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(54) Title: VACCINE

(57) Abstract: The present invention relates to antigens capable of raising host immune responses to parasites. In particular, the invention provides, vaccines for use in protecting against and/or reducing instances of parasite infection in avian hosts.

VACCINE

FIELD OF THE INVENTION

The present invention relates to antigens capable of raising host immune responses to parasites. In particular, the invention provides vaccines for use in protecting against and/or reducing instances of parasite infection in avian hosts.

BACKGROUND OF THE INVENTION

Infestation of laying-hen houses with the poultry red mite, *Dermanyssus gallinae* De Geer, costs the poultry industry an estimated €130 million per annum in the EU and infestation of production facilities with this pest has important animal welfare implications [1,2]. Poultry red mite infestation is a major health and welfare issue for laying hens as a result of anaemia, increased irritation and restlessness, feather-pecking and an increased incidence of cannibalism [1]. Poultry mites have also been implicated as intermediate hosts for a number of important diseases [3]. Controlling mite populations is currently a major problem to the egg-producing industry, with most acaricides affording only limited or short-lived reduction in the population of mites. Moreover, the withdrawal of current, effective acaricides (e.g. the organophosphate fenitrothion) along with the emergence of resistance to previously effective acaricides has exacerbated these problems with mite control [e.g. see 4]. Within Europe, varying constraints exist between countries as to the application of chemical acaricides. For example, in Sweden there are no registered compounds available for the control of ectoparasites. In a 2004 survey of UK poultry units, 87.5 % of the farms that responded reported infestation with *D. gallinae* [5].

The poultry red mite is referred to as a temporary parasite, since it is only found on the host when feeding, with the majority of its lifecycle spent concealed away in cracks and crevices of the poultry house structure. For this reason, red mite are a particular problem in free range or

barn systems as opposed to caged, since a greater number of potential hiding places can be sought. In addition, EU legislation requiring the replacement of conventional cages with "enriched" cages (containing a nesting area, a roosting perch and a litter/ scratch pad) by January 2012 is likely to exacerbate the mite problem by providing more refugia for the parasites in the cages. Under optimal conditions, the lifecycle of the poultry red mite can be completed within a week, so large populations can be rapidly established. This, in conjunction with the mites' ability to occupy small spaces, makes control measures extremely difficult and there is an urgent need for alternative methods of mite control.

As an alternative control strategy, vaccination offers advantages including prolonged efficacy, freedom from chemical residues/environmental pollution and reduced risk of resistance. It is now recognised that vaccines to blood-feeding parasites can result in effective and sustainable control [6,7] including the commercial tick vaccine developed using the protective Bm86 immunogen [7]. Previous work [8,9] along with work published from other research groups [10,11] has established that vaccination against the poultry red mite, using both native and recombinant antigens, is feasible.

Previous studies have identified fractions of crude antigen preparations which raise protective responses in poultry to *D. gallinae* [8].

However, despite the wealth of research in this field a suitable vaccine against *D. gallinae* has yet to be identified. Moreover, vaccines comprising specific or single antigen candidates which are cost effective to manufacture and which offer robust protective immunity, do not exist.

The present invention seeks to obviate the problems associated with the prior art.

SUMMARY OF THE INVENTION

The present invention is based on the finding that specific antigens derived from *Dermanyssus gallinae* (*D. gallinae*: poultry red mite) can be used to raise immune responses in avian species, prone or susceptible to, infection or infestation with/by the same. Furthermore, the inventors have discovered that the immune response raised is protective and may prevent or facilitate the clearance or eradication of a *D. gallinae* infection/infestation, in or from, an avian host. Furthermore, given the significant health problems associated with *D. gallinae* infections/infestations, the present invention may not only be used to reduce populations of *D. gallinae* infection/infestation in avian hosts, but it may also be used as a means of indirectly addressing the numerous secondary diseases and/or conditions associated with *D. gallinae* infections/infestations.

As such a first aspect of this invention provides one or more *Dermanyssus gallinae* (*D. gallinae*) antigens, for use in raising an immune response in an avian species.

In one embodiment, the antigens for use according to the first aspect of this invention are provided in the form of a composition.

In a second aspect, the invention provides the use of one or more *Dermanyssus gallinae* (*D. gallinae*) antigens, in the manufacture of a medicament for raising an immune response in an avian species.

In a third aspect, the invention provides a method of raising an immune response in an avian species, said method comprising administering one or more *Dermanyssus gallinae* (*D. gallinae*) antigens to said avian species.

The terms "avian", "avian species" or "avian host" as used herein are intended to encompass all avian species prone or susceptible to *D. gallinae* infection or infestation. In one

embodiment the "avian species" encompassed by this invention include, for example, those collectively known as poultry or fowl. In other embodiments these terms extend to include domesticated or game bird species such as, for example, chicken, pheasant, grouse, turkey, guineafowl and/or duck species. In one embodiment, the terms "avian", "avian species" or "avian host" extend to commercially important or farmed bird species.

Advantageously, the antigens and compositions for use, uses, medicaments and methods provided by this invention may be exploited in order to raise immune responses in chickens (*Gallus gallus domesticus*). In this way, the invention provides antigens and compositions for use, medicaments and methods which may be exploited to prevent, reduce and/or treat the occurrence of *D. gallinae* infections/infestations in chickens.

D. gallinae is a blood feeding avian ectoparasite and is exposed to host immunoglobulin when taking blood meals. Without wishing to be bound by theory, the inventors hypothesise that by administering one or more *D. gallinae* antigens to an avian, antibodies specific to (or selective for) the one or more *D. gallinae* antigens are produced in the avian's blood. In this way, when taking blood meals, *D. gallinae* parasites are exposed to the anti- *D. gallinae* antigen antibodies which adversely affect, debilitate, destroy, kill or inactivate the *D. gallinae* parasites. Again, without wishing to be bound by theory, mechanisms involved in the antibody mediated debilitation, destruction, killing and/or inactivation of the *D. gallinae* parasites may involve antibody mediated cell cytotoxicity processes and/or complement pathways.

It should be understood that while this specification makes general reference to "antibodies" and "specific" or "selective" antibodies, these terms encompass antibodies (and active or epitope binding fragments thereof) which bind to the antigens described herein (or

epitopes thereof) and/or antibodies (and active or epitope binding fragments thereof) which exhibit a degree of selectivity, specificity and/or affinity therefor.

Furthermore, it should be understood that the term "antibody" may relate to polyclonal antibodies or monoclonal antibodies. Antibodies of this type are discussed later.

In one embodiment, the invention relates to vaccines or vaccine compositions comprising one or more *D. gallinae* antigens for raising immune responses in avian species. In this invention, vaccines may be used prophylactically to prevent the establishment of a *D. gallinae* infection/infestation on an avian host or to reduce, ameliorate or eradicate an established *D. gallinae* infection/infestation. Furthermore, vaccines or vaccine compositions comprising any of the *D. gallinae* antigens described herein may be used as a means to indirectly ameliorate, reduce the symptoms of or eradicate, a secondary complication, disease or condition associated with a *D. gallinae* infection/infestation. One of skill will appreciate that the term "indirectly" ensures the reader understands that while vaccines provided by this invention may not have a direct effect upon secondary complications associated with *D. gallinae* infection/infestation, any reduction in the population of a *D. gallinae* infection/infestation affected by the vaccine described herein, may, in turn, affect a reduction in instances of secondary complications associated with *D. gallinae* infections/infestations. These secondary complications may be associated with pathogens (e.g.bacteria including *Salmonella, Campylobacter* or *E.coli*, mycobacterial species such as *M.gallisepticum*, or viruses including avian influenza) carried on or within the mites [4] affecting the avian host, or may be the result of the detrimental health effects on the avian host or on humans within the mites' environment, of allergens produced by the mites [12].

Without wishing to be bound by theory, it is suggested that avian species administered vaccines or vaccine compositions according to this invention, may produce protective anti-*D. gallinae* antigen antibodies serving to debilitate, destroy, kill or inactivate *D. gallinae*. Additionally, and again without being bound to any particular theory, the inventors hypothesise that, depending on the specific *D. gallinae* antigen(s) exploited by this invention, the protective antibodies raised in the avian host may have an anti-fecundity effect – restricting parasite egg production and/or larval development.

As such, in one embodiment, the present invention provides one or more *D. gallinae* antigens and (vaccine) compositions comprising the same for use in raising an immune response in an avian species, wherein said antigen(s) is/are (an) antigen(s) involved in a *D. gallinae* fertility process. In other embodiments, the invention provides uses, medicaments and methods (as described above) wherein the one or more *D. gallinae* antigens is/are (an) antigen(s) involved directly or indirectly in a *D. gallinae* fertility process. It should be understood that the phrase "antigens directly or indirectly associated with a *D. gallinae* fertility process" may encompass antigens involved in, for example, mating, fertilisation, embryogenesis, vitellogenesis, sequestration of nutrients for embryonic development, embryonic development, gender-specific reproductive and developmental processes, sexual differentiation and maturation, sexually-differentiated somatic and germ cell processes, oogenesis, spermatogenesis, oocyte maturation and/or ovulation,

The compositions, including the vaccine composition, provided by this invention may be formulated as sterile pharmaceutical compositions comprising one or more of the antigens described herein and a pharmaceutical excipient, carrier or diluent. These composition may be formulated for oral, topical (including dermal and sublingual), parenteral (including

subcutaneous, intradermal, intramuscular and intravenous), transdermal and/or mucosal administration.

