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[Continued on next page]

(54) Title: ANTI-SALMONELLA ANTIBODIES AND USES THEREOF

(57) Abstract: The present disclosure provides anti-Salmonella antibodies or antibody fragments, such as camelid single domain antibodies (VHHs), along with associated nucleic acids, host cells and phages. Methods of reducing the presence of Salmonella in an animal or an animal environment, methods and formulations for treating Salmonella infection, and methods of detecting Salmonella are also described.

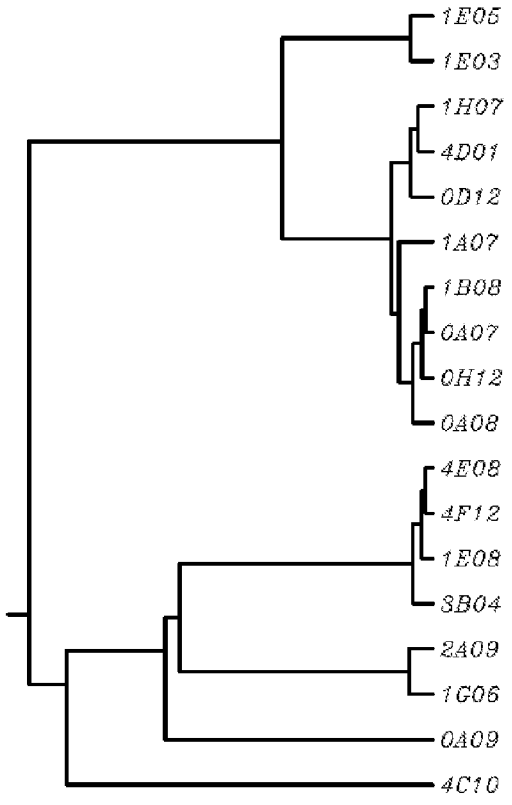

FIG. 2

WO 2016/125089 A1

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ANTI-SALMONELLA ANTIBODIES AND USES THEREOF

FIELD OF THE INVENTION

[0001] The field of the present invention relates generally to antibodies, fragments thereof, derivatives thereof, and to uses and applications of such antibodies. The antibodies and fragments described may be specifically directed against Salmonella.

BACKGROUND ART

[0002] Salmonellosis is one of the most commonly reported zoonotic diseases in humans. In the United States alone, it causes an estimated 1.3 million human food-borne illnesses and more than 500 deaths each year (Messens et al., 2013). Salmonella serotypes *enteritidis* and *typhimurium* are frequently detected in human infections (Ravel et al., 2010). Salmonellas are widely distributed in nature, and they are commonly carried by wild or farm-animal vectors. Poultry is known to be a major global reservoir of Salmonellas. Salmonella live in poultry gut as transient members of the intestinal microbial population without causing disease. Colonization of Salmonella does not usually affect poultry body weight gain or performance; thus, asymptomatic infection can increase the likelihood of zoonotic transmission to humans through the food chain (Hugas et al., 2014; Mazengia et al., 2014). Chicks can become infected vertically (from adults via the egg to the chick) or horizontally (from the environment, pests, or feed) (Cox et al., 2014; Rodriguez et al., 2006).

[0003] *Salmonella enterica* is one of the two main Salmonella species that causes gastroenteritis in humans. *S. enterica* is subdivided into 6 subspecies and almost all human infections are caused by subspecies I (*enterica*). More than 2600 serovars of *S. enterica* have been identified (Popoff and Le Minor, 1997); however, only a few of these serovars are responsible for most Salmonella infections in human and domestic animals (Porwollik et al., 2004).

[0004] The large and growing market for broiler chickens and eggs, and the emergence of antibiotic resistant strains of Salmonella have led to public health concerns, change in government regulation policies in Europe and North America and further demands to enact laws to control Salmonella levels in poultry (Hugas, et al., 2014).

[0005] Vaccination strategies in broiler chickens have shown sub-optimal results to-date mostly due to the short life span of the birds. Currently, two types of Salmonella vaccines are commercially available; an attenuated live vaccine and an inactivated vaccine. These vaccines are often administered to both breeder and layer flocks, but their effectiveness

depends on the targeted serovar, host species, and whether reduction rather than eradication is the objective (Doyle and Erickson, 2006). These vaccines do not eliminate initial colonization of the mucosal surfaces, particularly in the young bird (Dougan et al., 1988). Effective control depends upon a number of factors, including improved on-farm biosecurity, use of best practices in husbandry and use of vaccination and competitive exclusion products and feed additives. Preventive hygienic measures typically involve establishing effective farm-site biosecurity and poultry house sanitation protocols. Other more targeted strategies are being developed. For instance, a combination of Salmonella-specific lytic phages has been recently approved in Europe for applications in food packaging. Others have proposed and tested inclusion of bacteriocins and/or tailspike phage protein (Chakchouk-Mtibaa et al., 2014; Waseh et al., 2010) in the poultry feed for controlling Salmonella but to date none of these products has been commercialized.

[0006] It would be advantageous to provide antibodies or fragments thereof that assist in the reduction, prevention and/or treatment of Salmonella infection.

SUMMARY OF THE INVENTION

[0007] This disclosure refers to the development of camelid single domain antibodies (VHHs) that bind to Salmonella. VHHs are the smallest antigen binding fragments that can be readily expressed in bacteria or yeast in large quantities and at a significantly lower cost compared to conventional antibodies.

[0008] Accordingly, disclosed herein is an isolated antibody or antibody fragment comprising an amino acid sequence of any one of SEQ ID NOS:1-18, or a variant thereof. In one embodiment, the isolated antibody or antibody fragment binds directly to the exterior of Salmonella, optionally to Salmonella flagella.

[0009] In a preferred embodiment of the present disclosure, the Salmonella-binding antibody or antibody fragment comprises a CDR1 comprising an amino acid sequence of GRX_1FSX_2KP; a CDR2 comprising an amino acid sequence of ASX_3TGVST; and a CDR3 comprising an amino acid sequence of $AGTX_4RTLWGSKWRDX_5X_6EYEY$; wherein X_1 is T or S; X_2 is V or K; X_3 is F or Y; X_4 is T or L; X_5 is V or R; and X_6 is L or R.

[00010] In another preferred embodiment, the Salmonella-binding antibody or antibody fragment comprises a CDR1 comprising an amino acid sequence of GLDFSSYA; a CDR2 comprising an amino acid sequence of ISRFGGRL; and a CDR3 comprising an amino acid sequence of AADRRSGLGTSKEYDY.

[00011] In another preferred embodiment, the Salmonella-binding antibody or antibody fragment comprises a CDR1 comprising an amino acid sequence of GIIFSINA; a CDR2 comprising an amino acid sequence of ISAYDHT; and a CDR3 comprising an amino acid sequence of NVDEIRKF.

[00012] In another preferred embodiment, the Salmonella-binding antibody or antibody fragment comprises a CDR1 comprising an amino acid sequence of GRSFSLYG; a CDR2 comprising an amino acid sequence of ISGSGLATS; and a CDR3 comprising an amino acid sequence of AQRWTSGTIARATGEYGY.

[00013] In another preferred embodiment, the Salmonella-binding antibody or antibody fragment comprises a CDR1 comprising an amino acid sequence of GSIFSGDA; a CDR2 comprising an amino acid sequence of IGKEGDT; and a CDR3 comprising an amino acid sequence of ATFEERPQPSYVY.

[00014] In a preferred embodiment of the present disclosure, the isolated antibody or antibody fragment disclosed herein is modified for tolerance to one or more gut enzymes selected from the group consisting of pepsin, trypsin and chymotrypsin. In a further preferred embodiment, the antibody or antibody fragment disclosed herein comprises a detectable label.

[00015] The present disclosure further provides a nucleic acid molecule encoding the isolated antibody or antibody fragment disclosed herein, a host cell comprising the nucleic acid molecule, and a bacteriophage comprising the nucleic acid or the polypeptide.

[00016] Another preferred aspect of the present disclosure is a method of reducing the presence of Salmonella in an animal or an animal environment comprising administering to the animal the isolated antibody or antibody fragment disclosed herein. In one preferred embodiment, the method further comprises administering an antibiotic, bacteriocin, or other plant- or animal-derived compound effective against Salmonella to the animal. In another preferred embodiment, the method further comprises administering a competing microbe to the animal together with an antibody or antibody fragment disclosed herein, optionally co-expressed or co-contained in a probiotic system. The antibody or antibody fragment may be administered orally; the animal may be a chicken, optionally a laying hen or broiler chicken; and the animal environment may be a poultry farm.

[00017] Also disclosed is a method of reducing or preventing introduction of Salmonella into an animal environment comprising administering to an inductee animal the antibody or antibody fragment disclosed herein, prior to introducing the inductee animal into the animal environment.

[00018] Also disclosed is a method of treating a Salmonella infected subject comprising administering to the subject the isolated antibody or antibody fragment disclosed herein. In a preferred embodiment, the method of treating an infected subject further comprises administering to the subject antibiotic effective against Salmonella. The subject may be a livestock animal selected from the group consisting of a chicken, cow, or sheep, or the subject may be a human.

[00019] Also disclosed is a formulation for use in treating Salmonella infection comprising the isolated antibody or antibody fragment disclosed herein and a pharmaceutically acceptable excipient.

[00020] Further disclosed is a use of the isolated antibody or antibody fragment disclosed herein for treating Salmonella infection in a subject in need thereof.

[00021] Further disclosed is a method of detecting Salmonella in a sample comprising contacting the sample with the isolated antibody or antibody fragment disclosed herein, and detecting the presence of bound antibody or antibody fragment. In one preferred embodiment, the sample comprises a bodily fluid or fecal material. In another preferred embodiment, the sample comprises a food product or a surface swab from a food product.

[00022] Another aspect of the present disclosure is a kit for conducting the detection method, comprising the isolated antibody or antibody fragment disclosed herein and instructions for use in detecting Salmonella.

[00023] Another aspect provides use of the antibody or antibody fragment disclosed herein for preparation of a medicament for treatment of Salmonella infection.

BRIEF DESCRIPTION OF THE DRAWINGS

[00024] Further aspects and advantages will become apparent from the following description taken together with the accompanying drawings in which:

[00025] Figure 1 shows exemplary amino acid sequences of the anti-Salmonella antibodies and antibody fragments of the present disclosure and nucleic acid sequences encoding said antibodies and antibody fragments;

[00026] Figure 2 is a dendrogram showing amino acid sequence similarities between the anti-Salmonella antibodies and antibody fragments;

[00027] Figure 3 shows purified anti-Salmonella VHH domains on SDS-PAGE gel stained with Coomassie Brilliant Blue™ G250;

[00028] Figure 4 is a bar graph showing results of a binding assay for 18 different anti-Salmonella VHH domains to flagellin of Salmonella or whole cells of *Salmonella enterica*

strains: S. Heidelberg 918, S. Heidelberg 4643, S. Hadar 5643, or S. Hadar 5659 (binding measured at A450);

[00029] Figure 5 is a bar graph showing the results of a motility assay for *S. enterica* strain SG4904 in the presence of 4 different anti-Salmonella VHH domains (y-axis shows the diameter of the outgrowth of bacterial colonies);

[00030] Figure 6 is a bar graph showing the results of a cell proliferation assay of *S. enterica* strain SG4904 in the presence of 4 different anti-Salmonella VHH domains (bacterial growth rate determined by measuring OD_{600});

[00031] Figure 7 is a bar graph showing the activity of three VHHs against *S. enterica* serovar Hadar 5643 in a HeLa cell Salmonella internalization assay;

[00032] Figure 8 is a bar graph showing the activity of different concentrations of three VHHs against *S. enterica* serovar Hadar 5643 in a cultured chicken ileum or jejunum Salmonella internalization assay, as measured by Salmonella genome copy number (based on amplification of the single copy housekeeping gene *ttr*); and

[00033] Figure 9 is a scatter plot showing the effects of administering VHH 0A07 to non-SPF broiler chicks challenged with *S. enterica* serovar Hadar 5643 (CFU counts for ileum and jejunum sections are shown).

DETAILED DESCRIPTION

[00034] The present disclosure is based on the creation, isolation, and characterization of antibodies and antibody fragments that preferably have the ability to bind to the exterior of Salmonella cells. Features and uses of said antibodies and antibody fragments will now be described in greater detail. It will be appreciated that exemplary embodiments presented herein are within the scope of the present invention and are not intended as limiting. Reference is made to the Figures which relate to preferred embodiments of the present invention.

DEFINITIONS

[00035] The term "antibody" as used herein refers to a full length immunoglobulin that has the ability to bind to an antigen. The term "antibody fragment" as used herein refers to a less than full length portion of an immunoglobulin molecule—for example, a VHH domain—which retains the ability to bind to an antigen.

[00036] The term "VHH" or "VHH domain" as used herein refers to a single domain antibody derived from a heavy chain antibody raised in a camelid animal, such as a llama,

alpaca, or camel. Other terms for VHHs sometimes used in the art include but are not limited to: single domain antibodies (sdAbs), single variable domain antibodies, immunoglobulin single variable domains, heavy-chain variable domain antibodies, and Nanobodies™.

[00037] The term "isolated" or "purified" as used herein in association with a polypeptide, antibody, antibody fragment or a nucleic acid means a polypeptide, antibody, antibody fragment or nucleic acid that is substantially or essentially free of naturally associated molecules – for example, an isolated antibody that is substantially or essentially free of antibodies having different specificities.

[00038] The term "multimeric" or "multivalent" as used herein refers to having multiple antigen-binding locations on a polypeptide, typically from multiple copies of an antibody or antibody fragment, or from a plurality of similar but different such antibodies or antibody fragments.

