the diagnosis or detection of Clostridium difficile infection, the kit comprising at least one anti-toxins A and/or B antibody adapted for binding to at least one epitope of toxins A and/or B, and directions for diagnosing or detecting anti-toxin A and/or B antibody. Such at least one epitope may an amino acid sequence of any of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or any combination thereof.

EXAMPLES:

[0076] **Figure 7** shows the neutralisation effect on the cell rounding and dead of Caco-2 intestinal cell line by toxins A and/or B epitope antibodies formulation in presence of the supernatant of C-difficile NAP1/027 hypervirulent strain. Panel A) shows intact monolayer of Caco-2 cells after incubation with 25 µl of water (negative control). Panel B) shows disturbed and destroyed monolayer of Caco-2 cells, with a lot of cell rounding and dead cells after incubation with 25 µl of undiluted NAP1/027 strain C-difficile toxin A/B supernatant. Panel C) shows disturbed monolayer of Caco-2 cells, with cell rounding (arrows) and dead cells after incubation with 25 µl of 1:1000 dilution of NAP1/027 strain C-difficile toxin A/B supernatant. Panel D) shows preserved monolayer of Caco-2 cells after incubation with 25 µl of 1:1000 dilution of NAP1/027 strain C-difficile toxin A/B supernatant in the presence of a formulation containing 63 µg/ml of IBSCD1, IBSCD2, IBSCD3 and IBSCD4 toxins A/B epitope antibodies.

[0077] **Figure 8** shows the neutralisation effect on the cell rounding and dead of Caco-2 intestinal cell line by adding toxins A and/or B epitope antibodies formulation when in presence of the supernatant of C-difficile NAP1/027 hypervirulent strain and purified toxin A and toxin B. Panel A) shows complete rounding of cells, lost of adhesion and cells death after incubation with 400 ng/mL of purified toxin A. Panel B) shows reduction of cytotoxic effect, with decrease in rounding and cell death, after incubation with 400 ng/mL toxin A, preincubated with 63 µg/mL of IBSCD1, IBSCD2, IBSCD3 and IBSCD4 toxins A and/or B epitope antibodies. Panel C) shows disturbed monolayer of Caco-2 cells, with cell rounding and dead cells and without dome formation, after incubation with 25 µl of 1:100 dilution of NAP1/027 strain C-difficile toxin A/B supernatant. Panel D) shows preserved monolayer of Caco-2 cells after incubation with 25 µl of 1:100 dilution of NAP1/027 strain C-difficile toxin A and/or B supernatant in the presence of a formulation containing 125 µg/ml of IBSCD1, IBSCD2, IBSCD3 and IBSCD4 toxins A and/or B epitope antibodies. Panel E) shows complete rounding of cells, lost of adhesion and cell death after incubation with 10 ng/mL of purified toxin B. Panel F) shows reduction of cytotoxic effect, with decrease in rounding and cell death after incubation with 40 ng/mL toxin B, preincubated with 125 µg/mL of IBSCD1, IBSCD2, IBSCD3 and IBSCD4 toxins A and/or B epitope antibodies.

[0078] **Figure 9** shows cell death induced by C. difficile toxin A and B in Caco-2 cells and the improvement of cell viability by the blocking antibodies. Caco-2 cells were seeded in chamber slides in 200 µL of culture medium and incubated overnight at 37°C in CO_2 incubator. The following day, cell death was induced with toxin A and B and cells were incubated for 48h. Cells were washed with PBS and incubated with Annexin V and PI (1:10) for 15 min. Coverslips were mounted with mounting medium and DAPI. Images were taken by epifluorescence microscopy. Panel A) shows little or no cell death in cells treated with water. Panel B) shows little or no cell death due to a normal process of cell death in the presence of 125 µg/mL of IBSCD1, IBSCD2, IBSCD3 and IBSCD4 Toxins A and/or B epitope antibodies. Panel C) shows increased in number of green (or white), Annexin V labelled cells representing apoptotic cells, in the presence of 400 ng/mL toxin A. Panel D) shows a decrease in cell death with 400 ng/mL toxin A preincubated with 125 µg/mL of IBSCD1, IBSCD2, IBSCD3 and IBSCD4 toxins A and/or B epitope antibodies. Panel E) shows an increase in cell death with 40 ng/mL toxin B. Panel F) shows a decrease in cell death with 40 ng/mL toxin B preincubated with 125 µg/mL of IBSCD1, IBSCD2, IBSCD3 and IBSCD4 toxins A/B epitope antibodies. C. difficile toxins A and B induced programmed cell death and even necrosis (represented in white), while preincubation of toxins with IBSCD1, IBSCD2, IBSCD3 and IBSCD4 toxins A/B epitope antibodies deceased number of apoptotic cells in all conditions. Bleu, DAPI labelled cells represent live cells.

[0079] **Figure 10** shows the protection of the integrity of Caco-2 monolayer by the blocking antibodies against C. difficile toxin A and B. Panel A) shows 7-10 days post-confluent Caco-2 monolayer that was grown on porous 4 µm inserts. Only polarized monolayers of cell were used. At 6 h, the transepithelial electric resistance (TEER) of the Caco-2 monolayer rapidly decreased down to 67% when cells were stimulated with 400 ng/ml toxin A. Cells treated with toxin A preincubated with blocking antibodies IBSCD1, IBSCD2, IBSCD3 and IBSCD4 increased TEER up to 86 % compared to unstimulated cells, which were reported to one. Panel B) shows the rapid decrease of the transepithelial electric resistance (TEER) of the Caco-2 monolayer down to 77% when cells were stimulated with 40 ng/ml toxin B compared to unstimulated cells. Use of 125 µg/ml of blocking antibodies restored epithelial integrity up to 93 %. Panel C) shows that when cells were stimulates with supernatant of C. difficile NAP1/027 strain (1:10), TEER decreased to 61 % compared to unstimulated after 6 h. Preincubation of 125 µg/ml IBSCD1, IBSCD2, IBSCD3 and IBSCD4 toxins A and/or B

epitope antibodies with supernatant restored epithelial integrity up to 84 %. Results represent the mean SEM of three independent experiments performed in duplicate. Statistical analysis was done with 2 way ANOVA.

[0080] **Figure 11** shows the reduction of Clostridium difficile infection in vivo by the blocking antibodies against C-difficile toxin A and/or B. 6-8 weeks old female mice were housed in groups of 4 in sterile cages equipped with HEPA filters and containing sterile bedding. They had access to sterile food and water ad libitum. Mice are intrinsically resistant to CDI, so they were be pre-conditioned for three days with 250 mg/L clindamycin and 400 mg/L streptomycin in the drinking water, followed by an intraperitoneal (i.p.) injection of 1 mg clindamycin/mouse to disrupt their intestinal microbiota and make them susceptible to CDI. Twenty-four hours later, mice received by gavage 10e+5 spores of the epidemic CD strain R20291 to initiate the infection. Mice were gaved twice a day with 1mg of a preparation of IBSCD1, IBSCD2, IBSCD3 and IBSCD4 toxins A and/or B epitope antibodies in 0.1M carbonate buffer (pH 9.2) to neutralize the gastric acidity for 5 days. Control groups consist of uninfected mice, and infected but untreated mice. Panel A) shows the results from mice that were observed for a total of 7-10 days and clinical symptoms were monitored daily (diarrhea, weight loss, lethargy, etc.). Panel B) shows results from fresh fecal samples that were collected daily and homogenized in pre-reduced PBS, and CD were enumerated on agar plates. Clinical score end bacterial counts were the higher in untreated mice in comparison with uninfected mice. Mice receiving IBSCD1, IBSCD2, IBSCD3 and IBSCD4 toxins A and/or B epitope antibodies had lower CDI score then untreated mice and lower bacterial counts.