The (vaccine) compositions described herein, may comprise a discrete dosage unit and may be prepared by any of the methods well known in the art of pharmacy. Methods typically include the step of bringing into association one or more of the *D. gallinae* antigens described herein with liquid carriers or finely divided solid carriers or both.

Compositions (the term "composition" including a vaccine compositions), suitable for oral administration wherein the carrier is a solid are most preferably presented as unit dose formulations such as boluses, capsules or tablets each containing a predetermined amount of one or more of the *D. gallinae* antigens of this invention. A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine an active compound (for example a *D. gallinae* antigen) in a free-flowing form such as a powder or granules optionally mixed with a binder, lubricant, inert diluent, lubricating agent, surface-active agent or dispersing agent. Moulded tablets may be made by moulding an active compound with an inert liquid diluent. Tablets may be optionally coated and, if uncoated, may optionally be scored. Capsules may be prepared by filling an active compound, either alone or in admixture with one or more accessory ingredients, into the capsule shells and then sealing them in the usual manner. Cachets are analogous to capsules wherein an active compound together with any accessory ingredient(s) is sealed in a rice paper envelope. An active compound may also be formulated as dispersible granules, which may for example be suspended in water before administration, or sprinkled on food. The granules may be packaged, e.g., in a sachet. Formulations suitable for oral administration

wherein the carrier is a liquid may be presented as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water liquid emulsion.

Compositions suitable for oral administration include controlled release dosage forms, e.g., tablets wherein an active compound (for example one or more *D. gallinae* antigens) is formulated in an appropriate release-controlling matrix, or is coated with a suitable release-controlling film. Such compositions may be particularly convenient for prophylactic use.

Composition and vaccine compositions formulated for parenteral administration include sterile solutions or suspensions of an active compound (for example one or more *D. gallinae* antigens) in aqueous or oleaginous vehicles.

Injectable compositions and vaccines may be adapted for bolus injection or continuous infusion. Such preparations are conveniently presented in unit dose or multi-dose containers, which are sealed after introduction of the formulation until required for use. Alternatively, an active compound (for example one or more *D. gallinae* antigens) may be in powder form that is constituted with a suitable vehicle, such as sterile, pyrogen-free water or PBS before use.

Compositions comprising one or more *D. gallinae* antigens may also be formulated as long-acting depot preparations, which may be administered by intramuscular injection or by implantation, e.g., subcutaneously or intramuscularly. Depot preparations may include, for example, suitable polymeric or hydrophobic materials, or ion-exchange resins. They may also include preparations or adjuvants known to enhance the affinity and/or longevity of the avian immune response, such as single or double emulsions of oil in water. Such long-acting compositions are particularly convenient for prophylactic use.

Compositions suitable (or formulated) for mucosal administration include compositions comprising particles for aerosol dispersion, or dispensed in drinking water. When dispensed

such compositions should desirably have a particle diameter in the range 10 to 200 microns to enable retention in, for example, the nasal cavity; this may be achieved by, as appropriate, use of a powder of a suitable particle size or choice of an appropriate valve. Other suitable compositions include coarse powders having a particle diameter in the range 20 to 500 microns, for administration by rapid inhalation through the nasal passage from a container held close up to the nose, and nasal drops comprising 0.2 to 5% w/v of an active compound in aqueous or oily solution or suspension.

It should be understood that in addition to the carrier ingredients mentioned above, the various compositions described herein may include, an appropriate one or more additional (pharmaceutically acceptable) carrier ingredients such as diluents, buffers, flavouring agents, binders, surface active agents, thickeners, lubricants, preservatives (including anti-oxidants) and the like, and substances included for the purpose of rendering the formulation isotonic with the blood of the intended recipient.

Pharmaceutically acceptable carriers are well known to those skilled in the art and include, but are not limited to, 0.1 M and preferably 0.05 M phosphate buffer or 0.8% saline. Additionally, pharmaceutically acceptable carriers may be aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Preservatives and other additives may also be present, such as, for example, antimicrobials, antioxidants, chelating agents, inert gases and the like.

Compositions suitable for topical formulation may be provided for example as gels, creams or ointments.

One of skill will appreciate that when treating or preventing ectoparasite infections/infestations, the generation of local (cutaneous or sub-cutaneous) immune responses may be particularly advantageous.

Compositions for veterinary use may conveniently be in either powder or liquid concentrate form. In accordance with standard veterinary formulation practice, conventional water-soluble excipients, such as lactose or sucrose, may be incorporated in the powders to improve their physical properties. Thus particularly suitable powders of this invention comprise 50 to 100% w/w and preferably 60 to 80% w/w of the active ingredient(s) (for example one or more *D. gallinae* antigens) and 0 to 50% w/w and preferably 20 to 40% w/w of conventional veterinary excipients. These powders may either be added to, for example, avian feed – perhaps by way of an intermediate premix, or diluted in animal drinking water.

Liquid concentrates of this invention suitably contain one or more *D. gallinae* antigens and may optionally further include an acceptable water-miscible solvent for veterinary use, for example polyethylene glycol, propylene glycol, glycerol, glycerol formal or such a solvent mixed with up to 30% v/v of ethanol. The liquid concentrates may be administered to the drinking water of animals.

In general, a suitable dose of the one or more *D. gallinae* antigens provided by this invention may be in the range of about 10 to about 1000 μg *per* bird. Furthermore, the one or more antigens described herein may be administered on about 2 to about 5 occasions over a period of about 1 to about 10 weeks. In one embodiment, each bird may be administered about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650,

700, 750, 800, 850, 900, 950 or 1000 μg of the one or more antigens described herein. Furthermore, each bird may be administered the antigen(s) on 2, 3, 4 or 5 occasions over a 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 week period. It should be understood that each bird may receive the same or a different dose of the *D. gallinae* antigen(s)on each administration occasion.

Transdermal administration may be achieved with the use of impregnated coverings dressings, bandages or the like or via the use of some form of transdermal delivery device.

It should be understood that the term "*D. gallinae* antigen(s)" may relate to any component of the *D. gallinae* organism which is capable of eliciting an immune response in an avian host – such components may be regarded as immunoreactive. For example, the term "*D.* gallinae antigen(s)" may encompass proteins and/or peptides (including polypeptides and short peptide chains of one or more amino acids) including for example, glycoproteins and/or glycopeptides derived from *D. gallinae*. In addition, the term "*D. gallinae* antigen(s)" may relate to carbohydrate molecules. In certain embodiments, the antigens for use in this invention may comprise cell membrane antigens or antigens which are expressed by *D. gallinae* and which are exposed to the host (i.e. avian) immune system. Such antigens may include antigens expressed in the gut or on/in cells/tissues of *D. gallinae*. It should also be understood that the term "*D. gallinae* antigens" further encompasses any fragments or immunogenic/antigenic fragments of the *D. gallinae* antigens described herein. One of skill will appreciate that the term "*D. gallinae*" may also encompass *D. gallinae* proteins, polypeptides, peptides and/or carbohydrates which are otherwise known as "immunogens".

In one embodiment, the antigens provided by this invention may be one or more antigens derived from cells or tissues involved in, or associated with, fertility (or fecundity) processes. In this way, antibodies which bind to or recognise antigens derived from cells or tissues involved in

or associated with fertility or fecundity processes may inhibit or otherwise negatively affect (i) the ability of *D. gallinae* to produce viable eggs and (ii) larval development.

Antigens according to this invention may be provided as preparations derived directly from *D. gallinae*. For example, antigens for use in this invention may be obtained from whole or fragmented parasites harvested from donor animals and/or from the environment or habitat of the avian. Donor animals may be naturally infected/infested animals or animals which have been deliberately (or experimentally) infected with *D. gallinae*. In one embodiment, *D. gallinae* from which antigens may be derived may be obtained from a number of different donor avian subjects – those avian subjects inhabiting the same or different environments and/or habitats. Where the avian species are farmed, the *D. gallinae* may be obtained from one or more sites within a farm (for example a particular pen, poultry house, cage or shed within the farm) and/or from one or more of the infected or infested avian hosts within said site.

It should be understood that a vaccine or vaccine composition intended for use on a specific population of avian subjects infected/infested with *D. gallinae* (or at risk of infection/infestation with the same), may comprise one or more *D. gallinae* antigens derived from (i) one or more *D. gallinae* collected or harvested from the environment, habitat or locale of the specific population to be vaccinated and/or (ii) one or more *D. gallinae* collected or harvested from an environment, habitat or locale linked or associated with the specific population to be vaccinated. By way of example, where a farm comprises a complex of sites, perhaps spread across a particular geographical area, the invention may exploit one or more *D. gallinae* antigen(s) obtained or harvested from one or more of the sites of the complex – these antigens could be used to provide a vaccine for use on those avian subjects farmed at each site of the complex.

One of skill may refer to vaccines of the type described in the two paragraphs above as "autologous vaccines". As such, the invention provides autologous vaccines comprising one or more *D. gallinae* antigens for use in raising immune responses in avian species.