[00039] The term "nucleic acid" as used herein refers to double stranded or single stranded DNA, RNA molecules or DNA/RNA hybrids. These molecules may be nicked or intact as found in living cells. The double stranded or single stranded nucleic acid molecules may be linear or circular. The duplexes may be blunt ended or have single stranded tails, for example, with sticky ends created by restriction endonucleases.

[00040] The term "variant" as used herein refers to an amino acid or nucleotide sequence having at least 80% identity or sequence homology with a subject amino acid or nucleotide sequence.

ANTIBODIES AND ANTIBODY FRAGMENTS

[00041] According to one aspect of the present disclosure, provided herein is an isolated antibody or antibody fragment comprising an amino acid sequence of any one of SEQ ID NOS:1-18, or a variant thereof. In a preferred embodiment, said antibody or antibody fragment binds to Salmonella. In a further preferred embodiment, said antibody or antibody fragment binds to the flagella of Salmonella. The aforementioned antibodies or antibody fragments are preferably derived from a collection of antibodies raised in alpacas that were immunized with heat inactivated Salmonella cells, as described in these Examples below. Each of SEQ ID NOS:1-18 corresponds to the amino acid sequence of an isolated VHH domain from said collection and said sequences are shown in Figures 1A, 1B, 1C, 1D, 1E and 1F.

[00042] VHH domains are comprised of framework regions interspersed with three complementarity determining regions (CDRs): CDR1, CDR2, and CDR3. CDR sequences are

known to be essential for the specificity of binding between antibodies (or antibody fragments) and antigens. Accordingly, in a preferred embodiment of the present disclosure, the antibody or antibody fragment disclosed herein comprises a CDR1 comprising an amino acid sequence of GLDFSSYA (SEQ ID NO:40); a CDR2 comprising an amino acid sequence of ISRFGGRL (SEQ ID NO:41); and a CDR3 comprising an amino acid sequence of AADRRSGLGTSKEYDY (SEQ ID NO:42). In another preferred embodiment, CDR1 comprises an amino acid sequence of GIIFSINA (SEQ ID NO:43); CDR2 comprises an amino acid sequence of ISAYDHT (SEQ ID NO:44); and CDR3 comprises an amino acid sequence of NVDEIRKF (SEQ ID NO:45). In another preferred embodiment, CDR1 comprises an amino acid sequence of GRSFSLYG (SEQ ID NO:46); CDR2 comprises an amino acid sequence of ISGSGLATS (SEQ ID NO:47); and CDR3 comprises an amino acid sequence of AQRWTSGTIARATGEYGY (SEQ ID NO:48). In a further preferred embodiment, CDR1 comprises an amino acid sequence of GSIFSGDA (SEQ ID NO:49); CDR2 comprises an amino acid sequence of IGKEGDT (SEQ ID NO:50); and CDR3 comprises an amino acid sequence of ATFEERPQPSYVY (SEQ ID NO:51).

[00043] Consensus sequences can be defined based on some of the above-specified CDR sequences, as described in the Examples below and illustrated in Table 2. In a preferred embodiment of the present disclosure, an isolated antibody or antibody fragment is herein provided that binds Salmonella and comprises a CDR1 comprising an amino acid sequence of GRX_1FSX_2KP (SEQ ID NO:37); a CDR2 comprising an amino acid sequence of ASX_3TGVST (SEQ ID NO:38); and a CDR3 comprising an amino acid sequence of $AGTX_4RTLWGSKWRDX_5X_6EYEY$ (SEQ ID NO:39); wherein X_1 is T or S; X_2 is V or K; X_3 is F or Y; X_4 is T or L; X_5 is V or R; and X_6 is L or R. Optionally, CDR1 comprises an amino acid sequence of GRTFSVKP (SEQ ID NO:52); CDR2 comprises an amino acid sequence of ASFTGVST (SEQ ID NO:53); and CDR3 comprises an amino acid sequence of AGTTRTLWGSKWRDVLEYEY (SEQ ID NO:54). Optionally, CDR1 comprises an amino acid sequence of GRSFSVKP (SEQ ID NO:55); CDR2 comprises an amino acid sequence of ASFTGVST (SEQ ID NO:53); and CDR3 comprises an amino acid sequence of AGTLRTLWGSKWRDRREYEY (SEQ ID NO:56). Optionally, CDR1 comprises an amino acid sequence of GRTFSKKP (SEQ ID NO:57); CDR2 comprises an amino acid sequence of ASYTGVST (SEQ ID NO:58); and CDR3 comprises an amino acid sequence of AGTTRTLWGSKWRDVLEYEY (SEQ ID NO:54).

[00044] Any of the antibody fragments described herein may be utilized in an isolated form, or may form a portion of a longer molecule, such as within an antibody, for example a

recombinant antibody, a chimeric antibody, a small molecule conjugated antibody, a human antibody, or a humanized antibody.

[00045] Furthermore, an antibody fragment may include, but is not limited to Fv, single-chain Fv (scFv; a molecule consisting of V_L and V_H connected with a peptide linker), Fab, Fab', and $F(ab')_2$, or single domain antibody (sdAb). SdAbs may be of camelid origin, and thus may be based on camelid framework regions; alternatively, the CDRs may be grafted onto the framework regions of other antibody domains, for example but not limited to VNAR, human V_H or human V_L framework regions.

[00046] The present invention includes modifications of the antibodies or antibody fragments disclosed herein, and may include amino acid variations, including conservative substitutions, additions or deletions, provided at least 80%, preferably at least 90%, identity or sequence homology is observed and provided such a modification results in a functional variant. In a preferred embodiment, the percent identity is set at 90% or greater, and thus it is to be understood that an identity of each VHH domain can, for example, be individually determined as 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% of specified sequences.

[00047] It will be appreciated that the present antibodies or antibody fragments can also be preferably produced in multimeric forms. For example, a dimer or pentamer can be formed. Antibody fragments, such as VHHs, that are used to form a multimer may be the same or different from each other. Pentavalent multimeric VHH domains, or pentabodies, may possess higher affinity binding to an antigen as compared with monovalent VHH domains. The five VHH domains need not be identical to one another, and as such may comprise VHH domains of different sequences.

[00048] The antibodies or antibody fragments described herein may preferably be modified for tolerance or resistance to one or more gut enzymes. Typical gut enzymes which may have a destructive effect on a polypeptide include pepsin, trypsin and chymotrypsin. Thus, resistance to these enzymes is advantageous, as the peptide would have more exposure time to bind with ambient Salmonella within the intestinal tract. Single domain antibodies are, in general, significantly more resistant to proteases than conventional antibodies. Furthermore, VHHs are known to be amenable to polypeptide engineering for optimization of biophysical features including heat and protease resistance (Hussack et al., 2014). Scaffold engineering of portions of the polypeptide both inside and outside of the CDR regions are known in the art and can confer increased target affinity, protease resistance, as well as thermal and low pH resistance. For example, to favour the entropy of binding, the extended flexible CDR3 loop

may be constrained with an interloop disulfide bond that connects CDR1 and CDR3, or CDR2 position 55 and CDR3 or FR2 at position 50 and CDR3 (Muyldermans, 2013; Conrath et al, 2003). The cysteine of CDR3 that participates in either disulfide bond formation mentioned above can occur or be placed at any position of the extended CDR3 loop (Conrath et al, 2003). The stability of a VHH can be increased by introducing cysteine at position 54 and 78 to form an additional disulfide bond. This disulfide bond is known to make VHHs highly resistant to degradation by pepsin or chymotrypsin (Hagihara et al, 2007; Saerens et al, 2008; Hussack et al, 2014).

[00049] The antibodies or antibody fragments described herein may preferably be labeled with an acceptable label and optionally a linker as needed. The label may be rendered detectable or may in itself be detectable, so that the presence of binding to Salmonella can be observed. The antibody or antibody fragment may be linked to a radioisotope, a paramagnetic label such as gadolinium or iron oxide, a fluorophore, Near Infra-Red (NIR) fluorochrome or dye, an echogenic microbubble, an affinity label (for example biotin, avidin, etc), enzymes, or any other suitable agent that may be detected by diagnostic imaging methods.

[00050] The antibodies or antibody fragments described herein may be produced in any of the ways known in the art. For example, antibodies or antibody fragments may be expressed in a cell containing an encoding expression vector. Such expression systems are well known in the art and many variations may be used. Examples of cell-based expression systems may be *Saccharomyces cerevisiae*, *Bacillus subtilis*, *Bacillus brevis, Bacillus megaterium, Lactobacillus* species, *Escherichia coli, Pichia pastoris*, *Aspergillus niger*, and mammalian-derived cell lines such as CHO, HEK, or HeLa cells. The expressed antibody or antibody fragment can be isolated from a solution of lysed cells or the polypeptide may be secreted into media and isolated directly therefrom. The antibody or antibody fragment may also be artificially synthesized. Examples of artificial protein synthesis include solid-phase peptide synthesis, liquid-phase peptide synthesis, and cell-free protein synthesis, also known as *in vitro* protein synthesis.

NUCLEIC ACIDS, CELLS, AND BACTERIOPHAGES

[00051] Nucleic acid molecules encoding the amino acid sequences described above are encompassed herein. In one embodiment, the nucleic acid molecule comprises a nucleic acid sequence of any one of SEQ ID NOS:19-36. These sequences are illustrated in Figures 1A, 1B, 1C, 1D, 1E and 1F. Given the degeneracy of the genetic code, a number of variant

nucleic acid sequences would have the effect of encoding the amino acid, as would be readily understood by one skilled in the art. The nucleic acid molecules of the present invention may be double stranded or single stranded DNA, RNA molecules or DNA/RNA hybrids. These molecules may be nicked or intact as found in living cells. The double stranded or single stranded nucleic acid molecules may be linear or circular. The duplexes may be blunt ended or have single stranded tails, for example, with sticky ends created by restriction endonucleases.

[00052] A host cell comprising the nucleic acid molecule encoding any one of the antibody or antibody fragment described herein would also be readily recognized by the skilled artisan. The host cell could be a bacterium, such as a desired strain of *E. coli*, a yeast cell, such as a desired strain of *Pichia pastoris*, a mammalian-derived cell line such as CHO, HEK, or HeLa cells, or any other host cell suitable for carrying the nucleic acid molecule of the present invention.

[00053] A bacteriophage comprising the antibody or antibody fragment described herein, and/or comprising the nucleotide molecule encoding the antibody or antibody fragment is also encompassed in the present invention.

METHODS, USES, FORMULATIONS, AND KITS

[00054] The antibodies or antibody fragments described herein can specifically bind to Salmonella and it will be appreciated that said antibodies or antibody fragments may be useful for a number of purposes, including reducing Salmonella, inhibiting Salmonella and/or detecting Salmonella in a subject.

[00055] Accordingly, provided herein are methods of reducing the presence of Salmonella in an animal or an animal environment comprising administering to the animal an antibody or antibody fragment disclosed herein.

[00056] Within an individual animal, reducing the presence of Salmonella may comprise reducing contamination on the surface of the animal, or within the gastrointestinal tract of an animal. Should an animal be systemically infected, the method described herein could be used for reducing the presence of Salmonella.

[00057] The environment of an animal can preferably relate to the animal's immediate surroundings, such as the walls or floors of a cage or facility, the feeding or watering apparatuses within an animal compound, the bedding materials found in an animal compound, or simply the fecal material present external to the animal within the animal's confines.

[00058] Administering to an animal the antibody or antibody fragment described herein can preferably be for the purpose of reducing or inhibiting the presence of Salmonella within the animal to which the antibody or antibody fragment is administered, or an offspring of such an animal, or within the flock, cage or barn in which the animal lives. Reducing Salmonella within the animal's gastrointestinal tract is one way to reduce contamination within the animal's environment, leading to a safer food supply chain with lower incidence of contamination.

[00059] Co-administration of another substance that is effective against Salmonella is also a possible strategy for reducing Salmonella in an animal environment. For example, administering to the animal an antibiotic either at the same time as a co-formulation or at an adjacent time to the delivery of the antibody or antibody fragment can have an additive effect or may have a synergistic effect. The result of which may be a reduced likelihood of Salmonella contamination, but also reduced usage of antibiotic (i.e., fractional usage of antibiotic with synergistic efficacy). A bacteriocin effective against Salmonella can also be provided to the animal with the antibody or antibody fragment for an additive or synergistic effect. In addition to, or as an alternative to bacteriocin, any other plant- or animal-derived compound, such as a small molecule, peptide, or protein, that has an effect against Salmonella may be used together with the antibody or antibody fragment described herein. A competitive microbe may also be provided to the animal concurrently with the antibody or antibody fragment in order to possibly achieve an additive or a synergistic effect. The competitive microbe may be used together with the antibody or antibody fragment described herein as part of a probiotic system. Within such a probiotic system, the antibody or antibody fragment may be co-administered with the competitive microbe, or may be delivered sequentially. Expression of the antibody or antibody fragment within a probiotic system of the polypeptide described herein may also be undertaken.

[00060] The antibody or antibody fragment described herein may be administered orally to a subject. Oral delivery permits the polypeptide to be delivered within the water or food supply to an animal, and is less noticeable or stressful to an animal than an injection. Gavage is also an acceptable oral route when highly accurate delivery of an oral dosing regime is desirable. Other routes of administration can also be considered, such as inhalation, intranasal, gel-based or by spray, *in ovo*, topically or by injection such as intravenous, subcutaneous, intramuscular, intraorbital, intraocular, intradermal, gel-based, spray or rectal delivery route. The antibody or antibody fragment may be administered directly or within a phage or host microorganism.