[0081] **Figure 12** shoes the reduction of mucosal damage in murine colon by the blocking antibodies against C-difficile toxin A and/or B. 6-8 weeks old female mice were housed in groups of 4 in sterile cages equipped with HEPA filters and containing sterile bedding. They had access to sterile food and water ad libitum. Mice are intrinsically resistant to CDI, so they were be pre-conditioned for three days with 250 mg/L clindamycin and 400 mg/L streptomycin in the drinking water, followed by an intraperitoneal (i.p.) injection of 1 mg clindamycin/mouse to disrupt their intestinal microbiota and make them susceptible to CDI. Twenty-four hours later, mice received by gavage 10e+5 spores of the epidemic CD strain R20291 to initiate the infection. Mice were gaved twice a day with 1mg of a preparation of IBSCD1, IBSCD2, IBSCD3 and IBSCD4 toxins A and/or B epitope antibodies in 0.1M

carbonate buffer (pH 9.2) to neutralize the gastric acidity for 5 days. Control groups consist of uninfected mice, and infected but untreated mice. At day ten, colons were extracted and embedded in paraffin. Hematoxylin & Eosin (H&E) staining was performed. Panel A) shows normal H&E staining of uninfected mice. Panel B) shows H&E staining of infected but untreated mice, which presented signs of mild inflammation. Panel C) shows H&E staining of infected mice treated with IBSCD1, IBSCD2, IBSCD3 and IBSCD4 toxins A and/or B epitope antibodies, which presented less severe mucosal damage then untreated mice. Black arrows indicates thickness of submucosal layer, immune cell infiltration and lost of epithelial layer.

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[0083] While illustrative and presently preferred embodiment(s) of the invention have been described in detail hereinabove, it is to be understood that the inventive concepts may be otherwise variously embodied and employed and that the appended claims are intended to be construed to include such variations except insofar as limited by the prior art.

WHAT IS CLAIMED IS:

1) An isolated peptide consisting of the sequence set forth in SEQ ID NO: 1, said sequence comprising an epitope for anti-*Clostridium difficile* toxin A and B antibodies.

2) The isolated peptide of claim 1, wherein the isolated peptide is conjugated to a vaccine carrier.

3) The isolated peptide of claim 1 or 2, for immunizing an animal against *Clostridium difficile* infection.

4) A combination of peptides comprising the isolated peptide of claim 1, and at least one of i) an isolated peptide consisting of the sequence set forth in SEQ ID NO: 2, ii) an isolated peptide consisting of the sequence set forth in SEQ ID NO: 3, and (iii) an isolated peptide consisting of the sequence set forth in SEQ ID NO: 4.

5) The combination of peptides of claim 4, comprising the isolated peptide of claim 1, the isolated peptide consisting of the sequence set forth in SEQ ID NO: 2, the isolated peptide consisting of the sequence set forth in SEQ ID NO: 3 and the isolated peptide consisting of the sequence set forth in SEQ ID NO: 4.

6) The combination of peptides of claim 4, comprising the isolated peptide of claim 1 and the isolated peptide consisting of the sequence set forth in SEQ ID NO: 2.

7) The combination of peptides of any one of claims 4 to 6, wherein one or more of the isolated peptides are conjugated to a vaccine carrier.

8) The combination of peptides of any one of claims 4 to 7, for immunizing an animal against *Clostridium difficile* infection.

9) Use of the isolated peptide of claim 1 or 2 or the combination of peptides of any one of claims 4 to 7, for immunizing an animal against *Clostridium difficile* infection.

10) Use of the isolated peptide of claim 1 or 2 or the combination of peptides of any one of claims 4 to 7, for the manufacture of a medicament for immunizing an animal against *Clostridium difficile* infection.

11) A pharmaceutical composition for generating a neutralizing antibody directed against *Clostridium difficile* toxins A and B in an animal, said pharmaceutical composition

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comprising (a) the isolated peptide of claim 1 or 2 or (b) the combination of peptides of any one of claims 4 to 7, and a pharmaceutically acceptable excipient.

12) The pharmaceutical composition of claim 11, further comprising a pharmaceutically acceptable adjuvant.

13) The pharmaceutical composition defined in claim 11 or 12, for immunizing an animal against *Clostridium difficile* infection.

14) Use of the pharmaceutical composition defined in claim 11 or 12 for immunizing an animal against *Clostridium difficile* infection.

15) Use of the pharmaceutical composition defined in claim 11 or 12 for the manufacture of a medicament for immunizing an animal against *Clostridium difficile* infection.

16) The isolated peptide of claim 1 or 2, the combination of peptides of any one of claims 4 to 7, or the pharmaceutical composition of claim 11 or 12, for use in generating a neutralizing antibody directed against *Clostridium difficile* toxins A and B in a host.

17) The isolated peptide, combination of peptides or pharmaceutical composition for use according to claim 16, wherein the host is a mammal or a bird.

18) Use of (a) the isolated peptide of claim 1 or 2 (b) the combination of peptides of any one of claims 4 to 7 or (c) the pharmaceutical composition of claim 11 or 12, for generating a neutralizing antibody directed against *Clostridium difficile* toxins A and B in a host.

19) Use of (a) the isolated peptide of claim 1 or 2 (b) the combination of peptides of any one of claims 4 to 7 or (c) the pharmaceutical composition of claim 11 or 12, for the manufacture of a medicament for generating a neutralizing antibody directed against *Clostridium difficile* toxins A and B in a host.

20) The use according to claim 18 or 19, wherein the host is a mammal or a bird.

21) A purified antibody or an antigen-binding fragment thereof specifically binding to the isolated peptide of claim 1.

22) The antibody or antigen-binding fragment thereof of claim 21, which is a chimeric antibody, a veneered antibody, a humanized antibody or a single chain recombinant antibody.

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23) The antibody or antigen-binding fragment thereof of claim 21, which is a bird polyclonal antibody.

24) Use of the antibody or antigen-binding fragment thereof of any one of claims 21 to 23 for detecting pure *Clostridium difficile* toxin A and/or toxin B in cell culture.

25) Use of the antibody or antigen-binding fragment thereof of any one of claims 21 to 23 for detecting a presence of *Clostridium difficile* toxin A and/or toxin B produced by *Clostridium difficile* in cell culture.

26) The antibody or antigen-binding fragment thereof of any one of claims 21 to 23 for preventing or treating *Clostridium difficile* infection in a subject.

27) The antibody or antigen-binding fragment thereof of any one of claims 21 to 23 for preventing or treating *Clostridium difficile* toxin A and/or B intoxication in a subject.

28) Use of the antibody or antigen-binding fragment thereof of any one of claims 21 to 23, for the manufacture of a medicament for preventing or treating *Clostridium difficile* infection in a subject.

29) Use of the antibody or antigen-binding fragment thereof of any one of claims 21 to 23, for the manufacture of a medicament for preventing or treating *Clostridium difficile* toxin A and/or B intoxication in a subject.

30) The antibody or antigen-binding fragment thereof of claim 26 or 27, wherein the subject is a human.

31) The use of claim 28 or 29, wherein the subject is a human.