In order to obtain *D. gallinae* antigens, harevested or collected *D. gallinae* may be subjected to a homogenisation protocol to yield a homogenised suspension of *D. gallinae* components. The resulting homogenised *D. gallinae* suspension may then be subjected to one or more size/density separation techniques (such as centrifugation) so as to remove unwanted *D. gallinae* debris from useful antigen containing fractions of the suspension. The antigen containing fractions may then be subject to sterilisation procedures to render them suitable for use in this invention.

D. gallinae antigens for use in this invention may be further prepared for cold or freeze storage by the addition of one or more cryopreservative/cryoprotectant agents.

D. gallinae preparations produced in accordance with the methods described above may be regarded as "crude antigen preparations".

Crude antigen preparations of the type described above may be further processed in order to yield antigen fractions comprising fewer and more highly purified (or cleaner)/concentrated antigens or specific or select antigens. By way of example, techniques such as, for example, anion exchange, gel filtration and/or affinity chromatography may be used to prepare one or more fractions of, or to extract one or more specific antigens from, the crude antigen preparations described above.

In one embodiment, the present invention relates to specific *D. gallinae* antigens identified or characterised by PAGE analysis (i.e. migration) of *D. gallinae* antigen preparations

produced in accordance with the various methods described herein and mass spectrometry analysis of protein samples subjected to PAGE analysis.

In this regard the invention may comprise a *D. gallinae* antigen characterised by migration in SDS (i.e. reducing) PAGE techniques as having a size/weight of approximately 80KDa and/or a *D. gallinae* protein characterised by migration in SDS (i.e. reducing) PAGE techniques as having a size/weight of approximately 120KDa.

In one embodiment, the one or more *D. gallinae* antigens may comprise a vitellogenin protein, or pre-cursor or derivative/processed product thereof. In other embodiments, the one or more *D. gallinae* antigens may comprise a GP80-like protein or an immunogenic or antigenic fragment thereof.

In one embodiment, the one or more *D. gallinae* antigens provided by this invention comprise a sequence which is at least 30%, 40%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous or identical to any one of the sequences given as SEQ ID NOS: 1, 2 and 3 below. Further, it should be understood that the invention also relates to antigens comprising sequences which are at least 30%, 40%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous or identical to an immunogenic or antigenic fragment of any of the sequences given as SEQ ID NOS: 1, 2 or 3 below.

One of skill will appreciate that a level of sequence identity may be determined by comparing aligned amino acid sequences over a predetermined length so as to determine the number of positions at which an identical amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of amino acid residues in the length compared and multiplying the result by 100 to yield the percentage of sequence identity.

SEQ ID NO: 1 has the following sequence:

```
   1 VWLFVN_PST MRFFVLPLLL AAAASAEVFH FVGQHGQGST VYGVRGAVTV
  51 GAHQLTAEKT ALEYNGTLAV EQIREGEFLT KFTHFTVGKY NKLQRSVQDE
 101 TFDDLTPEEQ RVVKTLREPA VYEPHMQRPV RFFVKEGQIV RMEAEKEHPQ
 151 WSLNIFRSVL TLFQNQVSKP ATLAVPHVEY KYEDGITGNC KVQYEVFSLP
 201 EDVTVQGVFN LTKTKNYKDC LGRPVYLHLK DTQRGCAGVC DNHRPENFLA
 251 GYEEEITDYE LKPTPGCPVN QQRKDTLVTV QTVTKYNVSN GYLDEVRSEH
 301 TDIYRLYGGK LHVFTTLQLR LYGVAGPKIE EPKTVEIYKT LQLRLPHEED
 351 ELDIPVYALL REHTTQQQYG QHFQKYFEAV VQELLQLKDT QKQGQPEKQH
 401 YHSTAYLVEL VQAVSSMTEK ELKSIIPTIV HQAQPKQLTE EEHVRRQLWV
 451 ELLGKAGSKS AVKIIVELVK GKLLTPTEIR RVLQDVAAFQ SYPDTEMVEQ
 501 ILALCVKEQG LTATGKATAC VAAGKVLSKA CNSKVYQLAQ KHEQHKKTIN
 551 GKYQSIVQMQ EQKYTPETEP EAEEYRVTFG HLPVDPKLVC TPEKLQKYVS
 601 DLSHALHQAT DFKHVVAYIN GLAHVQKPEV LPELLGYVNG TASNLVHIHE
 651 QGEDIKEAVE FARHVAIVSL QHVAVKYPKE VNPIVRVVFE NTTEKVQTRI
 701 LAFDVWMDTQ PAQWEVEKVM QIANKDSSLE LTHYVYTALK TAMKAEEPCY
 751 QLLAQRVRAA WTQLRPFDLG SEFSHLRSKF YYDTVENYGI RGVWKVIASN
 801 TTILPFYTEA KVNQVRGPYK TTLFGAKLLV KGGDKVLEEL VGKDGLLERI
 851 AYALVGQIKT GPRQQNTEQL LKDIAQGMGL KREKDETPKA VLFWKLFSGD
 901 AVIPLDSHYI NELKQELLQT VTKFGKDGVT GHIVRVLVPT KAFHVEPSTI
 951 GLPIVHSTIH PVVLSVRYEN IKIHYGNQES RVAPKTLEIS GTVQPTILSF
1001 RQSRVFVSDK VGQKNPTVKT TDIKEFNVRL AFRVVYEHTP KRFRVHVKPV
1051 FDRVFHSGHC TELKLESAVL LKEELAAKTV EYDKCIKSLY QPIRRNHQIA
1101 GEWSGMMLRL TGESHQPWSG LPMFAPSVVS SEGILGAIIN RLSNKGMKHH
1151 TVSLYLETNN QQPITEWVAT IDVDSNVERL AKVPLSQQIT KVQKLKVQYA
1201 NRAQPLYPEL EPLVRKVESL LEKFETLDET TVEKLMLVKI EGLYQGQPKS
1251 TLKIAMKKIY NLEKTEQQYA LAAMHQESHK GLELSTNVSY PKIGSPFRYD
1301 PTFYAEDERM NGTLIVKLQS PQEQVFHVKF QATKSEEQLK ETEYEWFEVR
1351 CLAEQKAGKI MTDACRKAVL KDNSLDQLKI AVTVPRNVHP KIQTLAYKTL
1401 DLMKYMWYPK MQTEVAGLKQ REVLQALQHT EREVRISVNA TRESLWHLLY
1451 DVRVEMPFEN VTFSKVNIPG VRPAHMQLTT KEQLEHVYYR GQKDNVCVLG
1501 DKSVRTYDNV TFGLDVKTGC EYVLTRDTSS GTPDFTVTFQ VVKPDTFAKK
1551 IRVQLENTLV ELEPFTTTDR YITVVVNGTQ YQITFEKPVV FEYAAGKRVF
1601 LNVVDTSNVH HAPVITLYTE PKEVRVFFDG HSAKVFVVNK YKGNTKGVCG
1651 NNDNEQAHEF IGPNGKEYQH ANEFIASYGI GQACKVPAEN TREKLMETLK
1701 KEVEQIRRQE LIKKEKLRKE MQELERARNP QWMEQQEEQF WGEPLSTVSN
1751 EEWTTDSVEE QQLQRQVLKT AMSIENGHIC FSARPIATCK QGYKNHGVLR
1801 TERVESICLE KNEEAAIQAV QEIRAGQVVN IKSLEPYQKG RLFTMHKVPR
1851 CERHD_PE_N Q_LIQ
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SEQ ID NO: 2 has the following sequence:

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   1 QLKQQRLTQE QLVKEQAYPE YLRMIAYEDE YEKVTGRKPA TEKIEQALQR
  51 IVKTTEKHWS QIEPELREKL NVNNAQVRSI EYIITAIGQK KQPIAITGHA
 101 ILASTPSKQA RLAELTVDVE KHPISFEMQA VSAFAKAPQP FKAEIREQDQ
 151 RGILGFVAQL ETPKIGKKQY AGKIEMTKSE EQKQLMKRSI QQQPWYYRQC
 201 EEDKKEYTSE MSSACTRTRY HKSALNRVYA EIELPEEIPQ PIYNISRIIR
 251 DTLKVKLYGN LHATYNRKDM QQNKLQVELV YIDRFPAMHV ANLTIRTPVN
 301 EELHFERIAL PKALRPNSMW TLKEQIKAFL KNNRPEPVCV YNGKVIRTFD
 351 NVTVSLDTVK VGQKYLVARD NDEQSKFSIV ATKVQAAEES QTVLEVLLRD
 401 ATLIKLVPSV QKGVYKVHVN QTVTEVTPHK VQVYQYGPWA KHMLTLHVEE
 451 HKEGQDTLVV KVRDMHVHIV YDGKNFLIEI AGPQIKGQLT GLCGDLNHQH
 501 IDELTGPRGC HYEREEDFVR AFSFVPETNV AIEGEWVCPE GVHPRAASQM
```

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551 QISKKMEQQQ AAQQKKQLNI QQRQIVREEY NKMTPETKMI HQDGKVCYSV
601 DPVQACKPGL RAVETEMHTV AFVCLPIQHP MTAMIQREIQ VHGVIRNIPS
651 VMPGALEHIL YHQIKTPISC AN_TANMGQI KLFS
```

SEQ ID NO: 3 has the following sequence:

```
  1 SLVFLGAHPS NTASISTMRV LLTFVALAVA VSAAAYHGQK QVVIPDLFEG
 51 QREYVYNYRS LVATGLPLQS QRFAGLEKYG ILTVQVHEQH GTTKVLGLRL
101 GHVTSGSFDK EVEDVENRGI EGISHSIKEI VEKYGAVFVT IQDNEVQKIK
151 VPQGMPEEIV NIYRGIAALF TVGKPDTHVH GTESHPFTYQ GEHGEQLPVV
201 YRRQEKGIAG NFETTYEILS NPEHEYGYLN VTKTRNYLKK VGPDGRFFQN
251 GHDAHGCGPV CLTHKPEQID QNMQPDTTAW ETPVTEGCPV KFHPKKDLVE
301 AFTTYNYNMT VEQGGKIAVI HEAKGLDKKV LPLRKQQILT VSLLKVTLVE
351 VRPISQIWEE KAATKQYSDI TYRFPEGHKH DLAYLSLYSK GSKGEIVTEQ
401 ILPMLKALSK IIVSDNIELK SQTGDKVVQL TQALGVLTKT ELQSIWTVIG
451 EPVSEKTATE FEKVQRKVLV DIIALSGSND AAEFLVELIQ QERLTVLETV
501 HTLETLQKSI VKPTLTIIKQ LLNVCTETKF QKTRVVFSTA CIAFSEIVRH
551 NCEDYVVSQM TEAPKMQGEE KKHTVHSCGP HEYKAFIETI REKLYAAQEY
601 PQQVVYIQVL GRLAHPEALK ALVPYIYGEH EVIAQVEKMT RDNEHEDNSE
651 YVQFLRQVAI FALHHSVRKH GASVQPIVQG VYFNKKEDYE LRIAALSVLL
701 ATQPTEATFG RIVTELYKED NLEIASYTYS ALHALANSTL PCMKQSARRV
751 QNVLGAFPKK SYGIDYSKWG THTKYSPLMN FGYKGHWEIT QSNVSAIPRA
801 IYVGATANKG PFISTLGEFG MISKGLENLD RFVGQKGGVQ KIMENVMQRI
851 RRDARTFVGD ASVGRMLEEI ENAFDFNTEE NNEQLRAVVF GNVLGNEFYL
901 PVDKQFVTKV AEKVGEEMIK ILREEGSEKT LRYVRVLLPR TYVQVAPAVN
951 GLPVLMINRH PIVVSLALKD LKIRLGAEKE ELTLNPLTFA ASGLIQPTVY
1001 FTAFHTAMTI NPLETSHVGY GLRTIEQTYM SLPIDASIQY THQTKTLAFT
1051 FRPRFEKIFF HKTRAMTFKT EVLLISDVER PILDQYTVIK TQRKPYAIDR
1101 VFGEKLGMAL RVQGLTVNEN YADNLIYKIF TGQHTAVTSI LKGIANEWVL
1151 PRAWTVRIEQ NKQTPIDKVK VVLRLGDYLK DRMLTEQTEQ K
```

In one embodiment, the antigens and compositions for use, uses, medicaments and methods provided by this invention comprise one or more *D. gallinae* antigens comprising a sequence at selected from the group consisting of:

(a) SEQ ID NO: 1

(b) SEQ ID NO: 2

(c) SEQ ID NO: 3

(d) a sequence at least 30%, 40%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous or identical to any one of (a), (b) or (c); and

(e) a sequence at least 30%, 40%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous or identical to an immunogenic fragment of any one of (a), (b) or (c).

In one embodiment, the invention provides a vaccine or vaccine composition comprising one or more *D. gallinae* antigens comprising a sequence selected from the group consisting of:

(a) SEQ ID NO: 1

(b) SEQ ID NO: 2

(c) SEQ ID NO: 3

(d) a sequence at least 30%, 40%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous or identical to any one of (a), (b) or (c); and

(e) a sequence at least 30%, 40%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous or identical to an immunogenic fragment of any one of (a), (b) or (c).

wherein said vaccine or vaccine composition is for use in raising an immune response in an avian. In one embodiment, the avian species is a chicken (*Gallus gallus domesticus*). In a further embodiment, the immune response protects against *D. gallinae* infection/infestation.

In addition to providing specific peptide sequences (such as those given as SEQ ID NOS: 1-3 above), the invention further provides nucleic acid molecules encoding the same or fragments (preferably antigenic or immunogenic fragments) thereof. The nucleic acid may be DNA, RNA or a combination thereof and can include any combination of naturally occurring, chemically or enzymatically modified nucleotides. Furthermore, the nucleic acid may be double or single stranded. Within the scope of this invention are nucleic acid sequences that are

substantially complementary to any nucleic acid sequence encoding one of SEQ ID NOS: 1-3 described herein.

It should be understood that the term "substantially complementary" encompasses those nucleic acid molecules exhibiting a degree of sequence identity/homology with any nucleic acid sequence encoding SEQ ID NOS: 1-3 above or a fragment (especially an immunogenic fragment) thereof. A nucleic acid sequence having a level of identity or homology with a sequence encoding SEQ ID NO: 1, 2 or 3 (or an immunogenic fragment thereof) may exhibit at least 30%, 40%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity or homology with the full length nucleic acid sequence encoding SEQ ID NO: 1, 2 or 3 (or an immunogenic fragment thereof) or the relevant portion or fragment thereof.

One of skill will appreciate that a level of sequence identity may be determined by comparing aligned nucleic acid sequences over a predetermined length so as to determine the number of positions at which an identical nucleic acid base occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of nucleic acid bases in the length compared and multiplying the result by 100 to yield the percentage of sequence identity.

In view of the above, the present invention relates to antigens (and compositions comprising the same), uses (medicaments) and methods exploiting (i) proteins encoded by the sequences designated SEQ ID NOS: 1, 2 and 3 above as well as proteins encoded by nucleic acid sequences encoding any of SEQ ID NOS: 1, 2 or 3. Furthermore, the invention relates to antigens (and compositions), uses (medicaments) and methods exploiting any fragments, portions, variants, derivatives, analogues, homologues/orthologues of any of these sequences.

The invention may further relate to natural or artificially created variants or analogues of any of the protein/nucleic acid sequences described herein. Such variants may exhibit one or more amino acid/nucleic acid deletions, additions, substitutions and/or inversions, relative to, for example a reference sequence (such as for example sequences disclosed in this invention as SEQ ID NOS: 1, 2 or 3). In certain embodiments, the substitutions may represent conservative substitutions. One of skill will appreciate that a conservative substitution involves replacing one or more amino acids of a protein or peptide sequence with an alternate amino acid having similar properties and which does not substantially alter the physio-chemical properties and/or structure or function of the native (or wild type) protein.

As is well known in the art, the degeneracy of the genetic code permits substitution of one or more bases in a codon without alteration to the primary amino acid sequence. As such, genetic degeneracy may be exploited in order to yield variant nucleic acid sequences which encode peptide or protein sequences of SEQ ID NOS; 1, 2 and/or 3.

The nucleic acid molecules provided by this invention may take the form of nucleic acid constructs or vectors such as, for example a cloning or expression cassettes/vectors including phage vectors for use as described, for example in EP1370284. The term "nucleic acid constructs" or "vector" may further encompass constructs intended for use as DNA vaccines.

Vectors provided by this invention may be capable of directing the expression of nucleic acid sequences encoding *D. gallinae* antigens in, for example, bacterial, fungal, animal (including avian species and mammalian) and/or insect cells.

Accordingly, a fifth aspect of this invention provides a vector, preferably an expression vector, comprising a nucleic acid sequence encoding a *D. gallinae* antigen having a sequence selected from the group consisting of:

(a) SEQ ID NO: 1

(b) SEQ ID NO: 2

(c) SEQ ID NO: 3

(d) a sequence at least 30%, 40%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous or identical to any one of (a), (b) or (c); and

(e) a sequence at least 30%, 40%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous or identical to an immunogenic fragment of any one of (a), (b) or (c).

Expression vectors suitable for use in this aspect of the invention may further comprise one or more promoter sequences capable of directing expression in prokaryotic or eukaryotic cells such as, for example, avian, mammalian, fungal, bacterial, plant and/or insect cells.

A vector provided by this invention may be circular or linear, single stranded or double stranded and can include DNA, RNA or a combination or modification thereof. Furthermore, vectors of this invention may be, for example, plasmids, cosmids or viral vectors (for example retroviral or bacteriophage vectors). Vectors provided by this invention may further comprise selection or marker elements, for example antibiotic resistance genes and/or optically detectable tags. A large number of suitable vectors are known and further information may be obtained from Pouwels et al. Cloning Vectors: a Laboratory Manual (1985 and supplements), Elsevier, N.Y.; and Rodriquez, et al. (ads.) Vectors: a Survey of Molecular Cloning Vectors and their Uses, Buttersworth, Boston, Mass (1988) – both of which are incorporated herein by reference.

As such, in addition to techniques in which *D. gallinae* antigens are extracted or purified directly from harvested or collected *D. gallinae*, antigens to be exploited in this invention may be obtained using recombinant technology. In one embodiment, an expression vector comprising

one or more nucleic acid sequences encoding a *D. gallinae* antigen (such as any of those described herein) may be used to produce one or more recombinant *D. gallinae* antigens.

Accordingly, in a further aspect, the present invention provides host cells transfected or transformed with a vector as described herein. Eukaryotic or prokaryotic cells, such as, for example avian, plant, insect, mammalian, fungal and/or bacterial cells, may be transfected with one or more of the vectors described herein. One of skill in this field will be familiar with the techniques used to introduce heterologous or foreign nucleic acid sequences, such as expression vectors, into cells and these may include, for example, heat-shock treatment, use of one or more chemicals (such as calcium phosphate) to induce transformation/transfection, the use of viral carriers, microinjection and/or techniques such as electroporation. Further information regarding transformation/transfection techniques may be found in Current Protocols in Molecular Biology, Ausuble, F.M., ea., John Wiley & Sons, N.Y. (1989) which is incorporated herein by reference.

In view of the above, the present invention further provides a process for the production of a *D. gallinae* antigen encoded by a sequence of SEQ ID NO: 1, 2 or 3 (or an immunogenic fragment thereof) and for use in raising an immune response in an avian, said method comprising the step of (a) transforming a host cell with a nucleic acid sequence according to this invention or transfecting a host cell with a nucleic acid construct of the invention; (b) culturing the cells obtained in (a) under conditions in which expression of the nucleic acid (or rather a protein encoded thereby) takes place; and (c) isolating the expressed recombinant protein or peptide from the cell culture and/or the culture supernatant.

Recombinant proteins/peptides produced according to the method described above may be partially purified from the host cell before being used in a vaccine or vaccine composition. Where the polypeptide is secreted from the host cell, the cells may be separated from the media

by centrifugation. In such a situation, the supernatant, which contains the secreted polypeptide, may be used directly as a vaccine, or in a vaccine composition. Alternatively, the polypeptide may be partially purified from this supernatant, for example using affinity chromatography.

In one embodiment, any of the *D. gallinae* antigens described herein (whether directly isolated from harvested or collected *D. gallinae* or recombinantly produced) may be admixed with another component, such as another polypeptide and/or an adjuvant, diluent or excipient. Vaccines or vaccine compositions provided by this invention may, for example, contain viral, fungal, bacterial or other parasite antigens used to control other avian diseases/infections or infestations. For example, the vaccine or vaccine composition may be included within a multivalent vaccine which includes antigens against other avian (for example chicken) diseases. These vaccines may include, for example, the vaccines and target organisms listed in Table 1, below.

Vaccine name	Manufacturer	Target organism
89/03	MSD AH [Intervet]	Avian Infectious bursal disease virus [IBDV, Gumboro disease virus]
ABRONCHOVAX	Laboratoire National Vétérinaire [LANAVET]	Avian infectious bronchitis virus [IBV]
ADENIPRAVAC ND/IB	Laboratorios HIPRA S.A.	Avian infectious bronchitis virus [IBV] , Egg drop syndrome virus [EDS virus, Avian adenovirus], Newcastle disease virus [NDV, Avian paramyxovirus, APMV-1]
ADVENT Coccidiosis Control	Viridus Animal Health L.L.C. [Novus International Inc.]	Eimeria acervulina, Eimeria tenella, Eimeria maxima
AE+Pox	MSD AH [Intervet]	Avian encephalomyelitis virus [AEV] , Fowl pox virus
AE-POX VACCINE NOBILIS	MSD AH [Intervet]	Fowl pox virus, Hepatovirus
Aerovac AI	Investigación Aplicada, S.A. de C.V.	Avian influenza virus [AI]
Anemovac	Investigación Aplicada, S.A. de C.V.	Chicken infectious anaemia virus [CIAV]
Angara Disease Vaccine	Sindh Poultry Vaccine Centre (SPVC)	Adenovirus

ART VAX	MSD AH [Intervet]	Bordetella avium (Attenuated)
Artri-Vet	Laboratório Bio-vet S.A.	Avian reovirus (S 1133)
Avian Cholera Vaccine	Veterinary Serum and Vaccine Research Institute	Pasteurella multocida
Aviffa RTI	Merial	Avian rhinotracheitis virus [Avian metapneumovirus] (VCO3) (Attenuated)
AviPro 101 Coryza	Lohmann Animal Health GmbH & Co. KG	Haemophilus [Avibacterium] paragallinarum [Infectious coryza virus]
AviPro 104 MG	Lohmann Animal Health GmbH & Co. KG	Mycoplasma gallisepticum (S 6)
AviPro 109 SE4	Lohmann Animal Health GmbH & Co. KG	Salmonella Enteritidis (8, 14B, 23, 24)
AviPro ILT	Lohmann Animal Health GmbH & Co. KG	Gallid herpesvirus 1 [GaHV-1, Avian herpesvirus 1, Infectious Laryngotracheitis virus, ILTV]
AviPro MD BIVAC	Lohmann Animal Health GmbH & Co. KG	Marek's disease virus
AviPro SALMONELLA DUO	Lohmann Animal Health GmbH & Co. KG	Salmonella Enteritidis (Sm24/Rif12/Ssq) (Attenuated), Salmonella Typhimurium (Nal2/Rif9/Rtt) (Attenuated)
AviPro THYMOVAC	Lohmann Animal Health GmbH & Co. KG	Chicken infectious anaemia virus [CIAV] (CUX-1)
BAK-MG	Bestar Laboratories Pte Ltd.	Mycoplasma
BDK-PA	Bestar Laboratories Pte Ltd.	Pasteurella anatipestifer
Bio Coccivet R	Laboratório Bio-vet S.A.	Eimeria acervulina , Eimeria brunetti , Eimeria maxima , Eimeria necatrix , Eimeria praecox , Eimeria tenella , Eimeria mitis
Bio Mark Vet C	Laboratório Bio-vet S.A.	Meleagrid herpesvirus 1 [MeHV-1, Marek's disease virus serotype 3, Turkey herpesvirus, HVT] (FC126)
Bio-SHS	Laboratório Bio-vet S.A.	Avian metapneumovirus [Avian/Turkey rhinotracheitis]
Bio-SHS Viva	Laboratório Bio-vet S.A.	Avian pneumovirus
CALAVAC REO	C.A. Laboratorios Asociados [CALA]	Reovirus (S1133)
CAV Vaccine	Intervet Australia Pty Ltd.	Chicken infectious anaemia virus [CIAV] (3711)
Chevipok	Chevita GmbH	Pigeon pox virus
Chevivac-P12	Ceva Animal Health Ltd.	Pigeon paramyxovirus 1 [PPMV-1]
Chicken Necrotic Enteritis Gel Vaccine	Veterinary Serum and Vaccine Research Institute	Clostridium [welchii] perfringens
Coli-Ave Oleosa	Laboratório Bio-vet S.A.	Escherichia coli (O1, O2, O35, O78) (Subunit)
COR-2	Merial	Coronavirus (PL84084, CR88121)

Deparmune	Ceva Santé Animale	Barbarie duck parvovirus (FM) , Derzsy disease virus [Goose parvovirus] (LB)
Difeterviruela Aviaria Inmuner	Laboratorios Inmuner S.A.I.Y.C.	Avian smallpox virus (Attenuated)
Dindoral SPF	Merial	Epizootic haemorrhagic disease [EHD] virus
Duck Hepatitis Virus Live Freezing Vaccine	Green Cross Veterinary Products Co. Ltd.	Duck hepatitis virus (DHV-HSB type I)
Duck Virus Enteritis Vaccine	International Duck Research Cooperative Inc.	Anatid herpesvirus 1 [AHV-1, Duck herpesvirus 1]
Fowl Pox Vaccine Living	Ventri Biologicals [Venkateshwara Hatcheries Private Ltd.]	Fowl pox virus
FOWL POX VACCINE, LIVE, I. P. (Vet.)	BiO-MED Private Ltd.	Fowl pox virus (BM) (Attenuated)
Fowl Spirochaetosis Vaccine Inactivated	Ventri Biologicals [Venkateshwara Hatcheries Private Ltd.]	Borrelia anserina
Fowl typhoid	National Veterinary Institute Ethiopia	Salmonella Gallinarum (Attenuated)
HIPRAVIAR TRT	Laboratorios HIPRA S.A.	Turkey rhinotracheitis virus [Avian metapneumovirus]
Immucox Turkey	Imuvet Comercial Ltda.	Eimeria adenoeides , Eimeria meleagrimitis
Nobilis Erysipelas	MSD AH [Intervet]	Erysipelothrix rhusiopathiae (M2)
Nobilis OR Inac Emulsion for Injection for Chickens	MSD AH [Intervet]	Ornithobacterium rhinotracheale [ORT] (serotype A strain B3263/91)
Parduvak	IDT Biologika GmbH	Parvovirus
Parvokan	Merial	Derzsy disease virus [Goose parvovirus] (H) (Attenuated), Muscovy duck parvovirus (GM)
Pro'tect HE	Brinton Laboratories Inc.	Hemorrhagic enteritis virus
Virsin 424	Biovac Company Ltd.	Riemerella anatipestifer , Pasteurella multocida

Table 1. Example avian vaccines.

In a still further aspect, the present invention provides an avian population, for example a farmed population of chickens, treated, vaccinated or immunised with a vaccine or composition described herein, aid vaccine or composition comprising one or more of the *D. gallinae* antigens described herein.

One of skill will appreciate that the vaccines described in this invention may take the form of subunit-type vaccines where by one or more *D. gallinae* antigens are used to inoculate