[00061] As described above, modifications to the antibody or antibody fragment described herein may be made to increase efficacy of oral delivery. For example, scaffold engineering can be performed on portions of the antibody or antibody fragment within or outside of the CDR regions to confer protease resistance, as well as thermal and low pH resistance. However, and in addition, the form of antibody or antibody fragment delivery may also be altered with pharmaceutically acceptable coatings or excipients that provide a protective effect against gut enzymes, thermal or low pH effects. In this way, the sequence of the antibody or antibody fragment itself need not be modified, but rather the formulation prepared for oral delivery may itself be more optimal for the species of subject to which the antibody or antibody fragment is to be delivered. The antibody or antibody fragment may also be conjugated to small molecules such as cyclic peptides, macromolecules or polyethylene glycol to improve delivery or stability.

[00062] The dosage form may be of any type acceptable for antibody or antibody fragment delivery to animals. Coated forms and slow release forms could be used if desirable. Liquid, powder, crystal, gel, semi-solid, or tablet forms can be used.

[00063] The animal to which the antibody or antibody fragment may be delivered may preferably be a bird, such as a broiler chicken or laying hen. Other types of livestock animals, such as swine, cows, sheep, etc. may also benefit from the peptide if Salmonella is present in the animal's gut or surrounding environment. A preferred animal environment may be a barn or farm, such as a poultry farm.

[00064] In order to avoid contamination of an animal environment that is substantially free of Salmonella, a method is provided that aims to prevent introduction of a new contaminated animal or "inductee" animal into the environment, such as a barn. In such a method, the antibody or antibody fragment is administered to an inductee prior to introducing the inductee animal into the animal environment, such as a barn or farm. In this way, the animal could be cleared for the likelihood of contamination prior to taking up residence with the other animals who may have already received treatment.

[00065] The antibody or antibody fragment may also be administered to plants or plant-based materials by spraying or by other methods to reduce a level of contaminating Salmonella. Examples of such plants or plant-based materials include but are not limited to salads and spices.

[00066] Also disclosed is a method of treating a Salmonella infected subject comprising administering to the subject an antibody or antibody fragment disclosed herein. In a preferred embodiment, the method further comprises administering to the subject antibiotic effective

against Salmonella. The subject may be a livestock animal selected from the group consisting of a chicken, cow, or sheep, or the subject may be a human.

[00067] Also disclosed is a formulation for use in treating Salmonella infection comprising an antibody or antibody fragment disclosed herein and an excipient.

[00068] Also disclosed is a use of an antibody or antibody fragment disclosed herein for treating Salmonella infection in a subject in need thereof.

[00069] Also disclosed is a method of detecting Salmonella in a sample comprising contacting the sample with an antibody or antibody fragment disclosed herein, and detecting the presence of bound antibody or antibody fragment. In one preferred embodiment, the sample comprises a bodily fluid or fecal material. In another preferred embodiment, the sample comprises a food product or a surface swab from a food product.

[00070] For detection purposes, samples from a subject may comprise a bodily fluid or fecal material. The subject may be a human or a non-human animal. Samples of microbiota can be collected from the gastrointestinal (GI) tract or gut of a subject. Methods of sample collection are known to those skilled in the art. For example, microbiota samples may be obtained from stools, intestinal mucosal biopsies, gut lavage or combinations thereof. Collection can be performed during endoscopy by flushing a physiological solution, such as sterile saline solution or sterile water, onto the mucosa to remove the strongly adherent mucus layer overlying mucosal epithelial cells and the microbial community embedded within the mucus layer. Aspirates are then collected directly through an endoscope at a specific location in the gut and the samples are placed on ice.

[00071] Collection of gut microbiota can also be performed on stools. Collection of bacteria from stools is known in the art. In the case of fecal microbiota collection and analysis, fresh stools may be collected, immediately processed, and the processed materials can be stored at about -80°C.

[00072] For detection purposes, the subject may be a chicken, optionally a broiler chicken. Samples from such a subject may comprise intestinal fluid, carcass, feathers, skin, breast/leg meat rinses, as well as droppings from poultry or a bodily fluid, or rectal effluent. Samples may be taken from the environment such as the floor covering of barns, boots, wash/chill tanks or any other equipment used at a poultry processing facility as well as animal feed and water.

[00073] Once a sample has been collected, the presence of bound antibody or antibody fragment in said sample can be detected by carrying out any one of a number of binding assays or bound substrate detection procedures known in the art. Antibody or antibody

fragment binding can be measured directly or indirectly by using a tagged version of the antibody or antibody fragment, examples of which are described above. The step of detecting may be accomplished by any suitable method known in the art, for example, but not limited to: optical imaging, immunohistochemistry or molecular diagnostic imaging, ELISA, or other suitable method.

[00074] Also disclosed is a kit for conducting the detection method, comprising an antibody or antibody fragment disclosed herein and instructions for use in detecting Salmonella.

[00075] Exemplary embodiments of the present disclosure will now be described. These embodiments involve preparation and use of camelid single-domain antibodies (VHHs) specific for Salmonella, and are not intended as limiting.

EXAMPLES

[00076] **Example 1**

[00077] Immunization of Alpaca with different strains of Salmonella

[00078] To isolate VHH domains that target Salmonella, three alpacas were immunized with different strains of *Salmonella enterica*.

[00079] Three male alpacas (*Vicugna pacos*) were immunized subcutaneously with *Salmonella enterica* serovars. Five injections were performed in total. Each animal was injected with a mixture of 4 *Salmonella eneterica* strains ($1x10^9$ cfu from each) that were heat inactivated (30min at 65°C) and mixed with adjuvant (aluminum hydroxide, Alhydrogel™ 2%). The injection groups consisted of:

1- *S. Typhimurium* (SGSV1412, and SGSC4904), *S. Entritidis* (SGSC4901, SGSC3820);

2- *S. Newport* (SGSC4910), *S. Javiana* (SGSC4917), *S. Senftenberg* (SGSC2516), *S. Heidelberg* (SGSC4966); and

3- *S. Hadar* (SGSC4906), *S. Kentucky* (SGSC4914), *S. Infantis* (SGSC4905), S. *SaintPaul* (SGSC4920).

[00080] To prepare antigens for injection, Salmonella were cultured on LB agar plates. Approximately 1 x 10^9 cells were treated with heat at 65°C for 30 minutes in order to completely kill the bacteria. For each injection, a total of 4 x 10^9 killed cells were used with equal mixture of 4 different strains in a total volume of 0.4 ml. The contents were mixed with an equal volume of Alhydrogel™ (Sigma™). Injections were done at days 1, 15, 22, 29, and 36. Alpaca serum was analyzed for specific binding to a commercially available purified

flagellin from *S. Typhimurium* or the whole bacteria cells immobilized on the plates. Briefly, microtiter plates (Maxisorp™ plates) (Nalge Nunc International™, Rochester, NY) were coated overnight at 4ºC with 5 µg/ml of flagellin antigen in PBS. Wells were rinsed and blocked with 200 µl of 5 mg/ml Bovine Serum Albumin or 1% casein. Different dilutions of serum were added and incubated at room temperature for 1.5 h. Wells were washed with PBST (0.05% v/v Tween-20™), and incubated with goat anti-llama IgG (H+L) (1:1,000 in PBS) (Bethyl Laboratories™, Montgomery, TX) followed by Rabbit-anti-goat-HRP (1:5,000 in PBS) (Bethyl Laboratories™, Montgomery, TX). Signal was detected by adding 100 µl/well TMB peroxidase substrate (Kirkegaard and Perry Laboratories™, Gaithersburg, MD, USA). Reactions were stopped by adding 100 1M phosphoric acid and A_{450} was measured using a Bio-Rad™ ELISA plate reader.

[00081] **Example 2**

[00082] Phage display library constructions

[00083] A hyper-immunized alpaca VHH library was constructed based on RNA isolated from the lymphocytes of animals immunized as in Example 1.

[00084] A phage display library was constructed using a standard protocol (Arbabi Ghahroudi et al., 2009). Lymphocytes were collected from the blood using Lymphoprep™ Tubes (Axis-Shield™, Oslo, Norway). Total RNA was isolated from approximately 1 x 10^7 lymphocytes collected on day 36 post-immunization using RNAzol™ kit (Bioshop™, Burlington, Ontario, Canada). First-strand cDNA was synthesized with oligo(dT) primers from the SuperScript III First Strand™ cDNA synthesis kit (Invitrogen™, Burlington, Ontario, Canada) using 6 µg total RNA as template according to manufacturer's recommendations. Variable and part of the constant domains DNA were amplified using oligonucleotides MJ1-3 (sense) and two CH2 domain antisense primers, CH2 and CH2b3 (for primer sequences see Arbabi Ghahroudi et al., 2009; Baral et al. 2013) and heavy chain fragments (550-650 bp in length) were purified using the E.Z.N.A.™ Cycle Pure PCR purification Kit (Omega Bio-tek™, Norcross, Georgia, USA). The variable regions of heavy chain antibodies (IgG2 and IgG3) were re-amplified in a second PCR reaction using MJ7 and LP6-MJ8 primers (for primer sequences, see Baral et al. 2013). The amplified PCR products were purified with the Cycle Pure Kit™ (Omega Bio-tek™), digested with SfiI (Thermoscientific™, Toronto, Ontario, Canada), and re-purified using the same kit. Twelve micrograms of digested VHH fragments were ligated with 40 µg (1:3 molar ratio, respectively) Sfi-digested pADL-23c phagemid vector (Antibody Design Labs™, San Diego, California, USA) using T4 DNA ligase system and its

protocol (Promega™, Madison, Wisconsin, USA), transformed into commercial electrocompetent TG1 *E. coli* cells (Lucigen™ Corporation, Middleton, Wisconsin, USA), as described previously (Arbabi Ghahroudi et al., 2009), and a library size of 7.8 x 10^8 transformants was obtained. The VHH fragments from 50 colonies were PCR-amplified and sequenced to analyze the complexity of the library; all clones had inserts of expected sizes and were different from each other at their CDR regions as determined by sequencing of their encoding VHH fragments. The library was grown for 3-4 hours at 37 ºC, 250 rpm in 2 X YT/Carb-Glucose (1% w/v) medium. The bacterial cells were pelleted, resuspended in the same medium and stored as glycerol stock at -80ºC as described previously (Arbabi Ghahroudi et al., 2009).

[00085] Example 3

[00086] Screening phage display library to select for VHHs binding to Salmonella

[00087] The library screening (panning) was done through a sequential strategy using either whole, heat inactivated Salmonella bacteria or purified Salmonella flagellin protein (main component of flagella encoded by *fliC* gene) as a target. For the panning against whole Salmonella, the bacterial cells from 4 different strains were equally mixed and adjusted to an OD of 1 using PBS solution. The bacterial mixes were inactivated at 65ºC for 30 minutes and coated on a 96 well Maxisorb™ plate. To pan against flagellin protein (Flagellin from *Salmonella typhimurium*, purchased from Sigma™, cat#: SRP8029), flagellin solution (5ug/ml, dissolved in PBS solution) was coated on a 96 well Maxisorp™ plate. For each panning, BSA-PBS solution (0.5% of BSA dissolved in PBS solution) was also coated on the same 96-well Maxisorp™ plate as a pre-screening control. The coated plates were then incubated at 4ºC overnight. Next day, the coated wells were rinsed with PBS once and blocked with BSA-PBS solution for 2 h at 37ºC. The Salmonella phage library was diluted using BSA-PBS solution so that approximately 2x10^{12} phage particles were added first to the BSA-PBS wells and kept at 37ºC for 1 hour and then the supernatant was transferred to the Salmonella whole cell or flagellin wells. The phage particles were incubated in the Salmonella whole cell or flagellin wells for 2 hours at 37ºC and then washed 5 times with PBST containing 0.1% v/v Tween-20. The bound phages were eluted with 0.1 M triethylamine, neutralized with 1M Tris-HCL, PH 7.4 and incubated with exponentially growing TG1 cells in 10 ml of 2YT medium. After 30 min incubation at 37ºC, the cells were superinfected with 10^{11} M13KO7 helper phage (New England Biolabs™) for an additional 30 min. Two antibiotics, ampicillin (100 µg/L) and Kanamycin (25 µg/L), were added to the TG1 culture medium. Incubation continued at 37 ºC

for 16 hours, followed by selection of the phage infected TG1 cells. In the third day of panning, the amplified phage particles in culture supernatant were precipitated with polyethylene glycol (PEG) as described previously (Arbabi-Ghahroudi et al., 2009). Briefly, 10 ml of phage infected TG1 culture was centrifuged at 4000 rpm at 4ºC for 30 minutes, the supernatant was filtered with a 0.22 µm filter and mixed with 1/5 volume of PEG/NaCl (20% PEG, 2.5 M NaCl) on ice for 1 hour. The phages were pelleted by centrifugation at 4000 rpm at 4°C for 30 minutes. Finally, the enriched phage pool was suspended in 200 µl of PBS and ready for the next round of panning. Panning was continued for three more rounds following the same conditions except that washing was increased 7, 10 and 12 times with PBST for the second, third and fourth rounds of panning, respectively. After four rounds of panning, 96 randomly picked colonies were grown and subjected to phage ELISA screening.