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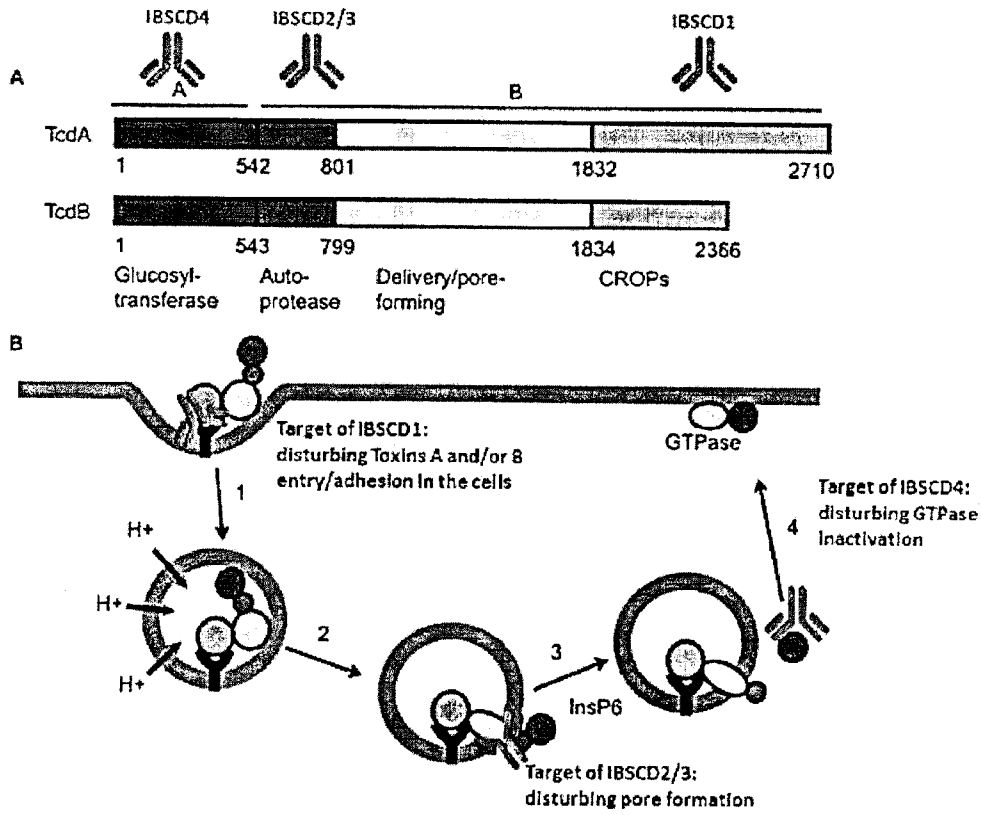
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B
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1
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TcdB
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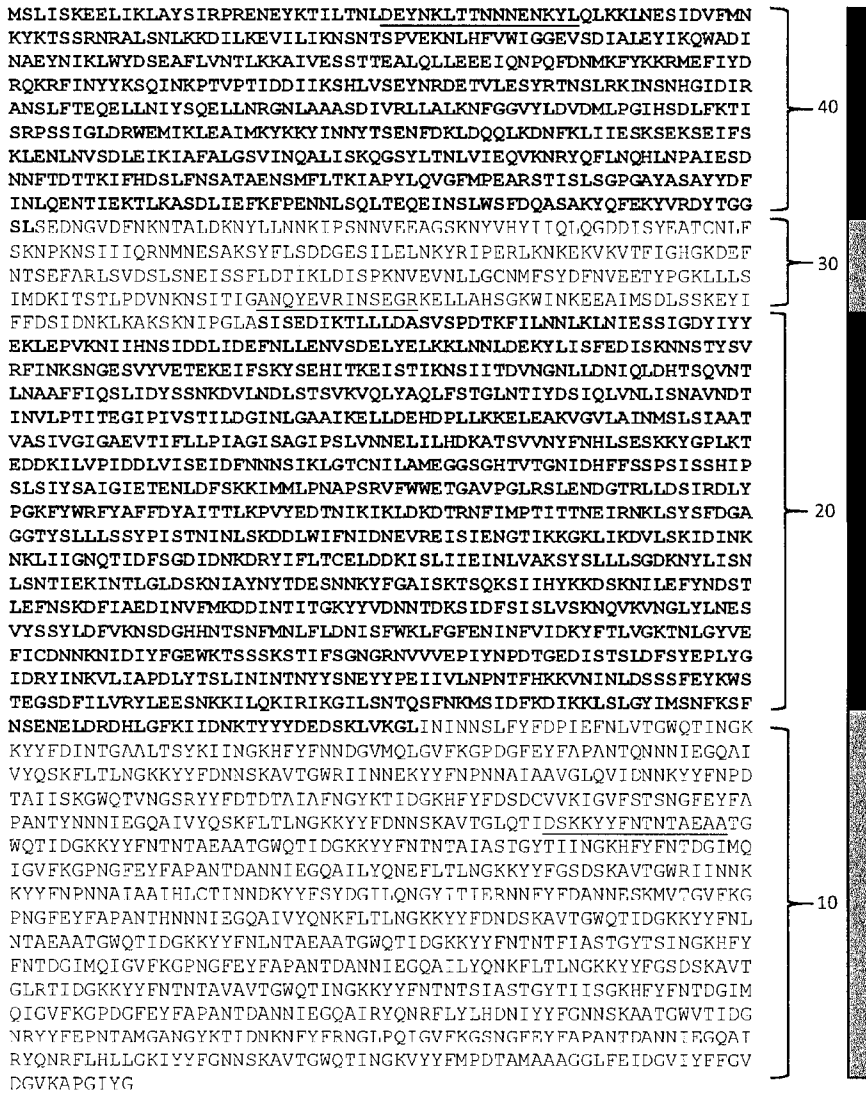
799
1834
2366
Glucosyl-transferase
Auto-protease
Delivery/pore-forming
CROPs
B
Target of IBSCD1:
disturbing Toxins A and/or B
entry/adhesion in the cells
GTPase
1
H+
H+
H+
2
3
InsP6
4
Target of IBSCD4:
disturbing GTPase
inactivation
Target of IBSCD2/3:
disturbing pore formation