an animal. Additionally or alternatively, the vaccine may comprise a nucleic acid molecule (known as a DNA vaccine) encoding one or more antigens encoded by SEQ ID NOS: 1, 2 or 3 or an immunogenic fragment thereof, to be expressed by the cells of an animal to be vaccinated. In this way, constitutive expression of *D. gallinae* antigens in a vaccinated host (such as, for example a vaccinated chicken) may elicit a constitutive protective immune response.

In addition to providing *D. gallinae* antigens for use in raising immune responses in animals, the present invention may also provide polyclonal and/or monoclonal antibodies (or antigen binding fragments thereof) that bind (or have affinity or specificity for) any of the *D. gallinae* antigens described. Production and isolation of polyclonal/monoclonal antibodies specific for protein/peptide sequences is routine in the art, and further information can be found in, for example "Basic methods in Antibody production and characterisation" Howard & Bethell, 2000, Taylor & Francis Ltd. Such antibodies may be used in diagnostic procedures, to, for example detect or diagnose *D. gallinae* infection/infestations in avian species, as well as for passive immunisation.

The present invention further provides a vaccine for use in preventing or controlling *D. gallinae* infection/infestation and associated diseases in avian hosts. The vaccine may be a polypeptide or polynucleotide vaccine.

The invention further provides a method for immunising an avian against *D. gallinae* infection/infestation and associated disease (for example secondary infections etc.), said method comprising the step of administering a vaccine of the invention to the avian.

DETAILED DESCRIPTION

The present invention will now be described in detail with reference to the following Figures which show:

Figure 1: SDS-PAGE to show protein profile of extracted antigens. Lane 1, 1/10 dilution of antigen, Lane 2, 1/100 dilution, Lane 3 Seablue plus 2 molecular mass marker.

Figure 2: Immunoblots of red mite vaccine preparation: panel A) Control – IgY taken from eggs that were not exposed to vaccination and panel B) IgY from eggs from hens vaccinated with mite antigens from a previous trial. Both panels: left to right lanes consist of molecular mass marker, then pre and post filtered antigen, respectively. Following filtration, no differences in antigenic profile were observed.

Figure 3: An Overview of the cages. Line 4 and 3 are back to back as is line 2 and line 1. Group 2 is the vaccinated group and Group 1 the control group. The gaps between group 2 and group 1 are empty cages that were treated to decrease mite migration between cages.

Figure 4: Mite numbers (/100) over a 4 month period. The control group (indicated by the blue line) are the mites that fed on hens that had been injected with adjuvant only. The vaccinated group (indicated by the red line) are the mites that fed on hens that had been injected with the vaccine antigen prepared in the same adjuvant.

Figure 5: A Western blot carried out on the antigen prepared using anti IgY extracted from the eggs collected from the vaccinated chickens and control chickens.

Figure 6: A Western blot carried out on antigen prepared using anti IgY extracted from the eggs collected from the chickens vaccinated with Formalin-inactivated antigen (Form), Beta propiolactone-inactivated antigen (BP) and control chickens (immunized with adjuvant only).

Methods

i) Antigen and vaccine preparation:

5mL of ice-cold phosphate buffered saline (PBS) was added to *ca.* 1mL of mites and homogenised on ice for 30sec. The homogenised mite sample was rested for 1 min on ice before repeating a second round of homogenisation for 30sec. The homogenised sample was then centrifuged for 20min @ 24000 xg @ 4°C. The supernatant (antigen) was filtered through a 0.22μm filter and divided into 1mL aliquots and stored at -80°C

Protein content of the sample was determined using the Pierce BCA protein assay, with bovine serum albumin (BSA) standards.

The protein profile of the extract was investigated by separation of the native extract on NuPAGE® Bis-Tris 4-12% gels, under reducing conditions, employing NuPAGE® MES SDS running buffer (Invitrogen) and the profile is shown in Figure 1.

An immunoblot was also performed on the mite extract, to determine the antibody reactivity of these antigens. The antibodies used for the blot were obtained from the eggs of hens previously vaccinated with red mite antigens, from a prior study. In addition, the impact of sterile filtration (to remove potentially harmful bacteria and sterilize the vaccine) on the antigen profile was also investigated, by comparing the immunoreactive antibody profiles before and after filtration (see Figure 2).

On the basis of the integrity of the proteins and their immunoreactivity, we proceeded to generate the vaccine. A total of 285 mgs (post filtration) of red mite protein was couriered on dry ice to

Ridgeway Biological Ltd, Compton, for formulation in an oil-in-water emulsion. Two equal batches of 250mls of the vaccine were produced (total 500 ml containing 285micrograms per 0.5ml dose), and, after confirmation of sterility, these two batches were couriered directly to Roslin Nutrition Ltd.

ii) Experimental plan and cage layout:

The trial involved a total of 768 Lohmann Brown hens, raised on-site at Roslin Nutrition Ltd. with no prior exposure to *D. gallinae*, consisting of two groups of hens. One group was assigned as the vaccine group (receiving the vaccine), and the second represented normal (receiving adjuvant alone) control hens. Neither group was treated with chemicals employed to control red mites during the duration of the study. All birds received two intra muscular injections at 12 and 17 weeks of age, before being assigned to cages. The vaccinated birds received 0.5ml of vaccine containing 285 micrograms of mite soluble protein/injection, with controls receiving an equal volume of adjuvant alone. After the second vaccine injection, the hens were placed in cages (4 per cage) in an isolated housing unit. This unit was free of red mites before the commencement of the study. Cages were placed together to form blocks consisting of four by three cages, and arranged to form two rows of blocks separated by a 1 metre passage. Each block was interspaced by empty cages lined with 'mite proof' plastic sheeting. Blocks consisted of either vaccinated or control hens, in an alternated pattern to reduce any 'space effect' within the housing unit between the two groups.

A diagram depicting the cage layout is shown in Figure 3.

The groups were designated by Roslin Nutrition as 'Group 1' and 'Group 2', and information on what treatment was given to each group was withheld from the scientists until after the mite counts were completed, to allow a "blind" trial.

Mite counts:

Throughout the period of study, red mite numbers were assessed by standard trapping and counting techniques. ADAS traps were placed into each cage 24 hours before collection, and introduced 2 weeks after the mite challenge. On collection traps were treated with 70% ethanol to kill the mites and placed in sealed containers. Mites were counted in a 'blind' fashion (i.e. without the counter's knowledge of which group was which), being designated with cage number only. On collection, mites were washed from the traps and containers with 70% ethanol into Petri dishes, before being counted. The mites were usually large enough to be visualized by the naked eye, and a dissecting microscope was only employed where there was doubt between distinguishing between debris and mite. Initial counts were very low (see results), but in the latter half of the study mite numbers increased substantially. In order to count these high numbers (over 500 mites/trap), the Petri dish was divided into equal portions by placing onto graticuled paper, the contents of a limited number of the portions were counted and the total number estimated by multiplication from these counts.

Production and welfare:

Egg production and general health parameters/observations of both groups were assessed on a regular basis throughout the study.

Further development of the vaccine towards industry compliance

The practical use of any autogenous vaccine will require Veterinary Medicine Directorate (VMD) approval. To obtain this approval we need to ensure freedom from adventitious agents. The most straightforward method of doing this is by "inactivation" of the vaccine by treatment of the mite extract with formalin or beta-propiolactone. Thus, the extract, prepared as described above ("Antigen and vaccine preparation") was treated with either formalin or beta-propiolactone prior to formulation with a standard autogenous vaccine preparation (containing inactivated *Escherichia coli*, *Pasteurella multocida, Mycoplasma gallisepticum* and *M. synoviae* antigens) by a commercial autogenous vaccine producing company, and injected into laying hens (in duplicate). Antibodies were extracted from the eggs laid by these hens and their immunoreactive profile was examined by immunoblot to establish whether comparable antigenicity was achieved between these inactivated extracts and the original extracts which had been used in the field trial described above. Ultimately, these antibodies will be mixed with hen blood and fed to red mites *via* an *in vitro* feeding device for their anti-mite effect, by determiningthe mortality over the following 5 day period.

Results

Antigen preparation and vaccination:

No problems were encountered during antigen preparation or formulation for the vaccine.

Mite counts:

Mite numbers were counted and recorded for each sampling date (see Table 1). A graph was then generated using the values that were recorded for each date (see Figure 4)

Table 1: The sum of the different sets of cages (6 cages in one set)

	25/11/2010		10/12/2010		11/01/2011		28/01/2011		15/02/2011		01/03/2011		15/03/2011	
	Grp 2	Grp 1	Grp 2	Grp 1	Grp 2	Grp 1	Grp 2	Grp 1	Grp 2	Grp 1	Grp 2	Grp 1	Grp 2	Grp 1
set 1	2	6	0	0	19	170	53	495	2685	7713	8899	17134	4029	32377
set 2	18	2	0	0	237	5	961	280	3778	2616	9275	13157	5852	12940
set 3	4	8	0	1	2	28	141	706	2268	5947	11979	23809	3821	39439
set 4	14	12	1	1	573	309	1211	368	3468	4670	15081	15350	6090	14751
set 5	1	15	1	0	29	50	480	853	1391	4738	7247	18952	2448	17627
set 6	26	11	0	0	386	25	1774	168	2988	1537	9080	10580	6133	14331
set 7	14	12	1	0	45	14	235	103	2040	2974	5669	19636	3736	21417
set 8	14	5	1	4	339	18	547	86	3292	2722	9936	8880	3052	10767
Sum	**93**	**71**	**4**	**6**	**1630**	**619**	**5402**	**3059**	**21910**	**32917**	**77166**	**127498**	**35161**	**163649**

From these results, it can be seen that for the first few weeks after mite challenge, very few mites were recorded in the traps. However, at the beginning of February and approximately 9 weeks following the mite challenge, the mite counts increased and by the end of the study, substantial numbers were recorded. At this time, highly significant differences ($P>0.01$ and $P>0.001$ at time points 01/03/2011 and 15/03/2011, respectively) in these counts between the vaccinated and control groups were demonstrated, with mean numbers in the vaccinated group approximately four-fold less than those in the control group. Of particular note during these counts was the observation that there were markedly fewer nymphal (juvenile) mites in traps from the vaccinated group when compared to those in the control group, where they constituted a sizeable proportion (~50%) of the total number. Further quantitative studies are required to confirm these observations.

Immunological studies:

The antigen prepared for the vaccination was separated by electrophoresis and used in an immunoblot to determine the antigens to which the highest antibody response was mounted during the trial. Eggs were collected 2 weeks post vaccination (15/11/2010), one month post vaccination (11/01/2011), 2 month post vaccination (28/01/2011) and the last collection at the end of the study (15/03/2011). Immunoglobulin prepared from these eggs was used to probe the immunoblots of the vaccine antigens. These blots are shown in figure 5. Although strong antibody reactive bands are seen from both the control and vaccinated antibodies, those from the former are likely to be avian IgY (heavy and light chains) extracted from the mites which contained ingested chicken blood, detected with the secondary anti-IgY antibody. Following vaccination with mite antigens however, additional antigenic bands were observed, with two of

the most prominent being in the order of ~80KDa and 120KDa (arrowed in Fig 5). It is relevant that antibodies to both these proteins appear to decline after vaccination, but reappear and are strongly represented at the terminal stages of the study when there was a significant divergence in mite populations between the two groups.

The immunoreactive (reacting with chicken IgY derived from hens vaccinated with a PBS extract of *Dermanyssus gallinae*) bands on Western blots of PBS extract of *Dermanyssus gallinae* were aligned with the corresponding area on an SDS PAGE gel (1 dimensional) separation of the same extract. The bands were excised and analysed by MALDI-Tof to derive a peptide mass fingerprint which was used to interrogate an EST database of nucleic acid sequences derived from *D. gallinae* mRNA. The EST database was produced by and held at Moredun Research Institute. It is not publically available. The contigs with the highest scores (sequence coverage) for each of the immunoreactive bands were translated into amino acid sequence and used to interrogate the NCBI BLASTp (nr) public database to assign identity and function. Each of the bands possessed very high homology to invertebrate vitellogenins (See Example 1: $E< 1 \times 10^{-80}$).

Further development of the vaccine towards industry compliance

Antibodies were extracted from the eggs laid by hens which had been immunized with red mite extract, prepared as described above ("Antigen and vaccine preparation") and treated with either formalin or beta-propiolactone prior to formulation with a standard autogenous vaccine preparation (containing inactivated *Escherichia coli*, *Pasteurella multocida*, *Mycoplasma gallisepticum* and *M. synoviae* antigens) by Ridgeway Biological Ltd., Compton, Berkshire. The

immunoreactive profile was examined by immunoblot to establish whether comparable antigenicity was achieved between these inactivated extracts and the original extracts which had been used in the field trial described above. Following vaccination with inactivated mite antigens (using either formalin or beta propiolactone) antigenic bands were observed, with two of the most prominent being in the order of ~80KDa and 120KDa (Figure 6), and similar to the western blot profile observed with antibodies obtained from the vaccinated hens in the *in vivo* trial described above.

Conclusions

This trial has provided strong evidence that vaccination of hens with an autogenous vaccine prepared from poultry red mites can have a major effect on mite populations, and provide alternative control for this parasite. Inactivation of the antigens using formalin or beta propiolactone did not affect their immunogenicity, indicating the commercial viability and exploitation for an autogenous vaccine for the poultry red mite.

The results strongly suggest that antibodies generated following vaccination affected mite survival and/or mite fecundity. That the latter may be the case is suggested by our observation that fewer juvenile mites were present in the vaccinated group; such an observation is in accord with an 'anti-fecundity' effect influencing egg production or larval development.

This field trial has provided proof-of-concept data to support the use of as a vaccine to control poultry red mite.

Example 1

Three immunoreactive (reacting with chicken IgY derived from hens vaccinated with a PBS extract of *Dermanyssus gallinae*) bands on Western blots of PBS extract of *Dermanyssus gallinae* were aligned with the corresponding area on an SDS PAGE gel (1 dimensional) separation of the same extract. The bands were excised and analysed by MALDI-Tof to derive a peptide mass fingerprint which was used to interrogate an EST database of nucleic acid sequences derived from *D. gallinae* mRNA. The EST database was produced by and held at Moredun Research Institute. It is not publically available. The contigs with the highest scores (sequence coverage) for each of the immunoreactive bands were translated into amino acid sequence and used to interrogate the NCBI BLASTp (nr) public database to assign identity and function. The three proteins for identification are labeled JH 1-3:

>JH 1

Match to: **contig12013** Score: **344** Expect: **3.2e-30**
length=5596 numreads=1668
Translated in frame 5

Nominal mass (M_r): **212498**; Calculated pI value: **8.06**
NCBI BLAST search of contig12013 against nr
Unformatted sequence string for pasting into other applications

Variable modifications: Carbamidomethyl (C),Oxidation (M),Propionamide (C)
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Number of mass values searched: **81**
Number of mass values matched: **58**
Sequence Coverage: **33%**

Matched peptides shown in **Bold**