[00088] **Example 4**

[00089] Expression and purification of monomeric VHH

[00090] VHH against flagellin or whole Salmonella cells identified in Example 3 were PCR amplified from the pADL23 phagemid vector with BbsI1-VHH forward primer (5'-TATGAAGACACCAGGCCCAGGTAAAGCTGGAGGAGTCT–3') (SEQ ID NO:59) and BamHI-VHH reverse primer (5'-TTGTTCGGATCCTGAGGAGACGGTGACCTG-3') (SEQ ID NO:60). The PCR fragments were digested with the BbsI and BamHI restriction enzymes and ligated into the similarly digested pSJF2 expression vector (Arbabi-Ghahroudi et al., 2009). Upon ligation, all plasmids were transformed into electrocompetent *E. coli* (TG1 strain) and selected on LB agar plates containing carbenicillin. Colonies were screened by colony PCR for inserts and the DNA was sequenced. The sequences were aligned and categorized into 18 different groupings or classes; each represented by one or more clones and each representing the same amino acid sequence (Table 1). A single clone was randomly selected from each class. Nucleic acid and amino acid sequences of the 18 selected VHHs are shown in Figure 1. CDR1, CDR2, and CDR3 are underlined within each polypeptide sequence. As shown in Table 2, the 18 VHH sequences can be placed in 5 distinct groups based on sequence similarities in the CDR regions.

[00091] The VHHs all have the canonical amino acid residues found in Camelid family VHHs at positions 42 (F/Y), 49 (E/Q/A), 50 (R), and 52 (F/V/G/L) (Muyldermans, et al. 1994) according to the IMGT numbering scheme (Lefranc, et al. 2003). In addition, the CDR3 domains in these VHHs are generally larger than CDR3 domains in human VH proteins. For example, the CDR3 domain of Group 1 VHHs is 20 residues in size (Table 2).

[00092] Analysis using the full polypeptide sequences of the 18 VHHs reveals that these VHHs can be classified into 2 larger groupings (Figure 2). The first grouping includes 1E05, 1E03, 1H07, 4D01, 0D12, 1A07, 1B08, 0A07, 0H12 and 0A08; the second grouping includes 4E08, 4F12, 1E08, 3B04, 2A09, 1G06, 0A09 and 4C10.

[00093] VHH antibodies were expressed using the standard periplasmic expression method (Arbabi-Ghahroudi et al., 2009). VHH antibody 1E03 was expressed in *P. pastoris* because no expression was achieved using the *E. coli* expression method. After induction of protein expression, cell cultures were harvested at 6,000 rpm x 30 min (4ºC), the supernatant decanted, and the periplasmic contents extracted from the cell pellet. Briefly, the pellet of monomeric VHH was resuspended in 20 ml of ice cold TES (0.2 M Tris-HCl pH 8.0, 20% (w/v) sucrose, 0.5 mM EDTA) and incubated on ice for 30 min. Next, 30 ml of ice-cold 1/8 TES (diluted in dH_2O) was added, incubated an additional 30 min on ice, and the slurry centrifuged at 9,000 rpm for 30 min (4ºC). The resulting supernatant containing VHH was dialysed overnight against PBS and purified using Profinity™ IMAC resin from BioRad™, as per manufacturer's instructions, with phosphate-based elution buffer containing 500mM imidazole.

[00094] Purified protein fractions were pooled and dialyzed against PBS. Eluted fractions were analyzed by SDS-PAGE and Western blotting before being dialysed into PBS. Figure 3 shows an example of VHH polypeptides on an SDS-PAGE gel stained with Coomassie™ Brilliant Blue G250. The arrow points to the position of the polypeptides. Size of the polypeptides is about 24kD. Expected size is around 15kD. The discrepancy is caused by three tags (c-myc, AviTag™ and His_6) linked to the VHHs.

[00095] VHH concentrations were determined by absorbance measurements at 280 nm using theoretical MW and extinction coefficients calculated with the ExPASy ProtParam™ Tool (expasy.org/tools/protparam.html) according to Pace et al., 1995. The yield of the purified monomeric VHHs ranged from 1 to 20 mg/L bacterial culture.

[00096] **Example 5**

[00097] VHH Binding Assays

Microtitre plates (Maxisorp™ plates) (Nalge Nunc international™, Rochester, NY) were coated overnight at 4ºC with 2.5µg/mL flagellin (Sigma-Aldrich™ SRP8029) and 14 different heat inactivated (65°C, 30min) *Salmonella enterica* serovar whole cells. Binding of flagellin and 4 of the *S. enterica* strains is described in Example 1. Wells were rinsed with PBS pH 7.4 and blocked with 200ul of BSA-PBS solution for 1 hr. Twenty different VHH-containing phages were prepared as described in Example 1 and used to test their binding ability to

flagellin or 14 different Salmonella strains. Phages in 2YT medium supernatant (100µL) were added to blocked coated microtitre plates and incubated at room temperature for 1 hour. Wells were washed with PBST solution and incubated with HRP conjugated anti-M13 monoclonal antibody (1:5000 in PBST) (Sigma-Aldrich™ GE27-9421-01) at room temperature for half an hour. Wells were washed again and signal was detected using 50ul/well TMB peroxidase substrate (Kirkegaard and Perry Laboratories™, Gaithersburg, MD, USA). Reactions were stopped by adding 50ul/well of 1M hydrochloric acid and A450 was measured using a Cytation™ 5 (Biotek™) multimode reader. As shown in Figure 4, a number of the VHHs were determined to bind to one or more of Salmonella flagellin and/or the Salmonella serovar whole cells. For example, robust binding was observed for VHH 1E05 against all inactivated S. Hadar and S. Heidelberg strains tested. This assay provides only a qualitative or semi-quantitative assessment of the target binding of the VHHs. Furthermore, binding cannot be discounted for VHHs that were not show to bind according to this assay, as said assay is limited by the presentation of antigen on coated plates and by the variation in growth rates of phages displaying particular VHHs.

[00098] **Example 6**

[00099] Salmonella Motility Assays

[000100] Motility assays was performed as described previously (Kalmokoff et al., 2006). VHHs at a final concentration of 2µg/µl were plated with Salmonella strain 4904 ($1x10^7$) on Muller-Hinton™ media with 0.35% agar and incubated at 37^0C under microaerophilic conditions (5% O_2, 10% CO_2 and 85% N_2) for 18 hours. Bacterial motility was determined by measuring the diameter of the circle produced by the growing bacteria on the plate. Results are shown in Figure 5. It was found that VHH 1E08 significantly inhibited bacterial motility.

[000101] **Example 7**

[000102] Cell Proliferation Assays

[000103] Around $1x10^7$ Salmonella (strain name: 4904) cells were incubated in 100µl Muller-Hinton™ liquid medium with 0.2µg/µl VHHs in a 96 well plate and incubated at 37^0C under microaerophilic conditions (5% O_2, 10% CO_2 and 85% N_2) for 18 hours. Bacterial growth rate was determined by measuring optical density at 600 nm wavelength (Figure 6). Similar to the motility assay result, VHH 1E08 was found to significantly inhibit the growth of Salmonella cells.

[000104] **Example 8**

[000105] Salmonella Internalization Assays in HeLa Cells

[000106] **Aim:** to identify anti-Salmonella VHH domains that interfere with *Salmonella* colonization of epithelial cells. To colonize the gut, *Salmonella* must attach to the surface and enter the host epithelial cells, where it undergoes intracellular replication. Without wishing to be limited by theory, VHHs that interfere with this process are expected to prevent bacterial attachment and/or allow attachment but block the invasion of the host cell.

[000107] **Summary of the assay**: HeLa epithelial cells are challenged with GFP expressing *Salmonella* in the absence or presence of different VHHs. *Salmonella* is allowed to attach and enter the cells and subsequently unbound bacteria are removed. Attached but non-internalized *Salmonella* are eliminated by gentamicin, an antibiotic that does not penetrate epithelial cells. Intracellular growth of Salmonella is then tracked by using a fluorescence plate reader, which quantifies the increase of GFP fluorescence over time.

[000108] **Method:** HeLa cells were grown in 24-well plates containing 500 µl/well of DMEM + 10% FBS and incubated at 37°C in the presence of 5% CO_2 for 24 h (80-100% confluence). *Salmonella enterica* serovar Hadar 5643 transformed with GFP was grown on the same day (from an overnight culture) to an OD of approximately 0.5. The culture was centrifuged (5 min, 5000 rpm) and resuspended in DMEM without FBS, and the bacteria were pre-incubated with the VHHs at different concentration for 30 min at 37°C with gentle mixing. Prior to infection, cells were washed 3X with PBS. *Salmonella* and VHHs were added to the HeLa cells (MOI: 100 in 500 ul/well of DMEM without FBS). The plates were incubated for 1 h at 37°C in 5% CO_2. After 1 h of infection (*BEFORE GENTA samples-Figure 7*) cells were washed 3X with PBS to remove non-adherent bacteria. To permeabilize and lyse HeLa cells, 1 ml of 1% saponin was added. The plates were incubated for 15 min at 37°C in 5% CO_2. Bacteria were resuspended by pipetting up and down vigorously (approx. 10 times per well). Serial dilutions were plated on LB agar plates. The plates were incubated overnight at 37 °C to quantify viable intracellular bacteria. DMEM with 10% FBS + gentamicin (100 µg/ml, final conc.) was added to the rest of the plate. After 1 h of infection (*AFTER GENTA samples-Figure 7*) cells were washed 3X with PBS to remove non-adherent bacteria. To permeabilize and lyse HeLa cells, 1 ml of 1% saponin was added. The plates were incubated for 15 min at 37°C in 5% CO_2. Bacteria were resuspended by pipetting up and down vigorously (approx 10 times per well). Serial dilutions were plated on LB agar plates. The plates were incubated overnight at 37 °C to quantify viable intracellular bacteria. DMEM with 10% FBS + gentamicin

(10 ug/ml, final conc.) was added to the rest of the plates and they were incubated for 24 h at 37°C in 5% CO_2. All assays were performed in duplicate.

[000109] **Results:** Based on both colony counts and fluorescence quantification of GFP expression, VHH 0A07 showed the highest inhibition of intracellular *S. enterica* strain Hadar 5643 growth (Figure 7).

[000110] **Example 9**

[000111] Salmonella Internalization Assays in Chicken Ileum and Jejunum Cultures

[000112] **Aim:** to identify anti-Salmonella VHHs that interfere with Salmonella colonization of adult chicken intestine. To colonize the chick gut, Salmonella must attach to the surface and enter the host epithelial cells, where it undergoes intracellular replication. Without wishing to be limited by theory, VHHs that interfere with this process are expected to prevent bacterial attachment and/or allow attachment but block the invasion of the host cell.

[000113] **Method**: Intestinal jejuni and ilea were obtained from ten (2x5) 30-day old non-SPF chickens. The jejuni and ilea were washed and cut into 0.5 x 0.8 cm pieces. VHHs 0A07, 0A08, 1E03, 1H07 and 0H12 were pre-bound to *S. enterica* serovar Hadar 5643 for 30 min. The mixture was applied onto the intestinal sections and the bacteria were allowed to infect for 3 hours. Treatment with gentamicin removed extracellular bacteria. Genomic DNA was extracted from the infected sections and an established robust 5′ nuclease (TaqMan™) real-time PCR assay was used to detect *Salmonella* as previously described (Malorny et al., 2004).

[000114] **Results:** 7.5-log reduction in genome copy number was achieved with the 0A07, 1E03 VHH treatment at 50µg/mL for the *S. enterica* serovar Hadar 5643 infection of ileum sections (Figure 8). 6-log reduction in genome copy number was also achieved with the 1E03 VHH treatment on infection of jejunal sections. 4-log reduction in genome copy number was observed with the 1H07 VHH on infection of jejunum sections. No MIC was determined because no complete inhibition was achieved up to 50µg/mL. The results represent biological duplicates.

[000115] **Example 10**

[000116] Treatment of Broiler Chickens with VHHs

[000117] The objective of the study presented in this Example was to test the efficacy of VHH 0A07 in a *Salmonella enterica* challenge model by administration via oral gavage.

[000118] **Method:** Animal testing and data collection was carried out by Colorado Quality Research, Inc. (Wellington, CO). Commercial broiler chickens (Cobb 500 breed) were

supplied by Simmons Foods™ (Siloam Springs, AR). Chicks were received at about 1 day old and put on a non-medicated industry average diet. The birds were housed in concrete floor pens within an environmentally controlled facility. All birds were placed in clean pens containing clean pine shavings as bedding. Lighting was via incandescent lights and a commercial lighting program was used. Water and feed was provided *ad libitum* throughout the study. The test facility, pens and birds were observed at least twice daily for general flock condition, lighting, water, feed, and ventilation. Upon receipt and prior to placement, all birds were tagged in the back of the neck with uniquely numbered individual identification tags. Clinical observations of all birds were made once daily. These observations included body weight and feed intake measurements.

[000119] For the study, 60 one-day old chicks were randomly assigned to one of two treatment groups, Group A or Group B – each group consisting of 30 birds. Group A was the control group; birds within this group received untransformed, inactivated *E. coli* cells. In contrast, Group B birds received inactivated *E. coli* expressing VHH 0A07.

[000120] Upon arrival, the chicks were tagged, randomized, weighed, and placed into 6 pens (2 blocks of 3 pens; 10 birds per pen). The following morning, the birds were challenged by oral gavage. Specifically, birds were gavaged with 0.5 ml of *S. enterica* at a concentration of $1.0x10^8$ CFU/bird. Treatment was thereafter administered three times via oral gavage: at 1 h, 24 h, and 48 h post challenge gavage. Birds in Group A were gavaged with inactivated E. *coli*; Group B with inactivated E. *coli* expressing VHH 0A07 (1.5 mg per dose per bird in 1000 µl dH_2O). At 70 h, birds were weighed and euthanized.