FIGURE 1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INLQENTIEKTLKASDLIEFKFPENNLSQLTEQEINSLWSFDQASAKYQFEKYVRDYTGG
SLSEDNGVDFNKNTALDKNYLLNNKIPSNNVEEAGSKNYVHYIIQLQGDDISYEATCNLF
SKNPKNSIIIQRNMNESAKSYFLSDDGESILELNKYRIPERLKNKEKVKVTFIGHGKDEF
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IMDKITSTLPDVNKNSITIGANQYEVRINSEGRKELLAHSGKWINKEEAIMSDLSSKEYI
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EKLEPVKNIIHNSIDDLIDEFNLLENVSDELYELKKLNNLDEKYLISFEDISKNNSTYSV
RFINKSNGESVYVETEKEIFSKYSEHITKEISTIKNSIITDVNGNLLDNIQLDHTSQVNT
LNAAFFIQSLIDYSSNKDVLNDLSTSVKVQLYAQLFSTGLNTIYDSIQLVNLISNAVNDT
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NKLIIGNQTIDFSGDIDNKDRYIFLTCELDDKISLIIEINLVAKSYSLLLSGDKNYLISN
LSNTIEKINTLGLDSKNIAYNYTDESNNKYFGAISKTSQKSIIHYKKDSKNILEFYNDST
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WQTIDGKKYYFNTNTAEAATGWQTIDGKKYYFNTNTAIASTGYTIINGKHFYFNTDGIMQ
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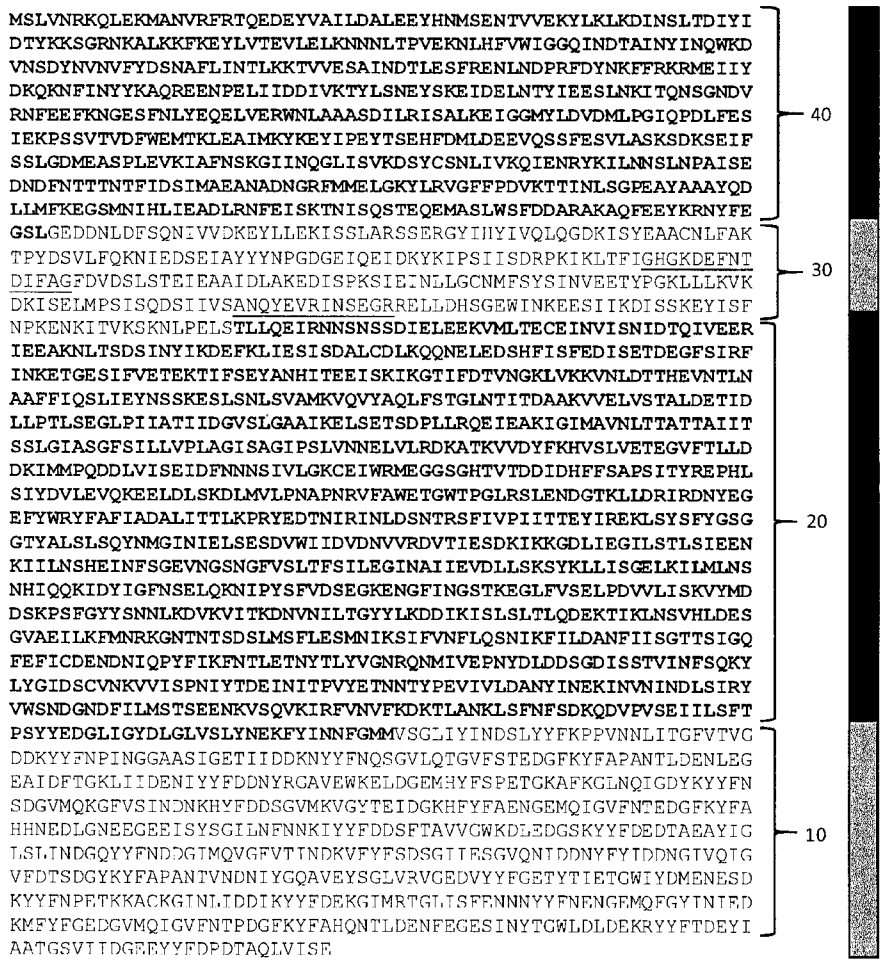
30
20
10

FIGURE 2A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NPKENKITVKSKNLPELSTLLQEIRNNSNSSDIELEEKVMLTECEINVISNIDTQIVEER
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INKETGESIFVETEKTIFSEYANHITEEISKIKGTIFDTVNGKLVKKVNLDTTHEVNTLN
AAFFIQSLIEYNSSKESLSNLSVAMKVQVYAQLFSTGLNTITDAAKVVELVSTALDETID
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SSLGIASGFSILLVPLAGISAGIPSLVNNELVLRDKATKVVDYFKHVSLVETEGVFTLLD
DKIMMPQDDLVISEIDFNNNSIVLGKCEIWRMEGGSGHTVTDDIDHFFSAPSITYREPHL
SIYDVLEVQKEELDLSKDLMVLPNAPNRVFAWETGWTPGLRSLENDGTKLLDRIRDNYEG
EFYWRYFAFIADALITTLKPRYEDTNIRINLDSNTRSFIVPIITTEYIREKLSYSFYGSG
GTYALSLSQYNMGINIELSESDVWIIDVDNVVRDVTIESDKIKKGDLIEGILSTLSIEEN
KIILNSHEINFSGEVNGSNGFVSLTFSILEGINAIIEVDLLSKSYKLLISGELKILMLNS
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DSKPSFGYYSNNLKDVKVITKDNVNILTGYYLKDDIKISLSLTLQDEKTIKLNSVHLDES
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FEFICDENDNIQPYFIKFNTLETNYTLYVGNRQNMIVEPNYDLDDSGDISSTVINFSQKY
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VFDTSDGYKYFAPANTVNDNIYGQAVEYSGLVRVGEDVYYFGETYTIETGWIYDMENESD
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10
20
30
40