```
     1 VWLFVN_PST MRFFVLPLLL AAAASAEVFH FVGQHGQGST VYGVRGAVTV
    51 GAHQLTAEKT ALEYNGTLAV EQIREGEFLT KFTHFTVGKY NKLQRSVQDE
   101 TFDDLTPEEQ RVVKTLREPA VYEPHMQRPV RFFVKEGQIV RMEAEKEHPQ
   151 WSLNIFRSVL TLFQNQVSKP ATLAVPHVEY KYEDGITGNC KVQYEVFSLP
   201 EDVTVQGVFN LTKTKNYKDC LGRPVYLHLK DTQRGCAGVC DNHRPENFLA
   251 GYEEEITDYE LKPTPGCPVN QQRKDTLVTV QTVTKYNVSN GYLDEVRSEH
   301 TDIYRLYGGK LHVFTTLQLR LYGVAGPKIE EPKTVEIYKT LQLRLPHEED
   351 ELDIPVYALL REHTTQQQYG QHFQKYFEAV VQELLQLKDT QKQGQPEKQH
   401 YHSTAYLVEL VQAVSSMTEK ELKSIIPTIV HQAQPKQLTE EEHVRRQLWV
   451 ELLGKAGSKS AVKIIVELVK GKLLTPTEIR RVLQDVAAFQ SYPDTEMVEQ
   501 ILALCVKEQG LTATGKATAC VAAGKVLSKA CNSKVYQLAQ KHEQHKKTIN
```