[000121] Tissue samples were immediately collected from the euthanized birds for microbiological assays. Jejunum and ileum materials were composited into one sample per bird. Collected samples were transported on icepacks to Microbial Research Inc. (Fort Collins, CO) for microbiological assays.

[000122] **Result:** Administration of VHH 0A07 significantly decreased the levels of *Salmonella enterica* serovar Hadar 5643 in chick jejunum and ileum by $0.7log_{10}$ ($p=0.029$) (Figure 9). The geometric mean and median of Salmonella load in control Group A (not expressing VHH 0A07) was $2.2x10^4$ and $3.1x10^4$, respectively. The geometric mean and median of Salmonella load in Group B (expressing VHH 0A07) was $5.2x10^3$ and $1.2x10^4$, respectively.

TABLES

Table 1: Anti-Salmonella VHHs

The following are the numbered clones identified within each class of VHHs.

Class	Colony
1	**4E08**, 4E12, 4F10,4G04,4G10,4H05
2	4A03,4A04,4A07,4A09,4A11,4B01,4B02,4H01,**1E08**
3	**0A09**
4	1E10,0C07,4C09,4D02,4D03,1C11,0A11,2C07,**0A08**,0F11,0G08,0G12
5	0G11,**1B08**
6	1A08,1C10,**0A07**,0B08,0B10,0B11,0C11,0E09,0E11,0F12,0H10
7	**1E05**
8	**2A09**
9	0E07,1B07,1C08,1D07,1D08,1F10,1G08,0A10,0A12,0C10,0D07,0D09,0D10,0E08,0E12,0F07,0G09,0H11,**0H12**
10	**3B04**,3B07,4A05,4A06,4E02,4E06,4E10,4E11,4F02,4F03,4F06,4F09,4F11,4G05,4G06,4G08,4G09,4G11,4H02,4H06,4H08,4H11,4H12,0B07
11	**1H07**,0F10,2A02,1E11,3A01,3A02,3A03,3A04,3A05,3A06,3A07,3A08,3A09,3A10,3A11,3B01,3B02,3B03,3B05,3B06,3B08,3B09,3B10,3B11,4A01,4A12,4B04,4B05,4B06,4B07,,4B08,4B09,4B10,4B11,4B12,4C01,4C02,4C03,4C04,4C05,4C06,4C07,4C08,4C11,4C12,4D04,4D05,4D06,4D07,4D08,4D09,4D10,4D12,4E01,4E03,4E04,4E05,4E07,4E09,4F01,4F04,4F05,4F07,4F08,4G02,4G03,4G07,4G12,4H03,4H04,4H07,4H09,4H10,1C09,1F07,1F08,2H05,0B12,0D11,0E10,0F08,0G07,0H07,0H09,
12	1B06,**1E03**
13	**1G06**,2A07,2B02,0C08
14	1G03,2H10,0C09,0D08,0F09,0G10,2B07,**1A07**
15	**4C10**,4G01
16	**4D01**,4D11
17	**4F12**
18	**0D12**

Table 2: Consensus amino acid sequences of CDR1, CDR2 and CDR3 of the five groups of anti-Salmonella VHHs

	CDR1	
Group 1	GRX_1FSX_2KP	0A08; 1B08; 0A07; 1E05; 0H12; 1H07; 1E03; 0F09; 4D01; 0D12
Group 2	GLDFSSYA	4F10; 1E08; 3B04; 4F12
Group 3	GIIFSINA	2A09; 1G06
Group 4	GRSFSLYG	0A09
Group 5	GSIFSGDA	4C10

X_1 = T or S; X_2 = V or K

	CDR2	
Group 1	ASX_3TGVST	0A08; 1B08; 0A07; 1E05; 0H12; 1H07; 1E03; 0F09; 4D01; 0D12
Group 2	ISRFGGRL	4F10; 1E08; 3B04; 4F12
Group 3	ISAYDHT	2A09; 1G06
Group 4	ISGSGLATS	0A09
Group 5	IGKEGDT	4C10

X_3 = F or Y

	CDR3	
Group 1	AGTX_4RTLWGSKWRDX_5X_6EYEY	0A08; 1B08; 0A07; 1E05; 0H12; 1H07; 1E03; 0F09; 4D01; 0D12
Group 2	AADRRSGLGTSKEYDY	4F10; 1E08; 3B04; 4F12
Group 3	NVDEIRKF	2A09; 1G06
Group 4	AQRWTSGTIARATGEYGY	0A09
Group 5	ATFEERPQPSYVY	4C10

X_4 = T or L; X_5 = V or R; X_6 = L or R

[000123] The scope of the claims should not be limited by the preferred embodiments set forth in the examples, but should be given the broadest interpretation consistent with the description as a whole.

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WHAT IS CLAIMED IS:

1. An isolated antibody or antibody fragment comprising an amino acid sequence of any one of SEQ ID NOS:1-18, or a variant thereof.

2. The antibody or antibody fragment of claim 1, wherein said antibody or antibody fragment specifically binds to Salmonella.

3. The antibody or antibody fragment of claim 2, wherein said antibody or antibody fragment specifically binds to flagella of said Salmonella.

4. An isolated antibody or antibody fragment that binds to Salmonella comprising: (i) a Complementarity Determining Region (CDR)1 comprising an amino acid sequence of GRX_1FSX_2KP (SEQ ID NO:37); (ii) a CDR2 comprising an amino acid sequence of ASX_3TGVST (SEQ ID NO:38); (iii) and a CDR3 comprising an amino acid sequence of $AGTX_4RTLWGSKWRDX_5X_6EYEY$ (SEQ ID NO:39);
wherein X_1 is T or S; X_2 is V or K; X_3 is F or Y; X_4 is T or L; X_5 is V or R; and X_6 is L or R.

5. An isolated antibody or antibody fragment that binds to Salmonella comprising: (i) a CDR1 comprising an amino acid sequence of GLDFSSYA (SEQ ID NO:40); (ii) a CDR2 comprising an amino acid sequence of ISRFGGRL (SEQ ID NO:41); and (iii) a CDR3 comprising an amino acid sequence of AADRRSGLGTSKEYDY (SEQ ID NO:42).

6. An isolated antibody or antibody fragment that binds to Salmonella comprising: (i) a CDR1 comprising an amino acid sequence of GIIFSINA (SEQ ID NO:43); (ii) a CDR2 comprising an amino acid sequence of ISAYDHT (SEQ ID NO:44); and (iii) a CDR3 comprising an amino acid sequence of NVDEIRKF (SEQ ID NO:45).

7. An isolated antibody or antibody fragment that binds to Salmonella comprising: (i) a CDR1 comprising an amino acid sequence of GRSFSLYG (SEQ ID NO:46); (ii) a CDR2 comprising an amino acid sequence of ISGSGLATS (SEQ ID NO:47); and (iii) a CDR3 comprising an amino acid sequence of AQRWTSGTIARATGEYGY (SEQ ID NO:48).

8. An isolated antibody or antibody fragment that binds to Salmonella comprising: (i) a CDR1 comprising an amino acid sequence of GSIFSGDA (SEQ ID NO:49); (ii) a CDR2 comprising an amino acid sequence of IGKEGDT (SEQ ID NO:50); and (iii) a CDR3 comprising an amino acid sequence of ATFEERPQPSYVY (SEQ ID NO:51).

9. The antibody or antibody fragment of any one of claims 1 to 8, modified for tolerance to one or more gut enzymes selected from the group consisting of pepsin, trypsin and chymotrypsin.

10. The antibody or antibody fragment of any one of claims 1 to 9, further comprising a detectable label.

11. A nucleic acid molecule encoding the antibody or antibody fragment as defined in any one of claims 1 to 10.

12. A host cell comprising the nucleic acid molecule as defined in claim 11.

13. A bacteriophage comprising the antibody or antibody fragment as defined in any one of claims 1 to 10.

14. A bacteriophage comprising the nucleic acid molecule as defined in claim 11.

15. A method of reducing the presence of Salmonella in an animal or an animal environment comprising administering to the animal the antibody or antibody fragment as defined in any one of claims 1 to 10.

16. The method of claim 15, further comprising administering an antibiotic, bacteriocin, or other plant- or animal-derived compound effective against Salmonella to the animal.

17. The method of claim 15, further comprising administering a competing microbe to the animal together with the antibody or antibody fragment as defined in any one of claims 1 to 10, optionally co-expressed or co-contained in a probiotic system.

18. The method of any one of claims 15 to 17, wherein the antibody or antibody fragment is administered to the animal orally.

19. The method of any one of claims 15 to 18, wherein the animal is a chicken.

20. The method of claim 19, wherein the chicken is a laying hen or a broiler chicken.

21. The method of any one of claims 15 to 20, wherein the animal environment is a poultry farm.

22. A method of reducing or preventing introduction of Salmonella into an animal environment comprising administering to an inductee animal the antibody or antibody fragment as defined in any one of claims 1 to 10, prior to introducing the inductee animal into the animal environment.

23. A method of treating a Salmonella infected subject comprising administering to the subject the antibody or antibody fragment as defined in any one of claims 1 to 10.

24. The method of claim 23, further comprising administering to the subject an antibiotic effective against Salmonella.

25. The method of claim 23 or 24, wherein the subject is a livestock animal selected from the group consisting of a chicken, cow, or sheep.

26. The method of claim 23 or 24, wherein the subject is a human.

27. A formulation for use in treating Salmonella infection comprising the antibody or antibody fragment as defined in any one of claims 1 to 10, and a pharmaceutically acceptable excipient.

28. Use of the antibody or antibody fragment as defined in any one of claims 1 to 10 for treating Salmonella infection in a subject in need thereof.

29. A method of detecting Salmonella in a sample comprising contacting the sample with the antibody or antibody fragment as defined in any one of claims 1 to 10, and detecting the presence of bound antibody or antibody fragment.

30. The method of claim 29, wherein the sample comprises a bodily fluid or fecal material.

31. The method of claim 29, wherein the sample comprises a food product or a surface swab from a food product.

32. A kit for conducting the method as defined in any one of claims 29 to 31, comprising the antibody or antibody fragment as defined in any one of claims 1 to 10 and instructions for use in detecting Salmonella.

33. Use of the antibody or antibody fragment as defined in any one of claims 1 to 10 for preparation of a medicament to treat Salmonella infection in a subject in need thereof.

4E08

Polypeptide sequence (SEQ ID NO:1):

HVQLVESGGGLVQAGGSLRLSCAASGLDFSSYAMGWFRQAPGEEREYVAGISRFGGRLYYADSVKGRFTISRDNA
KNTVYLQMNSLKPEDTAIYHCAADRRSGLGTSKEYDYWGQGTQVTVSS

Nucleic acid sequence (SEQ ID NO:19):

CATGTGCAGCTGGTGGAGTCTGGGGGAGGATTGGTGCAGGCTGGGGGCTCTCTGAGACTCTCCTGTGCAGC
CTCTGGACTGGACTTCAGTAGCTATGCCATGGGCTGGTTCCGCCAGGCTCCAGGAGAGGAGCGTGAGTACGT
AGCAGGTATTAGTAGATTTGGTGGTAGGCTCTACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGA
GACAACGCCAAGAACACGGTGTATCTGCAAATGAACAGTCTGAAACCTGAGGACACGGCCATTTATCACTGT
GCAGCCGATAGACGGTCGGGGTTGGGGACCAGTAAGGAGTATGACTACTGGGGCCAGGGGACCCAGGTCA
CCGTCTCCTCA

1E08

Polypeptide sequence (SEQ ID NO:2):

QVQLVESGGGLVQAGGSLRLSCAASGLDFSSYAMGWFRQAPGEEREYVAGISRFGGRLYYADSVKGRFTISRDNA
KNTVYLQMNSLKPEDTAIYHCAADRRSGLGTSKEYDYWGQGTQVTVSS

Nucleic acid sequence (SEQ ID NO:20):

CAGGCTCAGGTACAGCTGGTGGAGTCTGGGGGAGGATTGGTGCAGGCTGGGGGCTCTCTGAGACTCTCCTG
TGCAGCCTCTGGACTGGACTTCAGTAGCTATGCCATGGGCTGGTTCCGCCAGGCTCCAGGAGAGGAGCGTGA
GTACGTAGCAGGTATTAGTAGATTTGGTGGTAGGCTCTACTATGCAGACTCCGTGAAGGGCCGATTCACCATC
TCCAGAGACAACGCCAAGAACACGGTGTATCTGCAAATGAACAGTCTGAAACCTGAGGACACGGCCATTTAT
CACTGTGCAGCCGATAGACGGTCGGGGTTGGGGACCAGTAAGGAGTATGACTACTGGGGCCAGGGGACCCA
GGTCACCGTCTCCTCA

0A09

Polypeptide sequence (SEQ ID NO:3):

QVQLVESGGGLVQAGGSLRLSCAASGRSFSLYGMGWFRQAPEKEREFVAAISGSGLATSYVDSVKGRFTISRDNA
KNTVYLQMNSLKPEDTAVYYCAQRWTSGTIARATGEYGYWGQGTQVTVSS

Nucleic acid sequence (SEQ ID NO:21):

