a second enzyme activity in the form of a cysteine protease which appears to play a role in the release of the effector domain into the cytosol after translocation. The *C. difficile* binary toxin modifies cell actin by a mechanism which involves the transfer of an ADP-ribose moiety from NAD onto its target protein.

Treatment of *C. difficile* infection currently relies on antibiotics of which metronidazole and vancomycin constitute those of choice. However, these antibiotics are not effective in all cases and 20-30% of patients suffer relapse of the disease. Of major concern is the appearance in the UK of more virulent strains which were first identified in Canada in 2002. These strains, which include those belonging to PCR ribotype 027, toxinotype III, cause CDI with a directly attributable mortality more than 3-fold that observed previously.

New therapeutics are therefore required especially urgently since the efficacy of current antibiotics appears to be decreasing.

Accordingly, there is a need in the art for new therapies/therapeutics capable of specifically addressing *C. difficile* infection (CDI). This need is addressed by the present invention, which solves one or more of the above-mentioned problems.

Summary of Invention

In an aspect, the present invention provides an antibody composition comprising polyclonal ovine antibodies, for use in the prevention or treatment of *C. difficile* infection wherein the antibodies bind to a *C. difficile* toxin; optionally wherein said prevention or treatment is by oral delivery of the antibody composition.

In another aspect, the present invention provides a pharmaceutical composition for oral delivery, comprising the antibody composition as described herein, together with at least one component selected from a buffer, excipient and/or salt; optionally including one or more means for protecting said antibody composition from trypsin and/ or chymotrypsin and/ or stomach acid.

In another aspect, the present invention provides the use of the antibody composition as described herein or a pharmaceutical composition as described herein in the manufacture of a medicament for prevention or treatment of *C. difficile* infection, optionally wherein said medicament is to be administered via oral administration.

In another aspect, the present invention provides a pharmaceutical composition as described herein, for use in the prevention or treatment of *C. difficile* infection.

In another aspect, the present invention provides a method of producing ovine antibodies for use in the oral antibody composition as described herein, or for use in a pharmaceutical composition as described herein, wherein the ovine antibodies are elicited by a sheep in response to an immunogen comprising a *C. difficile* toxin or a fragment thereof.

In more detail, ~~a first aspect of the present invention provides~~disclosed are ovine antibodies, for oral use in the prevention or treatment of CDI. Said oral therapy provides a simple treatment/ prevention/ suppression of CDI with unexpected efficacy and/ or with reduced side-effects. In ~~another aspect~~one example, the ~~invention~~disclosure provides an antibody composition comprising the ovine antibodies, in a form suitable for oral use in the prevention or treatment of CDI. In one ~~embodiment~~example, the ovine antibodies are polyclonal antibodies.

In use, the antibodies of the ~~invention~~disclosure bind to a *C. difficile* toxin or a fragment thereof, preferably neutralising the biological activity of the toxin or fragment thereof. Accordingly, the antibodies of the present ~~invention~~disclosure are capable of preventing or treating CDI, and/ or preferably also preventing a relapse in a patient.

The antibody therapy of the present ~~invention~~disclosure provides a distinct advantage over other therapies in that it is able to inhibit the biological action of one or more of the toxins of *C. difficile*, whilst having a minimal or low immunogenic effect on a patient. Moreover, the antibodies of the present ~~invention~~disclosure can be produced with very high toxin-neutralising titres. Thus, the ovine antibodies can be readily obtained and can protect

the patient against the pathological effects produced by *C. difficile* with minimal or no side-effects. The antibodies of the present ~~invention~~disclosure may also be used prophylactically to prevent the onset of CDI.

The principal targets of the present ~~invention~~disclosure are *C. difficile* toxins or fragments thereof. Suitable *C. difficile* toxins, to which the antibodies of the ~~invention~~disclosure may bind to and/or neutralise, include any *C. difficile* toxins that cause or are associated with CDI or a symptom thereof. In a further ~~embodiment~~example, the antibodies of the invention bind to and/or neutralise one or more type of *C. difficile* toxin selected from the following: *C. difficile* Toxin A or a fragment thereof, *C. difficile* Toxin B or a fragment thereof, and *C. difficile* Binary Toxin or a fragment thereof.