FIGURE 2B

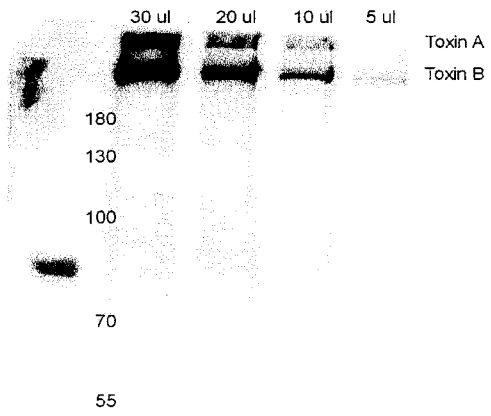


FIGURE 3

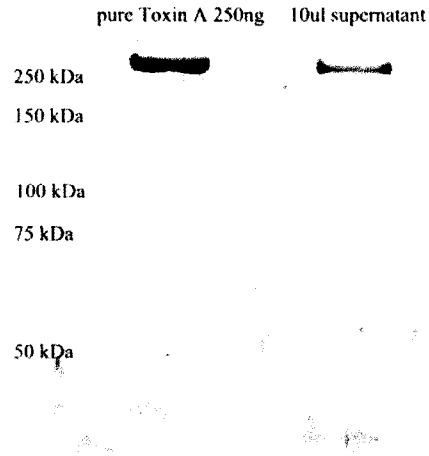


FIGURE 4

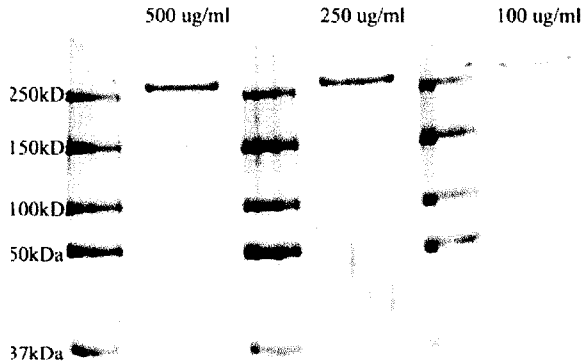


FIGURE 5

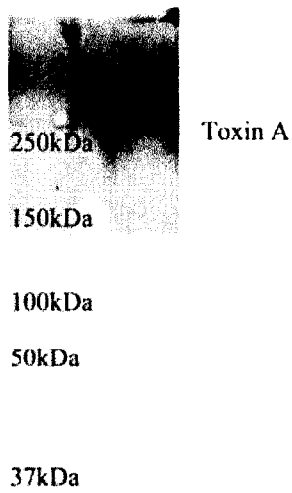


FIGURE 6