CAGGCTCAGGTACAGCTGGTGGAGTCTGGGGGAGGATTGGTGCAGGCTGGGGGGTCTCTGAGACTCTCCTG
TGCAGCCTCTGGACGCAGCTTCAGTCTTTATGGCATGGGCTGGTTCCGCCAGGCTCCAGAGAAGGAGCGTGA
GTTTGTAGCAGCTATTAGCGGGAGTGGACTTGCGACAAGTTATGTAGACTCCGTGAAGGGCCGATTCACCAT
CTCCAGAGACAACGCCAAGAACACGGTGTATCTGCAAATGAACAGCCTGAAACCTGAGGACACGGCCGTTTA
TTACTGTGCCCAGAGATGGACCAGCGGCACTATAGCGAGAGCCACGGGGGAGTATGGCTACTGGGGCCAGG
GGACCCAGGTCACCGTCTCCTCA

FIG. 1A

0A08

Polypeptide sequence (SEQ ID NO:4):

QVQLVESGGGLVQAGGSLRLSCTDSGRTFSVKPMGWFRQAPGKEREFVAAASFTGVSTFYADSVKDRFTIFRDKD
KNAMDLQINSLKPEDTGAYYCAGTTRTLWGSKWRDVLEYEYWGQGTQVTVSS

Nucleic acid sequence (SEQ ID NO:22):

CAGGCTCAGGTACAGCTGGTGGAGTCTGGGGGAGGATTGGTGCAGGCTGGGGGCTCTCTGAGACTCTCCTG
TACAGACTCTGGACGCACCTTCAGTGTAAAACCCATGGGCTGGTTCCGGCAGGCTCCAGGGAAGGAGCGTGA
GTTTGTAGCAGCTGCAAGTTTTACTGGTGTGAGCACATTCTACGCAGACTCCGTGAAGGACCGATTCACCATC
TTCCGAGACAAGGACAAGAACGCGATGGATCTGCAAATTAACAGCCTGAAACCTGAGGACACGGGCGCGTA
TTACTGTGCAGGAACCACCCGAACATTATGGGGTAGTAAATGGAGAGATGTTCTTGAATACGAATATTGGGG
CCAGGGGACCCAGGTCACCGTCTCCTCA

1B08

Polypeptide sequence (SEQ ID NO:5):

QVQLVESGGGLVQAGGSLRLSCTDSGRTFSVKPMGWFRQAPGMEREFVAAASFTGVSTFYADSVKDRFAIFRDK
DKNTMDLQINSLKPEDTGAYYCAGTTRTLWGSKWRDVLEYEYWGQGTQVTVSS

Nucleic acid sequence (SEQ ID NO:23):

CAGGCTCAGGTACAGCTGGTGGAGTCTGGGGGAGGCTTGGTGCAGGCTGGGGGCTCTCTGAGACTCTCCTG
TACAGACTCTGGACGCACCTTCAGTGTAAAACCCATGGGCTGGTTCCGGCAGGCTCCAGGGATGGAGCGTGA
GTTTGTAGCAGCTGCAAGTTTTACTGGTGTGAGCACATTCTACGCAGACTCCGTGAAGGACCGATTCGCCATC
TTCCGAGACAAGGACAAGAACACGATGGATCTGCAAATTAACAGCCTGAAACCTGAGGACACGGGCGCGTAT
TACTGTGCAGGAACCACCCGAACATTATGGGGTAGTAAATGGAGAGATGTTCTTGAATACGAATATTGGGGC
CAGGGGACCCAGGTCACCGTCTCCTCA

0A07

Polypeptide sequence (SEQ ID NO:6):

QVQLVESGGGLVQAGGSLRLSCTDSGRTFSVKPMGWFRQAPGMEREFVAAASFTGVSTFYADSVKDRFTIFRDK
DKNTMDLQINSLKPEDTGAYYCAGTTRTLWGSKWRDVLEYEYWGQGTQVTVSS

Nucleic acid sequence (SEQ ID NO:24):

CAGGCTCAGGTACAGCTGGTGGAGTCTGGGGGAGGATTGGTGCAGGCTGGGGGCTCTCTGAGACTCTCCTG
TACAGACTCTGGACGCACCTTCAGTGTAAAACCCATGGGCTGGTTCCGGCAGGCTCCAGGGATGGAGCGTGA
GTTTGTAGCAGCTGCAAGTTTTACTGGTGTGAGCACATTCTACGCAGACTCCGTGAAGGACCGATTCACCATC
TTCCGAGACAAGGACAAGAACACGATGGATCTGCAAATTAACAGCCTGAAACCTGAGGACACGGGCGCGTAT
TACTGTGCAGGAACCACCCGAACATTATGGGGTAGTAAATGGAGAGATGTTCTTGAATACGAATATTGGGGC
CAGGGGACCCAGGTCACCGTCTCCTCA

FIG. 1B

1E05

Polypeptide sequence (SEQ ID NO:7):

QVQLVESGGGLVQAGGSLSLSCEDSGRSFSVKPMAWFRQAPGLEREFVAAASFTGVSTFYADSVKDRYTIFREKD
NNTVYLQMNSLQPEDTGAYYCAGTLRTLWGSKWRDRREYEYWGQGTQVTVSS

Nucleic acid sequence (SEQ ID NO:25):

CAGGCTCAGGTACAGCTGGTGGAGTCTGGGGGGGGATTGGTGCAGGCTGGGGGCTCTCTGAGTCTCTCCTG
CGAAGACTCTGGACGCTCCTTCAGTGTAAAGCCCATGGCCTGGTTCCGGCAGGCTCCAGGGCTGGAGCGTGA
GTTTGTAGCAGCTGCAAGTTTCACTGGTGTGAGCACATTCTATGCAGACTCCGTGAAGGACCGATACACCATC
TTCAGAGAGAAGGACAATAACACGGTGTATCTGCAAATGAACAGCCTACAACCTGAGGACACGGGCGCGTAT
TATTGTGCAGGAACCCTCCGAACGCTATGGGGTAGTAAATGGCGGGATCGTCGTGAATACGAATATTGGGGC
CAGGGGACCCAGGTCACCGTCTCCTCA

2A09

Polypeptide sequence (SEQ ID NO:8):

QVQLVESGGGLVQPGGSLRLSCAASGIIFSINAMGWYRQAPGKQRELVARISAYDHTNYADSVKGRFTISRDNAK
NTVYLQMNSLKPEDTAVYYCNVDEIRKFWGQGTQVTVSS

Nucleic acid sequence (SEQ ID NO:26):

CAGGCTCAGGTACAGCTGGTGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCCTG
TGCAGCCTCTGGAATTATCTTCAGTATCAATGCCATGGGGTGGTATCGCCAGGCTCCAGGGAAGCAGCGCGA
GTTGGTCGCACGTATTAGTGCTTATGATCATACAAACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCC
AGAGACAACGCCAAGAACACGGTGTATCTGCAAATGAACAGCCTGAAACCTGAGGACACGGCCGTCTATTAC
TGTAATGTAGATGAAATACGGAAATTCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA

0H12

Polypeptide sequence (SEQ ID NO:9):

QVQLVESGGGLVQPGGSLRLSCTDSGRTFSVKPMGWFRQAPGMEREFVAAASFTGVSTFYADSVKDRFTIFRDK
DKNTMDLQINSLKPEDTGAYYCAGTTRTLWGSKWRDVLEYEYWGQGTQVTVSS

Nucleic acid sequence (SEQ ID NO:27):

CAGGCTCAGGTACAGCTGGTGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCCTG
TACAGACTCTGGACGCACCTTCAGTGTAAAACCCATGGGCTGGTTCCGGCAGGCTCCAGGGATGGAGCGTGA
GTTTGTAGCAGCTGCAAGTTTTACTGGTGTGAGCACATTCTACGCAGACTCCGTGAAGGACCGATTCACCATC
TTCCGAGACAAGGACAAGAACACGATGGATCTGCAAATTAACAGCCTGAAACCTGAGGACACGGGCGCGTAT
TACTGTGCAGGAACCACCCGAACATTATGGGGTAGTAAATGGAGAGATGTTCTTGAATACGAATATTGGGGC
CAGGGGACCCAGGTCACCGTCTCCTCA

FIG. 1C

3B04

Polypeptide sequence (SEQ ID NO:10):

QVKLEESGGGLVQAGGSLRLSCAASGLDFSSYAMGWFRQAPGEEREYVAGISRFGGRLYYADSVKGRFTISRDNA
KNTVYLQMNSLKPEDTAIYHCAADRRSGLGTSKEYDYWGQGTQVTVSS

Nucleic acid sequence (SEQ ID NO:28):

CAGGTAAAGCTGGAGGAGTCTGGGGGAGGATTGGTGCAGGCTGGGGGCTCTCTGAGACTCTCCTGTGCAGC
CTCTGGACTGGACTTCAGTAGCTATGCCATGGGCTGGTTCCGCCAGGCTCCAGGAGAGGAGCGTGAGTACGT
AGCAGGTATTAGTAGATTTGGTGGTAGGCTCTACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGA
GACAACGCCAAGAACACGGTGTATCTGCAAATGAACAGTCTGAAACCTGAGGACACGGCCATTTATCACTGT
GCAGCCGATAGACGGTCGGGGTTGGGGACCAGTAAGGAGTATGACTACTGGGGCCAGGGGACCCAGGTCA
CCGTCTCCTCA

1H07

Polypeptide sequence (SEQ ID NO:11):

QVKLEESGGGLVQAGGSLRLSCTDSGRTFSKKPMGWFRQAPGMEREFVAAASYTGVSTFYADSVKDRFTIFRDKD
KNTMDLQINSLKPEDTGAYYCAGTTRTLWGSKWRDVLEYEYWGQGTQVTVSS

Nucleic acid sequence (SEQ ID NO:29):

CAGGTAAAGCTGGAGGAGTCTGGGGGAGGATTGGTGCAGGCTGGGGGCTCTCTGAGACTCTCCTGTACAGA
CTCTGGACGCACCTTCAGTAAAAAACCCATGGGCTGGTTCCGGCAGGCTCCAGGGATGGAGCGTGAGTTTGT
AGCAGCTGCAAGTTATACTGGTGTGAGCACATTCTATGCAGACTCCGTGAAGGACCGATTCACCATCTTCAGA
GACAAGGACAAGAACACGATGGATCTGCAAATTAACAGCCTGAAACCTGAGGACACGGGCGCGTATTATTGT
GCAGGAACCACCCGAACATTATGGGGTAGTAAATGGCGAGATGTCCTTGAATACGAATATTGGGGCCAGGG
GACCCAGGTCACCGTCTCCTCA

1E03

Polypeptide sequence (SEQ ID NO:12):

QVKLEESGGGLVQAGGSLSLSCEDSGRSFSVKPMAWFRQAPGLEREFVAAASFTGVSTFYADSVKDRYTIFREKDN
NTVYLQMNSLQPEDTGAYYCAGTLRTLWGSKWRDRREYEYWGQGTQVTVSS

Nucleic acid sequence (SEQ ID NO:30):

CAGGTAAAGCTGGAGGAGTCTGGGGGAGGATTGGTGCAGGCTGGGGGCTCTCTGAGTCTCTCCTGCGAAGA
CTCTGGACGCTCCTTCAGTGTAAAGCCCATGGCCTGGTTCCGGCAGGCTCCAGGGCTGGAGCGTGAGTTTGT
AGCAGCTGCAAGTTTCACTGGTGTGAGCACATTCTATGCAGACTCCGTGAAGGACCGATACACCATCTTCAGA
GAGAAGGACAATAACACGGTGTATCTGCAAATGAACAGCCTACAACCTGAGGACACGGGCGCGTATTATTGT
GCAGGAACCCTCCGAACGCTATGGGGTAGTAAATGGCGGGATCGTCGTGAATACGAATATTGGGGCCAGGG
GACCCAGGTCACCGTCTCCTCA

FIG. 1D

1G06

Polypeptide sequence (SEQ ID NO:13):

QVKLEESGGGLVQPGGSLRLSCAASGIIFSINAMGWYRQAPGKQRELVARISAYDHTNYADSVKGRFTISRDNAKN
TVYLQMNSLKPEDTAVYYCNVDEIRKFWGQGTQVTVSS

Nucleic acid sequence (SEQ ID NO:31):

CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCCTGTGCAGC
CTCTGGAATTATCTTCAGTATCAATGCCATGGGGTGGTATCGCCAGGCTCCAGGGAAGCAGCGCGAGTTGGT
CGCACGTATTAGTGCTTATGATCATACAAACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGAC
AACGCCAAGAACACGGTGTATCTGCAAATGAACAGCCTGAAACCTGAGGACACGGCCGTCTATTACTGTAAT
GTAGATGAAATACGGAAATTCTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA

1A07

Polypeptide sequence (SEQ ID NO:14):

QVKLEESGGGLVQPGGSLRLSCTDSGRTFSVKPMGWFRQAPGMEREFVAAASFTGVSTFYADSVKDRFTIFRDKD
KNTMDLQINSLKPEDTGAYYCAGTTRTLWGSKWRDVLEYEYWGQGTQVTVSS

Nucleic acid sequence (SEQ ID NO:32):

CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCCTGTACAGA
CTCTGGACGCACCTTCAGTGTAAAACCCATGGGCTGGTTCCGGCAGGCTCCAGGGATGGAGCGTGAGTTTGT
AGCAGCTGCAAGTTTTACTGGTGTGAGCACATTCTACGCAGACTCCGTGAAGGACCGATTCACCATCTTCCGA
GACAAGGACAAGAACACGATGGATCTGCAAATTAACAGCCTGAAACCTGAGGACACGGGCGCGTATTACTGT
GCAGGAACCACCCGAACATTATGGGGTAGTAAATGGAGAGATGTTCTTGAATACGAATATTGGGGCCAGGG
GACCCAGGTCACCGTCTCCTCA

4C10

Polypeptide sequence (SEQ ID NO:15):