Thus, in one ~~embodiment~~example, the antibody composition of the present ~~invention~~disclosure comprises ovine antibodies that bind to and/or neutralise *C. difficile* Toxin A (or a fragment thereof). In another ~~embodiment~~example, the antibody composition of the present ~~invention~~disclosure comprises ovine antibodies that bind to and/or neutralise *C. difficile* Toxin B (or a fragment thereof). In yet another ~~embodiment~~example, the antibody composition of the present ~~invention~~disclosure comprises ovine antibodies that bind to and/or neutralise *C. difficile* Binary Toxin (or a fragment thereof).

In another ~~embodiment~~example, the antibody composition of the present ~~invention~~disclosure comprises ovine antibodies that bind to and/or neutralise *C. difficile* Toxin A (or a fragment thereof) and to *C. difficile* Toxin B (or a fragment thereof). In another ~~embodiment~~example, the antibody composition of the present ~~invention~~disclosure comprises ovine antibodies that bind to and/or neutralise *C. difficile* Toxin A (or a fragment thereof) and to *C. difficile* Binary Toxin (or a fragment thereof). In yet another ~~embodiment~~example, the antibody composition of the present ~~invention~~disclosure comprises ovine antibodies that bind to and/or neutralise *C. difficile* Toxin B (or a fragment thereof) and to *C. difficile* Binary Toxin (or a fragment thereof).

The antibody composition of the present ~~invention~~disclosure may also comprise ovine antibodies that bind to and/or neutralise *C. difficile* Toxin A (or a fragment thereof), to *C. difficile* Toxin B (or a fragment thereof) and to *C. difficile* Binary Toxin (or a fragment thereof).

The antibodies of the present ~~invention~~disclosure interact with specific epitopes of the toxin. For example, an antibody can bind an epitope in the N-terminal domain (e.g. between amino acids 1-957) or the mid-region domains (e.g. between amino acids

958-1831) or the C-terminal repeat domains (e.g. between amino acids 1832-2710) of *C. difficile* Toxin A. For example, the antibody may bind to an epitope within amino acids 1832-2710 of *C. difficile* Toxin A. Similarly an antibody can bind an epitope in the N-terminal domain (e.g. between amino acids 1-955) or the mid-region domains (e.g. between amino acids 956-1831) or the C-terminal repeat domains (e.g. between amino acids 1832-2366) of Toxin B. For example, an antibody may bind to an epitope within amino acids 1832-2366 of Toxin B. In the case of the binary toxin antibodies may bind to the catalytic domain (Fragment A) or the receptor binding domain, which resides in the C-terminal portion of Fragment B (approx residues 400-870); and/ or to the N-terminal half of Fragment B (approx residues 1-400), which is involved in the binding and translocation of Fragment A into the cell.

In one ~~embodiment~~example, the *C. difficile* toxin is selected from one of toxinotypes 0 to XV. Preferred toxinotypes (plus example Ribotypes and Strains) are listed in the Table immediately below. The listed toxinotypes are purely illustrative and are not intended to be limiting to the present invention.

Toxinotype	Example Ribotypes	Example Strains	Reference
0	001, 106	VPI10463	Rupnik et al. (1998) J. Clinical Microbiol. 36: 2240-2247
I	003, 012, 102	EX623	
II	103	AC008	
III	027, 034, 075, 080	R20291, QCD-32g58	
IV	023, 034, 075, 080	55767	
V	066, 078	SE881	
VI	045, 063, 066	51377	
VII	063	57267	
VIII	017, 047	1470	
IX	019	51680	
X	036	8864	
XI	033	IS58, R11402	Rupnik et al. (2001) Microbiology 147: 439-447
XII	056	IS25	
XIII	070	R9367	
XIV	111	R10870	
XV	122	R9385	

Different antibodies of the present ~~invention~~disclosure may bind to and/or neutralise a *C. difficile* toxin from the same or from different strains of *C. difficile*. For example, the antibodies may bind to and/or neutralise one or more of the following: *C. difficile*

Toxin A - Toxinotype 0; *C. difficile* Toxin B - Toxinotype 0; *C. difficile* Toxin A - Toxinotype III; *C. difficile* Toxin B - Toxinotype III; *C. difficile* Toxin A - Toxinotype V; and/or *C. difficile* Toxin B - Toxinotype V. Preferably, a mixture of antibodies is employed, which bind to and/ or neutralise Toxins A and B from all or most of these Toxinotypes. An antibody of the present ~~invention~~disclosure may bind to an epitope in the N-terminal domain, the mid-region domain, and/or the C-terminal repeat domain of said strains of *C. difficile* Toxin A and/ or *C. difficile* Toxin B and/ or *C. difficile* Binary Toxin.