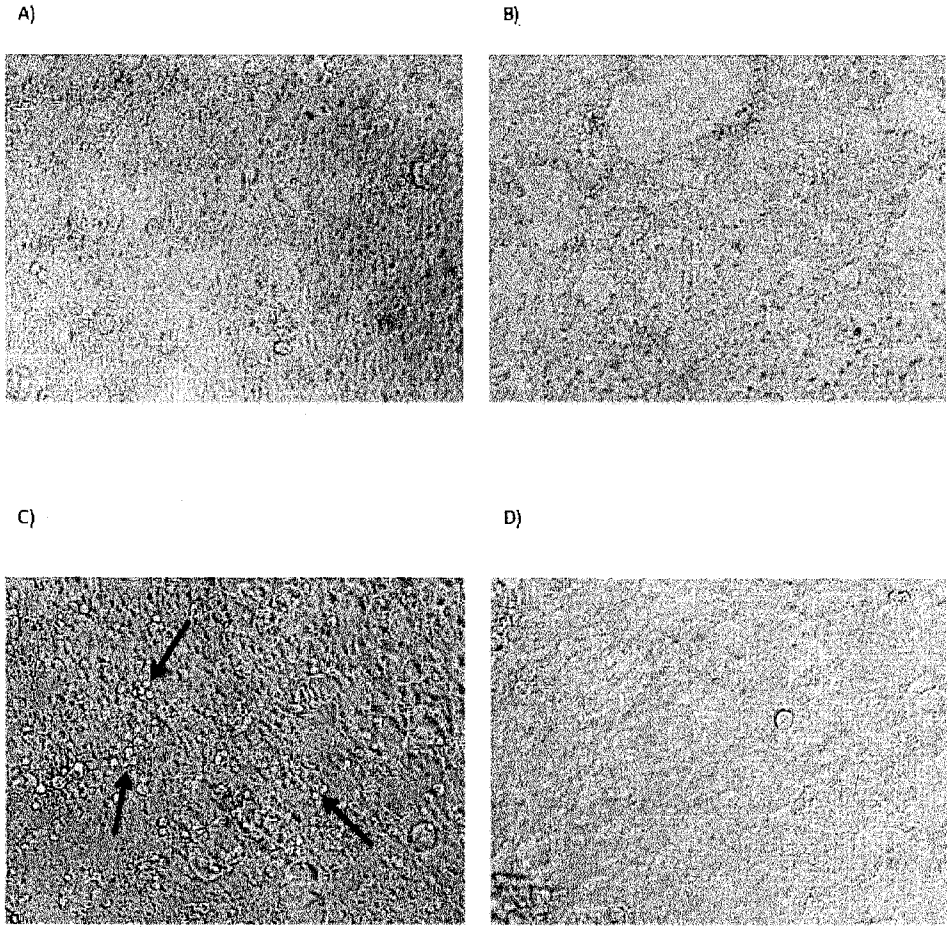


FIGURE 7

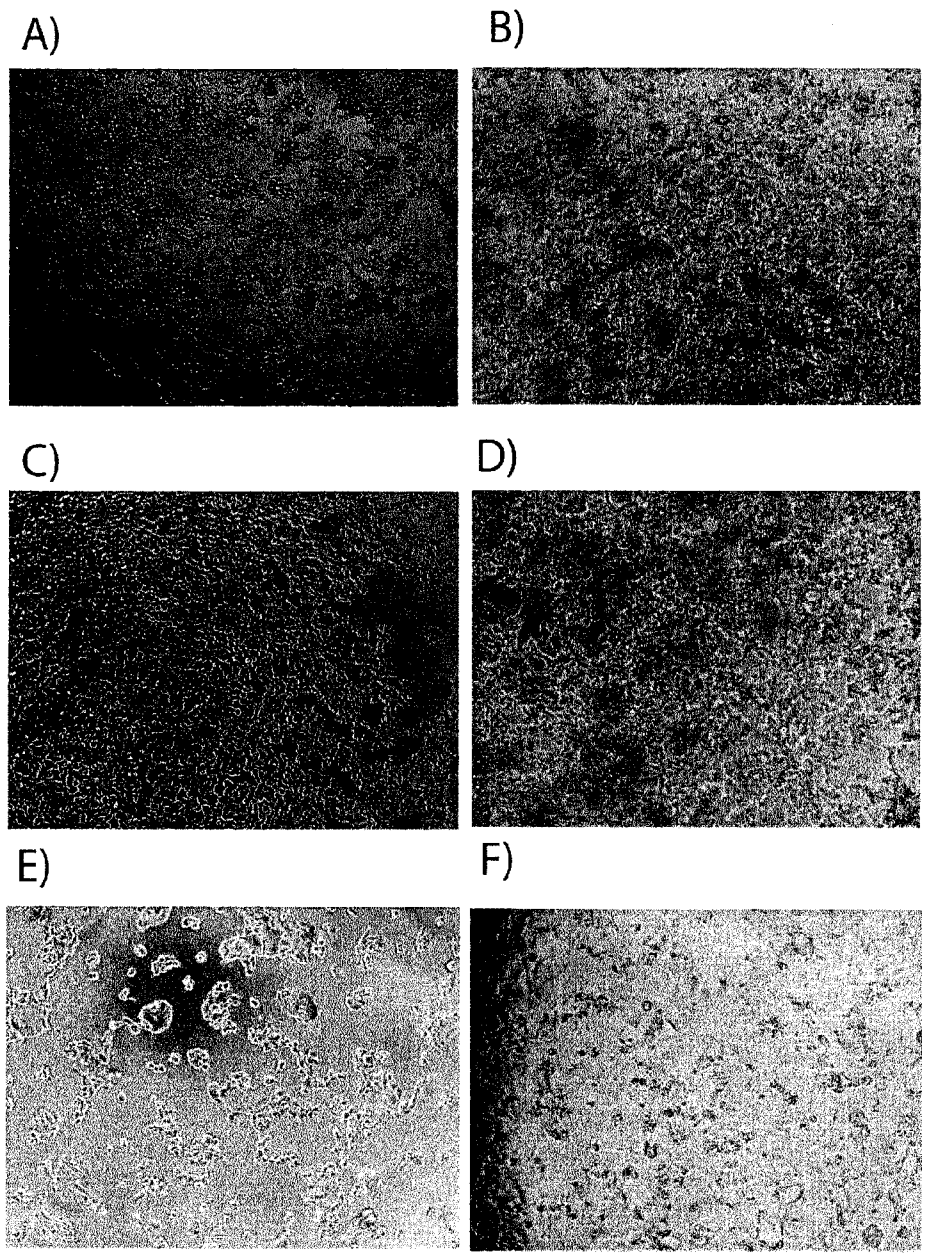


FIGURE 8

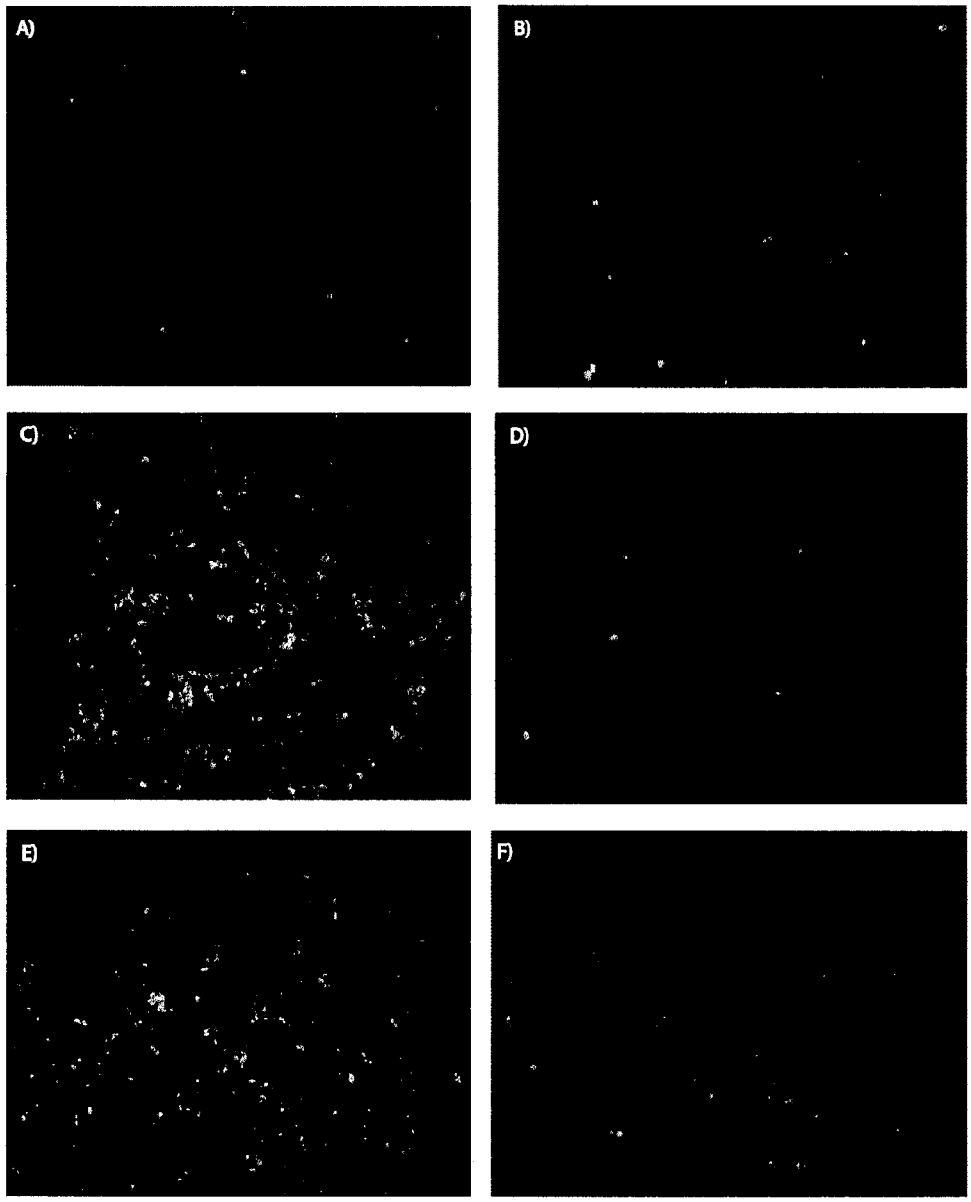
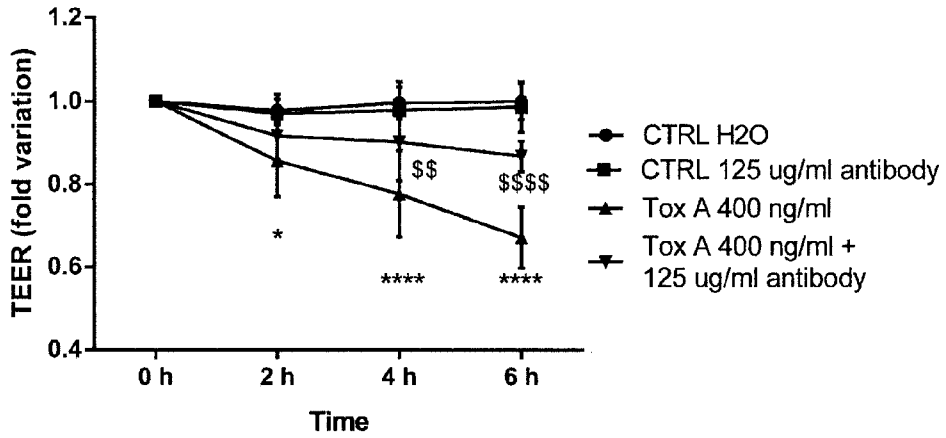
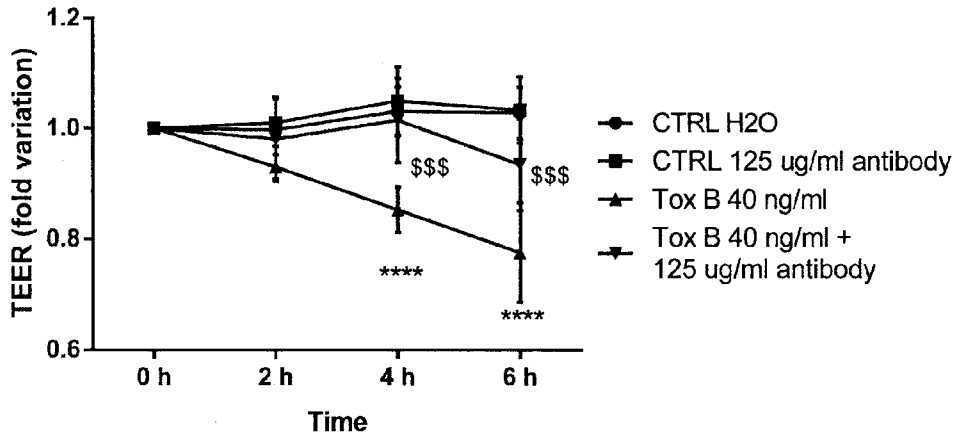


FIGURE 9

A)



B)



C)