QVKLEESGGGSVQAGGSLRLSCAVSGSIFSGDAMGWYRQAPGKKREYVALIGKEGDTVYADSVKGRFTISRDNAK
NTFYLQMNNLEPEDTARYICATFEERPQPSYVYWGPGTQVTVSS

Nucleic acid sequence (SEQ ID NO:33):

CAGGTAAAGCTGGAGGAGTCTGGGGGAGGCTCGGTGCAGGCTGGGGGGTCTCTGAGACTCTCCTGTGCAGT
CTCTGGAAGCATCTTCAGTGGTGATGCCATGGGCTGGTACCGCCAGGCTCCAGGAAAGAAGCGCGAGTATGT
CGCGTTAATTGGTAAGGAAGGTGACACAGTCTACGCAGACTCTGTGAAGGGCCGCTTCACCATCTCCAGAGA
CAATGCCAAGAACACGTTCTATCTACAAATGAACAACCTGGAACCTGAGGACACGGCCAGATATATTTGTGCG
ACATTCGAGGAGCGACCCCAACCATCGTATGTCTACTGGGGCCCGGGGACCCAGGTCACCGTCTCCTCA

FIG. 1E

4D01

Polypeptide sequence (SEQ ID NO:16):

QVKLVDSGGGLVQAGGSLRLSCTDSGRTFSKKPMGWFRQAPGMEREFVAAASYTGVSTFYADSVKDRFTIFRDK
DKNTMDLQINSLKPEDTGAYYCAGTTRTLWGSKWRDVLEYEYWGQGTQVTVSS

Nucleic acid sequence (SEQ ID NO:34):

CAGGTAAAGCTGGTGGATTCTGGGGGAGGATTGGTGCAGGCTGGGGGCTCTCTGAGACTCTCCTGTACAGA
CTCTGGACGCACCTTCAGTAAAAAACCCATGGGCTGGTTCCGGCAGGCTCCAGGGATGGAGCGTGAGTTTGT
AGCAGCTGCAAGTTATACTGGTGTGAGCACATTCTATGCAGACTCCGTGAAGGACCGATTCACCATCTTCAGA
GACAAGGACAAGAACACGATGGATCTGCAAATTAACAGCCTGAAACCTGAGGACACGGGCGCGTATTATTGT
GCAGGAACCACCCGAACATTATGGGGTAGTAAATGGCGAGATGTCCTTGAATACGAATATTGGGGCCAGGG
GACCCAGGTCACCGTCTCCTCA

4F12

Polypeptide sequence (SEQ ID NO:17):

QVQLVESGGGLVQAGGSLRLSCAASGLDFSSYAMGWFRQAPGEEREYVAGISRFGGRLYYADSVKGRFTISRDNA
KNTVYLQMNSLKPEDTAIYHCAADRRSGLGTSKEYDYWGQGTQVTVSS

Nucleic acid sequence (SEQ ID NO:35):

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGATTGGTGCAGGCTGGGGGCTCTCTGAGACTCTCCTGTGCAGC
CTCTGGACTGGACTTCAGTAGCTATGCCATGGGCTGGTTCCGCCAGGCTCCAGGAGAGGAGCGTGAGTACGT
AGCAGGTATTAGTAGATTTGGTGGTAGGCTCTACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGA
GACAACGCCAAGAACACGGTGTATCTGCAAATGAACAGTCTGAAACCTGAGGACACGGCCATTTATCACTGT
GCAGCCGATAGACGGTCGGGGTTGGGGACCAGTAAGGAGTATGACTACTGGGGCCAGGGGACCCAGGTCA
CCGTCTCCTCA

0D12

Polypeptide sequence (SEQ ID NO:18):

QVQLVESGGGLVQAGGSLRLSCTDSGRTFSKKPMGWFRQAPGMEREFVAAASYTGVSTFYADSVKDRFTISRDK
DKNTMDLQINSLKPEDTGAYYCAGTTRTLWGSKWRDVLEYEYWGQGTQVTVSS

Nucleic acid sequence (SEQ ID NO:36):

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGATTGGTGCAGGCTGGGGGCTCTCTGAGACTCTCCTGTACAGA
CTCTGGACGCACCTTCAGTAAAAAACCCATGGGCTGGTTCCGGCAGGCTCCAGGGATGGAGCGTGAGTTTGT
AGCAGCTGCAAGTTATACTGGTGTGAGCACATTCTATGCAGACTCCGTGAAGGACCGATTCACCATCTCCAGA
GACAAGGACAAGAACACGATGGATCTGCAAATTAACAGCCTGAAACCTGAGGACACGGGCGCGTATTATTGT
GCAGGAACCACCCGAACATTATGGGGTAGTAAATGGCGAGATGTCCTTGAATACGAATATTGGGGCCAGGG
GACCCAGGTCACCGTCTCCTCA

FIG. 1F

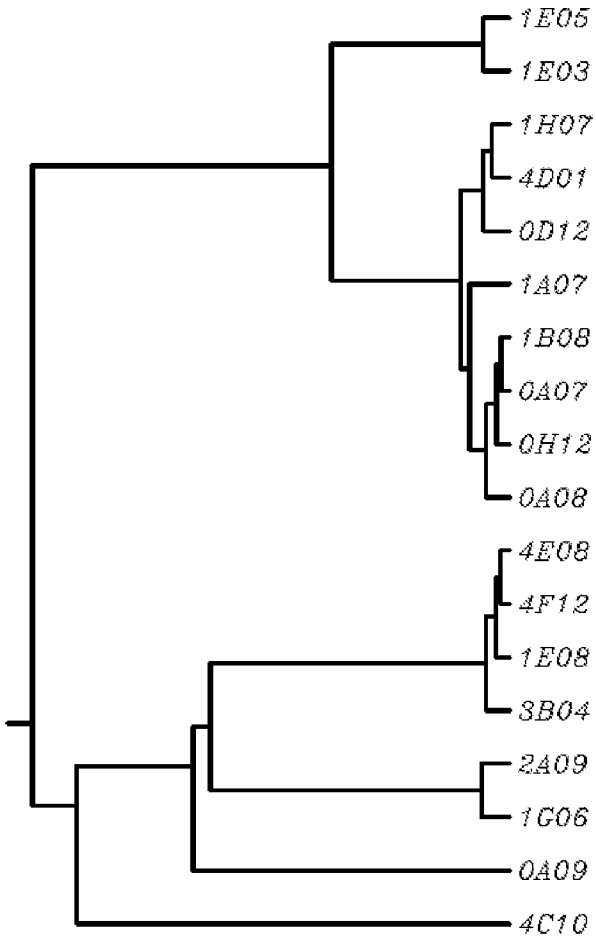
1E05
1E03
1H07
4D01
0D12
1A07
1B08
0A07
0H12
0A08
4E08
4F12
1E08
3B04
2A09
1G06
0A09
4C10

FIG. 2

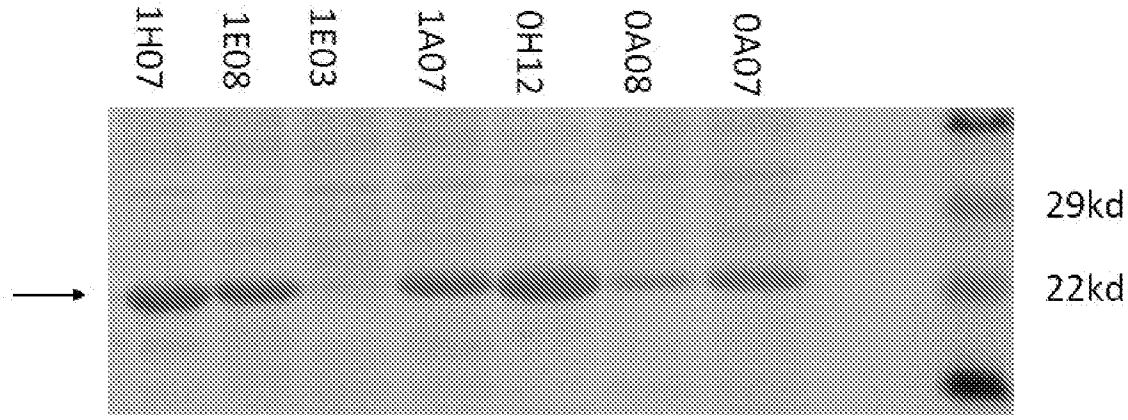

FIG. 3

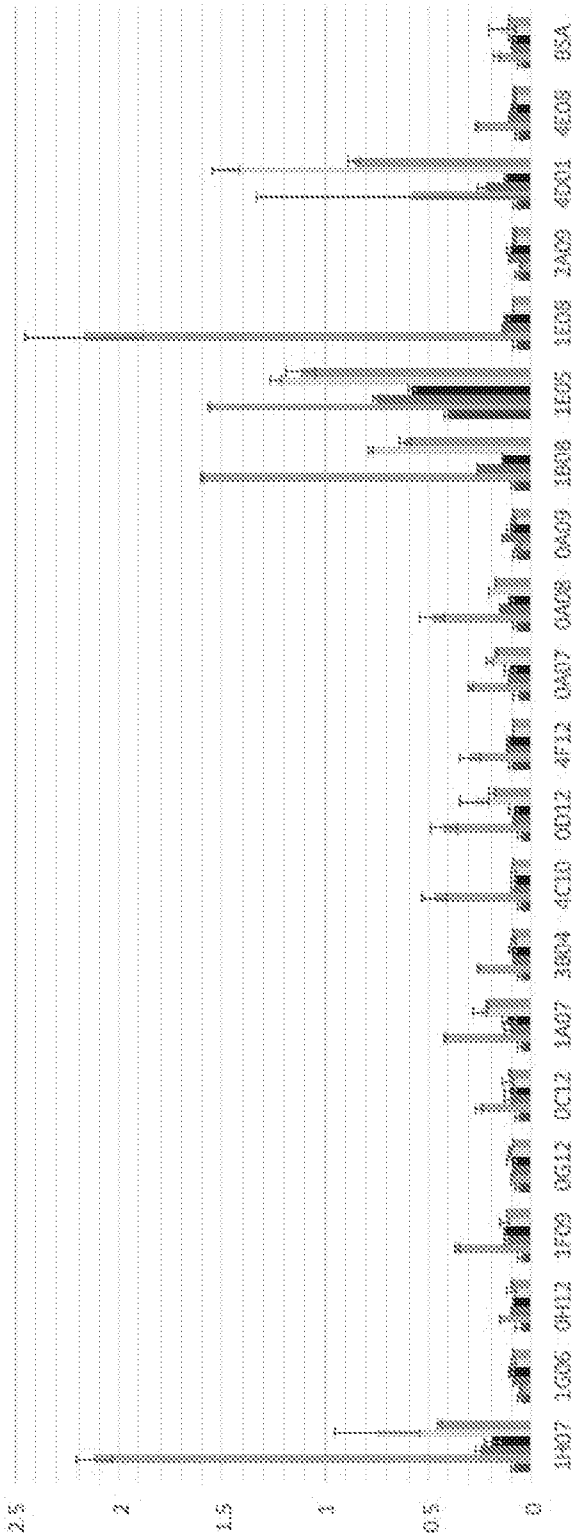
PBS
Flagellin
Heidelberg 918
Heidelberg 4643
Hadar 5643
Hadar 5652
2.5
2
1.5
1
0.5
0
1H07
1G06
0H12
1F09
0G12
0C12
1A07
3B04
4C10
0D12
4F12
0A07
0A02
0A09
1B08
1E05
1E06
3A09
4D01
4E06
BSA