In certain ~~embodiments~~examples, the antibodies of the present ~~invention~~disclosure may bind to and/or neutralise at least one *C. difficile* toxin comprising an amino acid sequence at least 80%, 85%, 90%, 95%, 98%, 99%, or more identical to SEQ ID NOs: 1-6, or a fragment thereof.

The ~~invention~~disclosure also embraces a corresponding method for prevention or treatment of CDI, said method comprising oral administration of the antibody composition of the present ~~invention~~disclosure to a patient. The patient can be infected with *C. difficile*, or have a symptom of *C. difficile* (e.g. mild self-limiting diarrhoea, abdomenal pain, fever and loss of appetite to life-threatening conditions such as pseudomembranous colitis and cytotoxic megacolon) or have a predisposition towards *C. difficile* infection (e.g. undergoing treatment with antibiotics, having experienced *C. difficile* and at risk of relapse, or exposed to a second individual who has shown the clinical symptoms associated with *C. difficile* infection). The present ~~invention~~disclosure thereby provides an effective means for preventing, suppressing or treating CDI (or a symptom thereof).

In one ~~embodiment~~example, said method of treating CDI comprises oral administration of the antibody composition of the present ~~invention~~disclosure to a patient infected with *C. difficile*, or suffering from the symptoms of CDI. This can be accomplished using a therapeutically effective amount of the antibodies. Such administration may be effected by repeated administrations of antibody compositions of the present invention, for a prolonged period of time. The antibody components of said compositions may be the same or different (in terms of their toxinotype specificity and/ or targeted binding region or epitope on a *C. difficile* Toxin), and administration can be concurrent or sequential, and can be effected in any order.

In another ~~embodiment~~example, said method of preventing CDI comprises oral administration of the antibody composition of the present ~~invention~~disclosure to a patient to provide passive immunity against CDI. This can be accomplished using a prophylactically effective

amount of the antibodies prior to the onset or in the very early stages of CDI. Such administration may be effected by repeated administrations of antibody compositions of the present ~~invention~~disclosure, for a prolonged period of time. The antibody components of said compositions may be the same or different (in terms of their toxinotype specificity and/ or targeted binding region or epitope on a *C. difficile* Toxin), and administration can be concurrent or sequential, and can be effected in any order.

In another ~~embodiment~~example, said method of treating CDI comprises administering antibody systemically (eg. once or twice per day, or once or twice or 3- or 4-times per every 3-4 days; for a short period of typically 1-2 weeks) followed by a more prolonged period of oral administration (eg. once or twice or 3- or 4- or 5- or 6-times per day, or once or twice or 3- or 4- or 5- or 6-times per every 3-4 days, or once or twice or 3- or 4- or 5- or 6-times per week) of antibody. In this ~~embodiment~~example, the systemically administered antibody is provided as a formulation suitable for that route and the orally administered antibody is provided in the form of a composition of the present invention. Such administration may be effected by giving one or more administrations of antibody via the systemic route followed by repeated oral administrations of antibody compositions of the present invention, for a more prolonged period of time. The antibody components of said compositions may be the same or different (in terms of their toxinotype specificity and/ or targeted binding region or epitope on a *C. difficile* Toxin).

In another ~~embodiment~~example, the above oral administration may be performed prior to or simultaneously with a corresponding systemic administration of said antibodies. Naturally, when administered systemically, the antibodies are formulated accordingly (eg. such formulations are typically provided as isotoxic aqueous formulations and do not require means for protection against stomach acid or stomach enzymes such as trypsin and/ or chymotrypsin).

In one ~~embodiment~~example, the subject to be treated or protected is a subject in one or more or the following category: hospitalised; over 65 or 70 years' old; receiving broad-range spectrum antibiotics; having previous CDI history/ infection; having close proximity to symptomatic CDI patients; having mild-to-moderate disease severity; presenting as asymptomatic but considered at high risk of relapse (eg. because of one or more relapse episodes); having close proximity to CDI outbreak areas or patients.

Detailed Description

Antibody preparation