```
 551 GKYQSIVQMQ EQKYTPETEP EAEEYRVTFG HLPVDPKLVC TPEKLQKYVS
 601 DLSHALHQAT DFKHVVAYIN GLAHVQKPEV LPELLGYVNG TASNLVHIHE
 651 QGEDIKEAVE FARHVAIVSL QHVAVKYPKE VNPIVRVVFE NTTEKVQTRI
 701 LAFDVWMDTQ PAQWEVEKVM QIANKDSSLE LTHYVYTALK TAMKAEEPCY
 751 QLLAQRVRAA WTQLRPFDLG SEFSHLRSKF YYDTVENYGI RGVWKVIASN
 801 TTILPFYTEA KVNQVRGPYK TTLFGAKLLV KGGDKVLEEL VGKDGLLERI
 851 AYALVGQIKT GPRQQNTEQL LKDIAQGMGL KREKDETPKA VLFWKLFSGD
 901 AVIPLDSHYI NELKQELLQT VTKFGKDGVT GHIVRVLVPT KAFHVEPSTI
 951 GLPIVHSTIH PVVLSVRYEN IKIHYGNQES RVAPKTLEIS GTVQPTILSF
1001 RQSRVFVSDK VGQKNPTVKT TDIKEFNVRL AFRVVYEHTP KRFRVHVKPV
1051 FDRVFHSGHC TELKLESAVL LKEELAAKTV EYDKCIKSLY QPIRRNHQIA
1101 GEWSGMMLRL TGESHQPWSG LPMFAPSVVS SEGILGAIIN RLSNKGMKHH
1151 TVSLYLETNN QQPITEWVAT IDVDSNVERL AKVPLSQQIT KVQKLKVQYA
1201 NRAQPLYPEL EPLVRKVESL LEKFETLDET TVEKLMLVKI EGLYQGQPKS
1251 TLKIAMKKIY NLEKTEQQYA LAAMHQESHK GLELSTNVSY PKIGSPFRYD
1301 PTFYAEDERM NGTLIVKLQS PQEQVFHVKF QATKSEEQLK ETEYEWFEVR
1351 CLAEQKAGKI MTDACRKAVL KDNSLDQLKI AVTVPRNVHP KIQTLAYKTL
1401 DLMKYMWYPK MQTEVAGLKQ REVLQALQHT EREVRISVNA TRESLWHLLY
1451 DVRVEMPFEN VTFSKVNIPG VRPAHMQLTT KEQLEHVYYR GQKDNVCVLG
1501 DKSVRTYDNV TFGLDVKTGC EYVLTRDTSS GTPDFTVTFQ VVKPDTFAKK
1551 IRVQLENTLV ELEPFTTTDR YITVVVNGTQ YQITFEKPVV FEYAAGKRVF
1601 LNVVDTSNVH HAPVITLYTE PKEVRVFFDG HSAKVFVVNK YKGNTKGVCG
1651 NNDNEQAHEF IGPNGKEYQH ANEFIASYGI GQACKVPAEN TREKLMETLK
1701 KEVEQIRRQE LIKKEKLRKE MQELERARNP QWMEQQEEQF WGEPLSTVSN
1751 EEWTTDSVEE QQLQRQVLKT AMSIENGHIC FSARPIATCK QGYKNHGVLR
1801 TERVESICLE KNEEAAIQAV QEIRAGQVVN IKSLEPYQKG RLFTMHKVPR
1851 CERHD_PE_N Q_LIQ
```

Contig12013 refers to a contiguous sequence assembled from EST data produced by and held at Moredun Research Institute. It is not publically available:

BLAST search of translated contigs 12013:

Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E value	Links
XP_002415224.1	vitellogenin, putative [Ixodes scapularis] >gb|EEC18889.1| vitellogenin, putative [Ixodes scapularis]	931	931	91%	0.0	G
AAW78557.2	vitellogenin [Dermacentor variabilis]	799	799	89%	0.0	
AAA92143.1	GP80 precursor [Rhipicephalus microplus]	520	520	64%	8e-145	

NB GP80 has been tested as a vaccine candidate against the tick *Rhipicephalus (Boophilus) microplus* (Tellam et al., Vet Parasitol. 2002 Jan 3;103(1-2):141-56)

>JH 2

Match to: **contig00171** Score: **101** Expect: **6.4e-06**
length=2053 numreads=831
Translated in frame 1

Nominal mass (M_r): **78467**; Calculated pI value: **8.82**
NCBI BLAST search of contig00171 against nr
Unformatted sequence string for pasting into other applications

Variable modifications: Carbamidomethyl (C),Oxidation (M),Propionamide (C)
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Number of mass values searched: **87**

Number of mass values matched: 23
Sequence Coverage: 38%

Matched peptides shown in **Bold**

1 QLKQQRLTQE QLVK**EQAYPE** **YLR**MIAYEDE YEKVTGRKPA TEK**IEQALQR**
51 IVKTTEK**HWS** **QIEPELREKL** **NVNNAQVRSI** **EYIITAIGQK** KQPIAITGHA
101 ILASTPSKQA RLAELTVDVE **KHPISFEMQA** **VSAFAK**APQP FKAEIREQDQ
151 RGILGFVAQL ETPKIGKKQY AGKIEMTKSE EQKQLMK**RSI** **QQQPWYYR**QC
201 EEDKK**EYTSE** **MSSACTR**TRY HKSALNRVYA EIELPEEIPQ PIYNISRIIR
251 DTLKVK**LYGN** **LHATYNR**KDM QQNK**LQVELV** **YIDRF**PAMHV ANLTIR**TPVN**
301 **EELHFER**IAL PK**ALRPNSMW** **TLKEQ**IKAFL **KNNRPEPVCV** **YNGK**VIRTFD
351 NVTVSLDTVK VGQKYLVARD NDEQSKFSIV ATK**VQAAEES** **QTVLEVLLR**D
401 ATLIKLVPSV QKGVYKVHVN QTVTEVTPHK **VQVYQYGPWA** KHMLTLHVEE
451 HKEGQDTLVV KVRDMHVHIV YDGK**NFLIEI** **AGPQIKGQLT** **GLCGDLNHQH**
501 **IDELTGPRGC** **HYEREEDFVR** **AFSFVPETNV** **AIEGEWVCPE** **GVHPRAASQM**
551 QISKKMEQQQ AAQQKKQLNI QQRQIVREEY NK**MTPETKMI** **HQDGK**VCYSV
601 DPVQACKPGL RAVETEMHTV AFVCLPIQHP MTAMIQREIQ VHGVIRNIPS
651 VMPGALEHIL YHQIKTPISC AN_**TANMGQI** KLFS

Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E value	Links
BAH02666.2	vitellogenin [Ornithodoros moubata]	310	310	91%	6e-82	
XP_002403966.1	vitellogenin, putative [Ixodes scapularis] >gb|EEC14774.1| vitellogenin, putative [Ixodes scapularis]	243	243	95%	7e-62	UG
ABW82681.2	vitellogenin-2 precursor [Dermacentor variabilis]	226	226	87%	7e-57	
BAE94323.1	vitellogenin fused with superoxide dismutase [Daphnia magna]	82.8	82.8	65%	2e-13	
BAD05137.1	vitellogenin fused with superoxide dismutase [Daphnia magna]	82.4	82.4	65%	2e-13	
ABS88989.1	vitellogenin [Rhipicephalus microplus]	79.0	79.0	48%	2e-12	
ADD73552.1	vitellogenin 2 [Paracyclopina nana]	79.0	79.0	61%	3e-12	
BAE94322.1	vitellogenin fused with superoxide dismutase [Daphnia magna]	79.0	79.0	65%	3e-12	
BAE94324.1	vitellogenin fused with superoxide dismutase [Daphnia magna]	79.0	79.0	65%	3e-12	
AAA92143.1	GP80 precursor [Rhipicephalus microplus]					

NB GP80 has been tested as a vaccine candidate against the tick *Rhipicephalus (Boophilus) microplus* (Tellam et al., Vet Parasitol. 2002 Jan 3;103(1-2):141-56)

>JH 3

Match to: **contig11897** Score: **112** Expect: **5.1e-07**
length=3575 numreads=721
Translated in frame 6

Nominal mass (M_r): **134318**; Calculated pI value: **7.89**
NCBI BLAST search of contig11897 against nr
Unformatted sequence string for pasting into other applications

Variable modifications: Carbamidomethyl (C),Oxidation (M),Propionamide (C)

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Number of mass values searched: **92**
Number of mass values matched: **30**
Sequence Coverage: **26%**

Matched peptides shown in **Bold**