FIG. 4

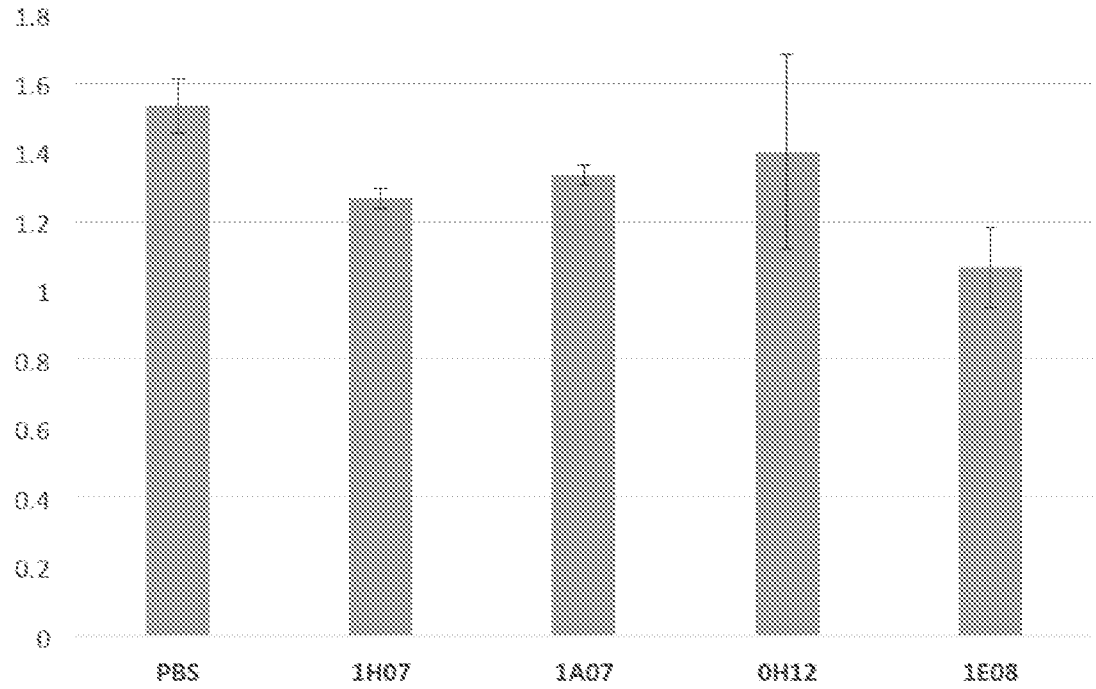

FIG. 5

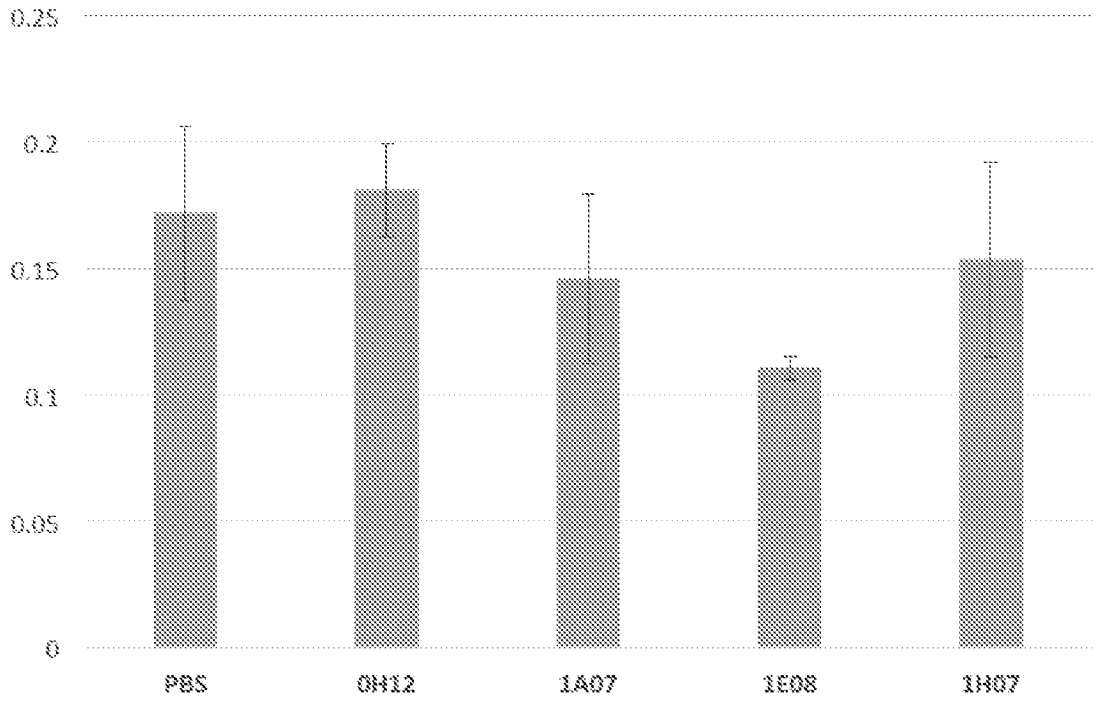

FIG. 6

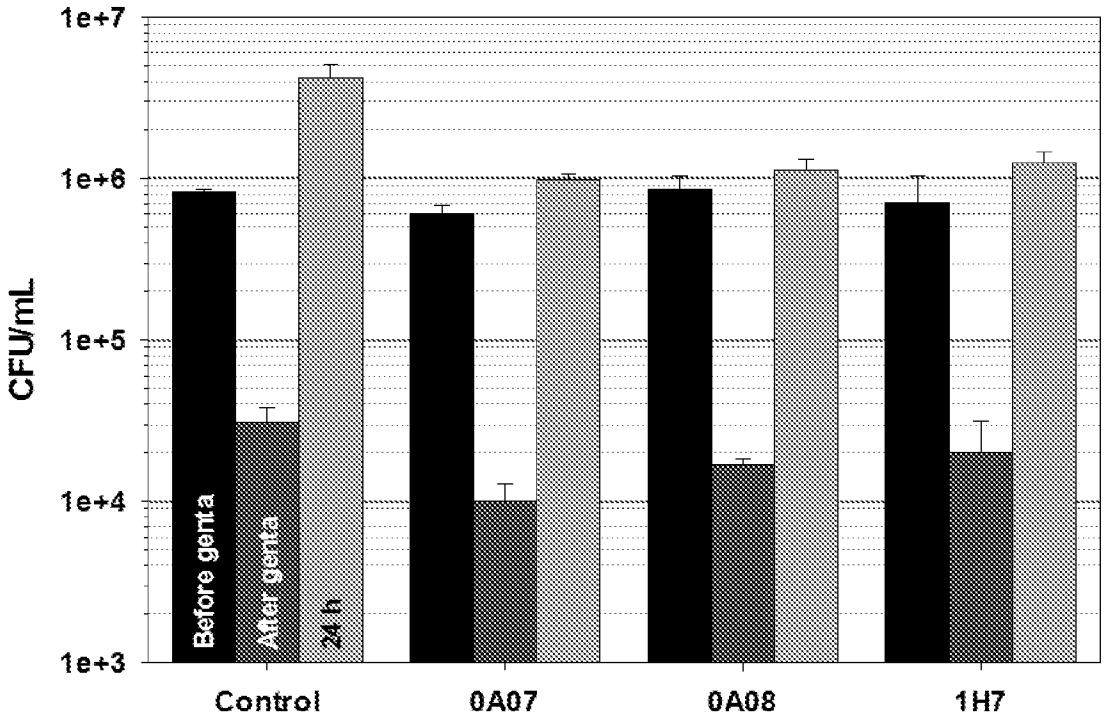
1e+7
1e+6
1e+5
1e+4
1e+3
CFU/mL
Before genta
After genta
24 h
Control
0A07
0A08
1H7

FIG. 7

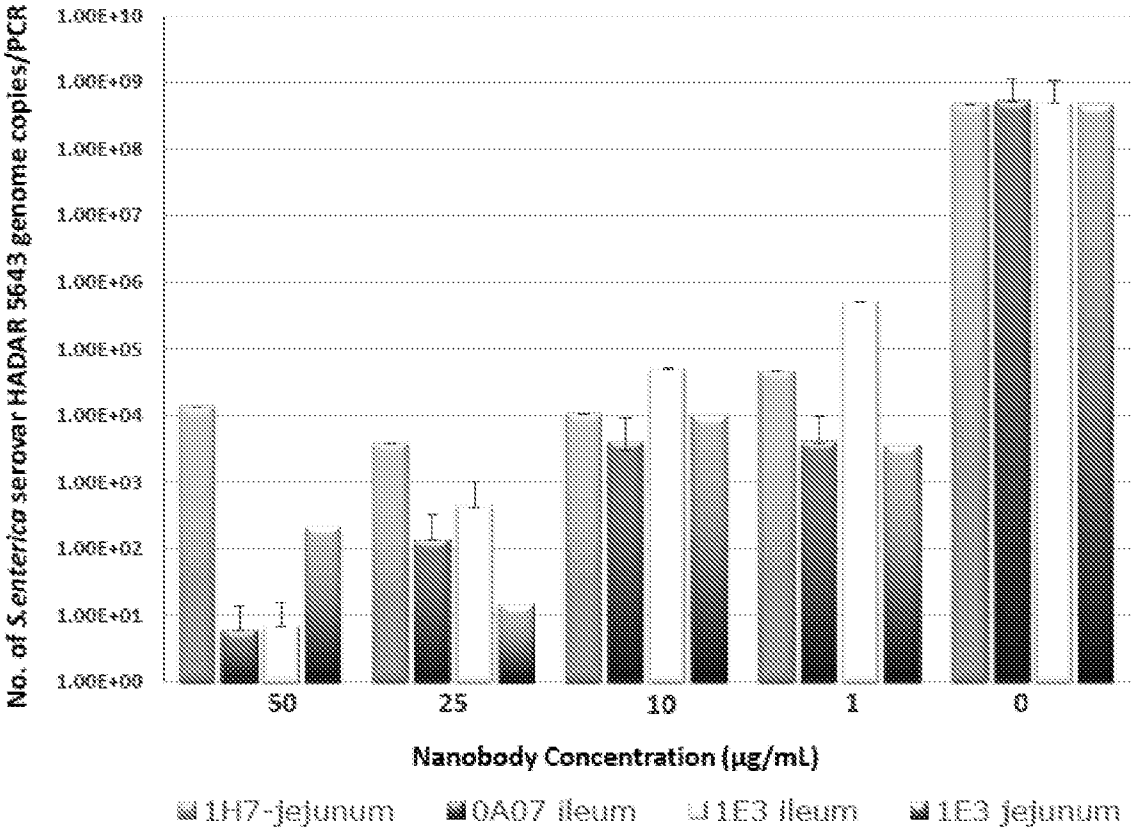
No. of S.enterica serovar HADAR 5643 genome copies/PCR
1.00E+10
1.00E+09
1.00E+08
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
50
25
10
1
0
Nanobody Concentration (µg/mL)
1H7-jejunum
0A07 ileum
1E3 ileum
1E3 jejunum

FIG. 8

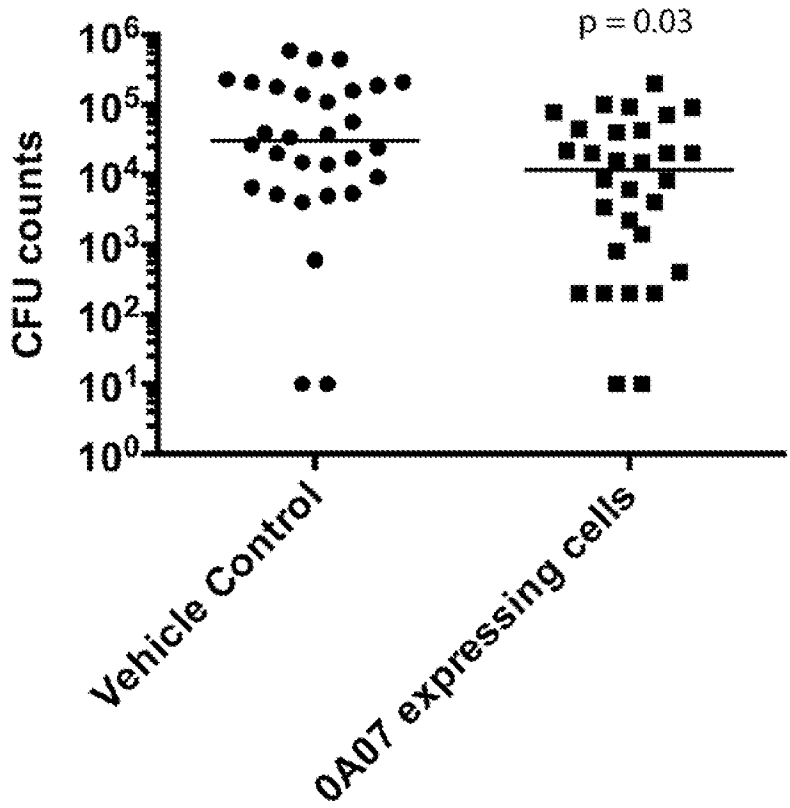
p = 0.03
CFU counts
10^6
10^5
10^4
10^3
10^2
10^1
10^0
Vehicle Control
0A07 expressing cells

FIG. 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2016/050546

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***C07K 16/12*** (2006.01), ***A61K 39/40*** (2006.01), ***A61P 31/04*** (2006.01), ***C12N 15/13*** (2006.01), ***C12N 7/01*** (2006.01), ***G01N 33/569*** (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
All IPCs

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Questel Orbit, Cipo library search tool, Scopus and GenomeQuest. Keywords searched: VHH, nanobody, single domain, antibody, flagellin, salmonella. Author/Applicant search: ABCELEX, RIAZI, WILLIAMS, SHAHINAS, BABAEI, YAN.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NOWACKA, A., *"Isolation of Salmonella Serovar-Specific SingleDomain Antibodies"*, A Thesis Presented to The University of Guelph, Ontario, Canada, 30 September, 2014 (30-09-2014). Downloaded from the internet on 5 March 2016. (https://atrium.lib.uoguelph.ca/xmlui/handle/10214/8503)	
A	HIRIART, Y. et al., *"Generation and selection of anti-flagellin monoclonal antibodies useful for serotyping Salmonella enterica"*, SpringerPlus 2013, 2:640, doi:10.1186/2193-1801-2-640.	
A	REMENTERIA, A. et al., *"Characterization of a Monoclonal Antibody Directed against Salmonella enterica Serovar Typhimurium and Serovar [4,5,12:i:_]"*, Applied and Environmental Microbiology, March 2009 (03-2009), Vol. 75, No. 5, pages 1345–1354, doi:10.1128/AEM.01597-08.	

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier application or patent but published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 15 April 2016 (15-04-2016)	Date of mailing of the international search report 15 April 2016 (15-04-2016)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 819-953-2476	Authorized officer Ali Abdallah (819) 639-7686

Box No. II **Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.: 15-26
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 15-26 are directed to a method for treatment of the human or animal body by surgery or therapy, which the International Searching Authority is not required to search under PCT Rule 39.1(iv). However, this Authority has carried out a search based on the alleged effect or purpose/use of the product defined in claims 1-10.

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III **Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

The claims are directed to a plurality of inventive concepts as follows: Each antibody is considered as a separate alleged invention. The only linking feature between the different antibodies is a VHH that specifically binds to flagella of salmonella. Said feature in known in the art (see document D1). Thus the claims must be limited to one inventive concept as set out in PCT Rule 13.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☑ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.