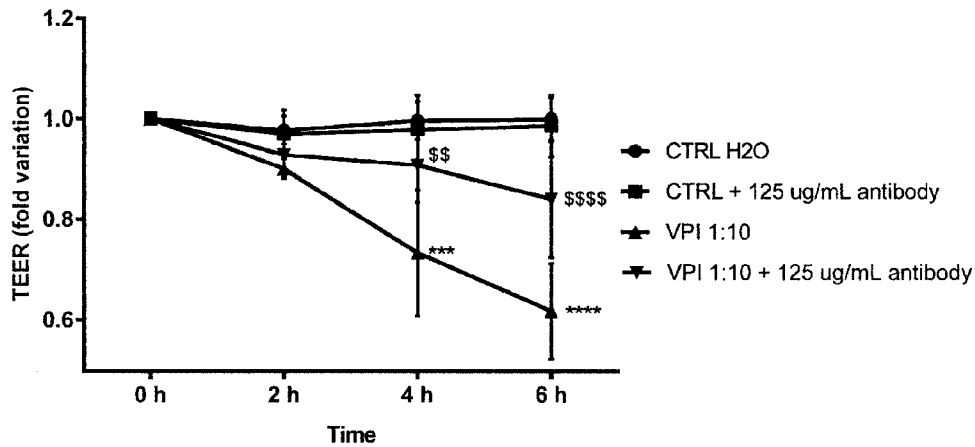
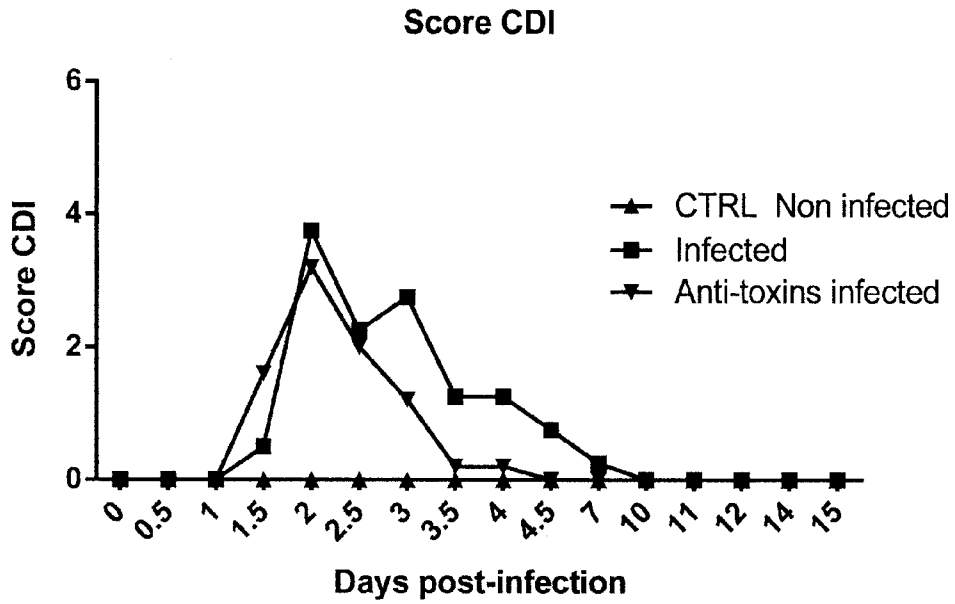


FIGURE 10

A


Score CDI
Score CDI
6
4
2
0
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
7
10
11
12
14
15
Days post-infection
CTRL Non infected
Infected
Anti-toxins infected

B

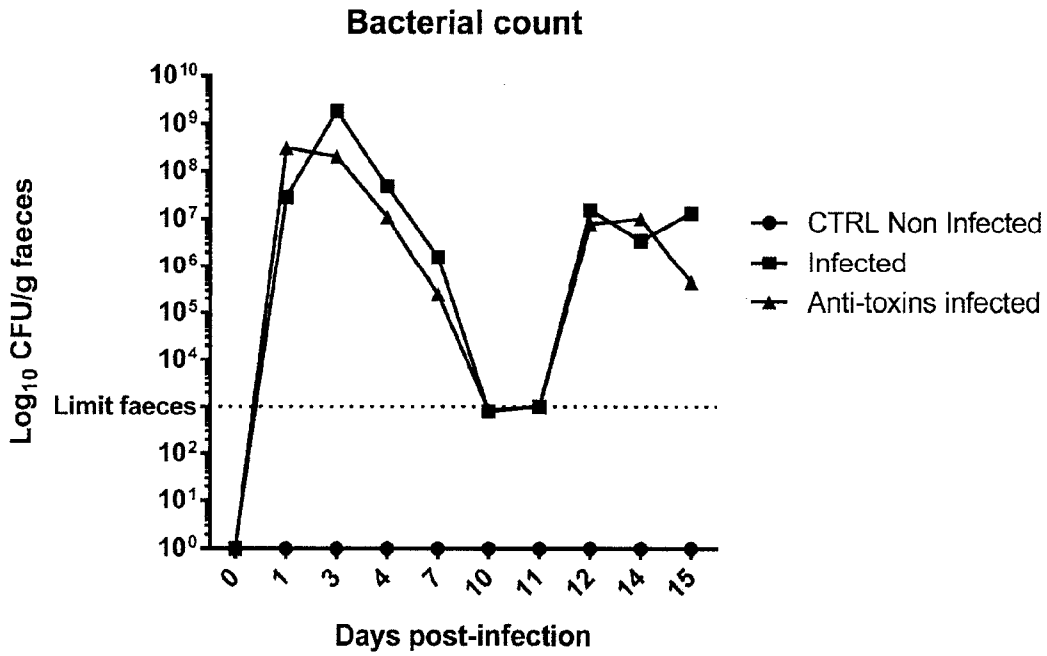

Bacterial count
Log10 CFU/g faeces
10^10
10^9
10^8
10^7
10^6
10^5
10^4
Limit faeces
10^2
10^1
10^0
0
1
3
4
7
10
11
12
14
15
Days post-infection
CTRL Non Infected
Infected
Anti-toxins infected

FIGURE 11

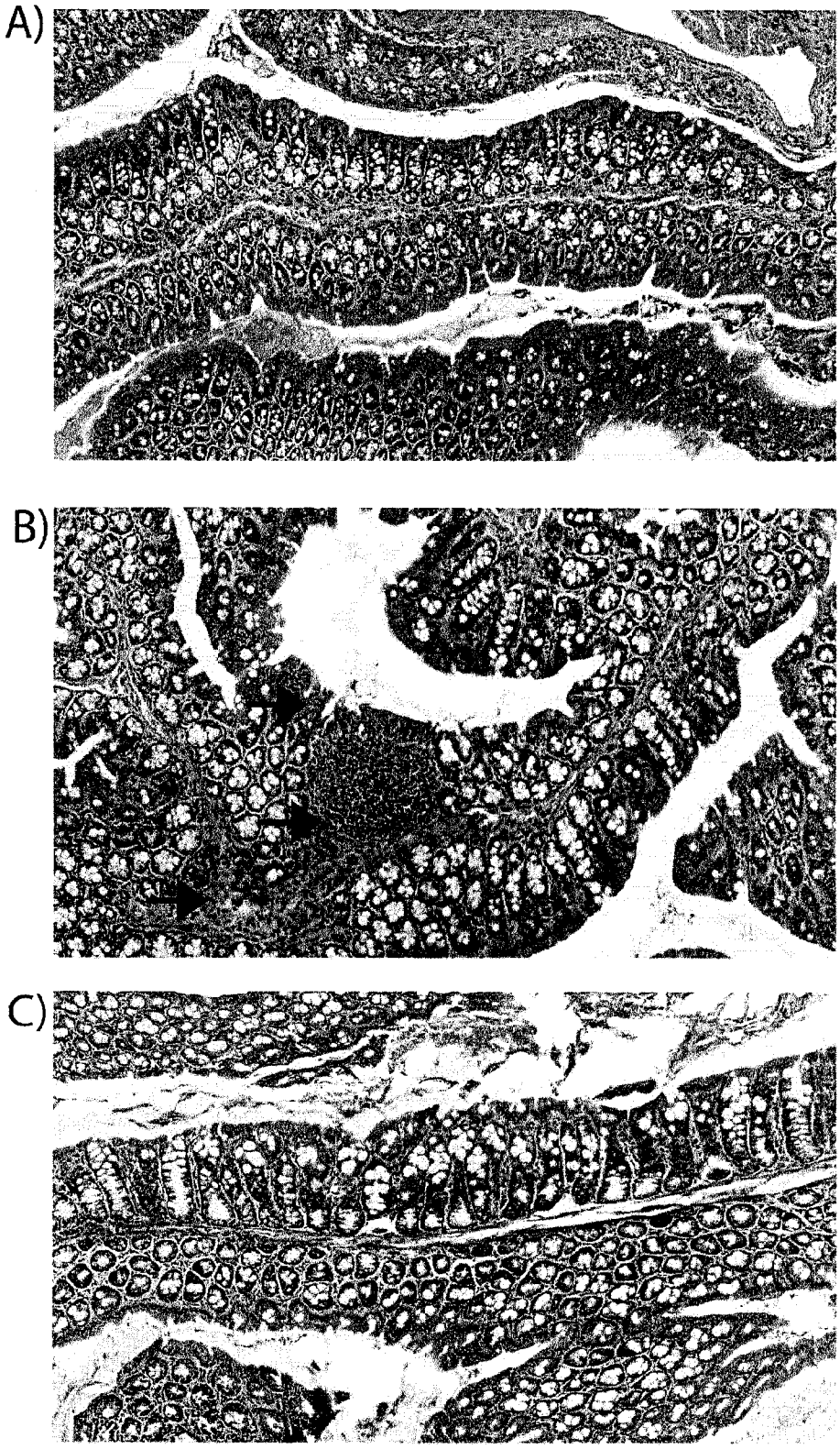
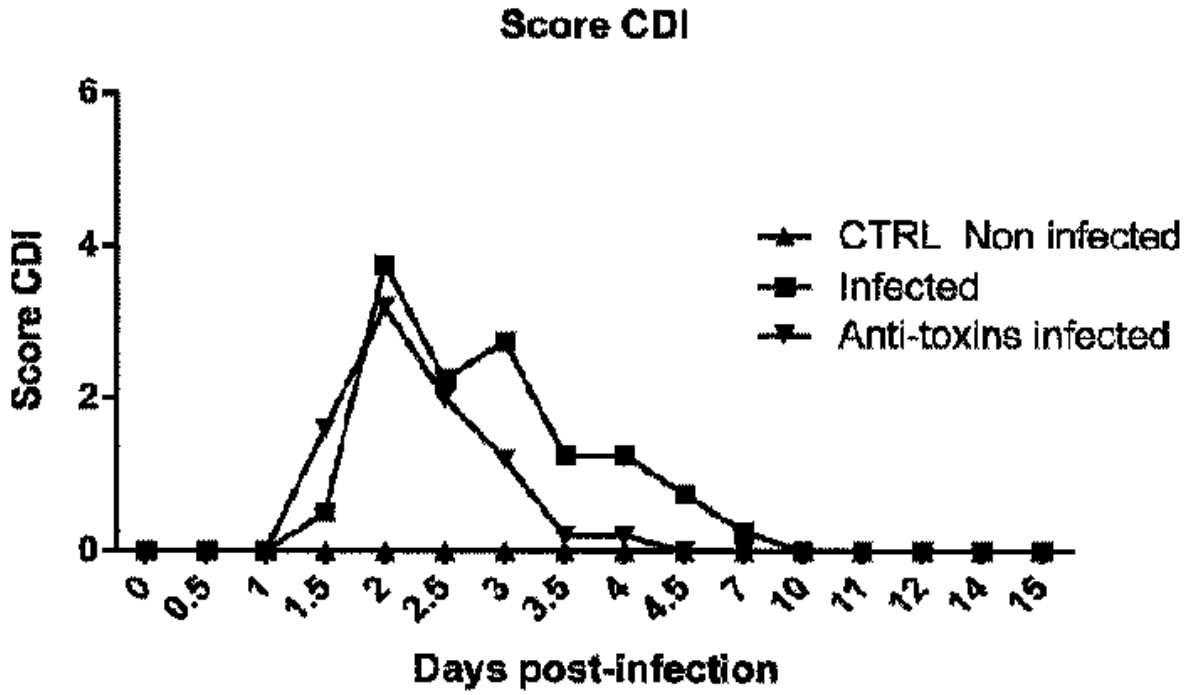
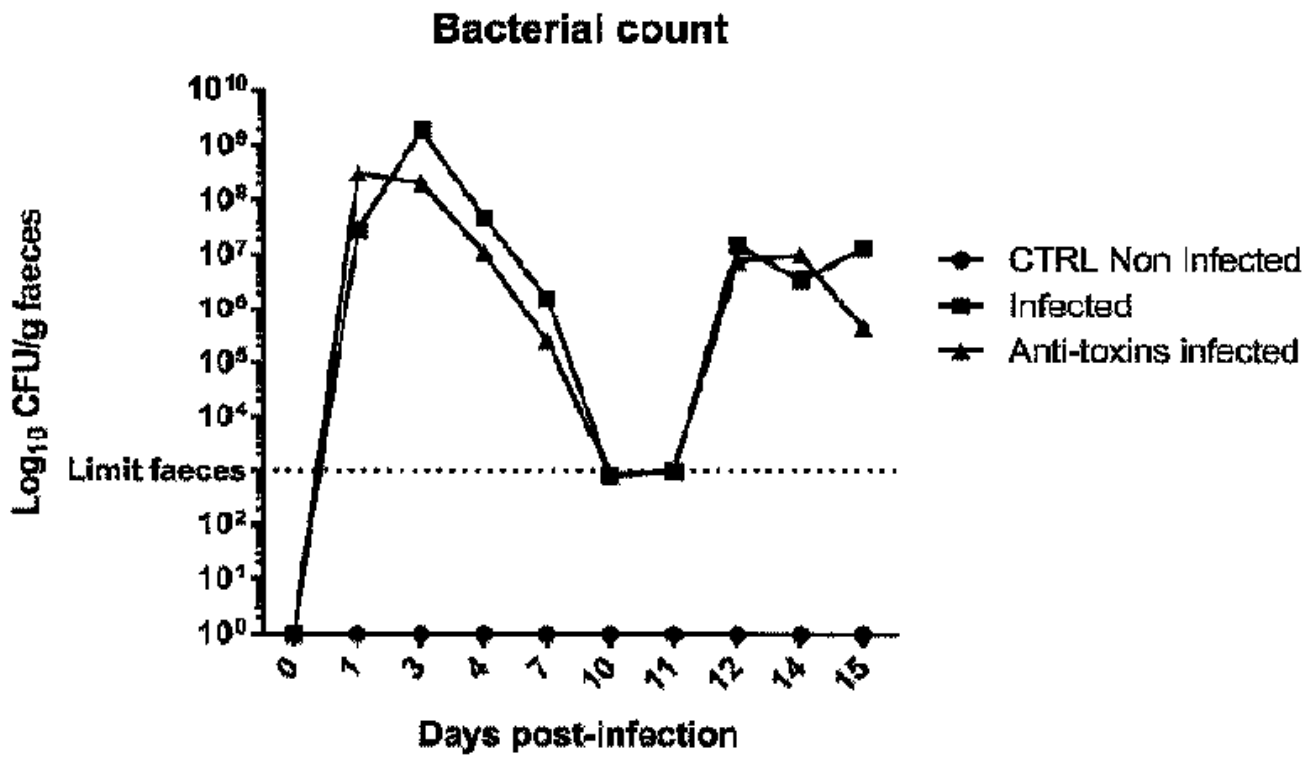


FIGURE 12

A


Score CDI
Score CDI
6
4
2
0
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
7
10
11
12
14
15
Days post-infection
CTRL Non infected
Infected
Anti-toxins infected

B


Bacterial count
Log10 CFU/g faeces
10^10
10^9
10^8
10^7
10^6
10^5
10^4
Limit faeces
10^2
10^1
10^0
0
1
3
4
7
10
11
12
14
15
Days post-infection
CTRL Non Infected
Infected
Anti-toxins infected