```
   1 SLVFLGAHPS NTASISTMRV LLTFVALAVA VSAAAYHGQK QVVIPDLFEG
  51 QREYVYNYRS LVATGLPLQS QRFAGLEKYG ILTVQVHEQH GTTKVLGLRL
 101 GHVTSGSFDK EVEDVENRGI EGISHSIKEI VEKYGAVFVT IQDNEVQKIK
 151 VPQGMPEEIV NIYRGIAALF TVGKPDTHVH GTESHPFTYQ GEHGEQLPVV
 201 YRRQEKGIAG NFETTYEILS NPEHEYGYLN VTKTRNYLKK VGPDGRFFQN
 251 GHDAHGCGPV CLTHKPEQID QNMQPDTTAW ETPVTEGCPV KFHPKKDLVE
 301 AFTTYNYNMT VEQGGKIAVI HEAKGLDKKV LPLRKQQILT VSLLKVTLVE
 351 VRPISQIWEE KAATKQYSDI TYRFPEGHKH DLAYLSLYSK GSKGEIVTEQ
 401 ILPMLKALSK IIVSDNIELK SQTGDKVVQL TQALGVLTKT ELQSIWTVIG
 451 EPVSEKTATE FEKVQRKVLV DIIALSGSND AAEFLVELIQ QERLTVLETV
 501 HTLETLQKSI VKPTLTIIKQ LLNVCTETKF QKTRVVFSTA CIAFSEIVRH
 551 NCEDYVVSQM TEAPKMQGEE KKHTVHSCGP HEYKAFIETI REKLYAAQEY
 601 PQQVVYIQVL GRLAHPEALK ALVPYIYGEH EVIAQVEKMT RDNEHEDNSE
 651 YVQFLRQVAI FALHHSVRKH GASVQPIVQG VYFNKKEDYE LRIAALSVLL
 701 ATQPTEATFG RIVTELYKED NLEIASYTYS ALHALANSTL PCMKQSARRV
 751 QNVLGAFPKK SYGIDYSKWG THTKYSPLMN FGYKGHWEIT QSNVSAIPRA
 801 IYVGATANKG PFISTLGEFG MISKGLENLD RFVGQKGGVQ KIMENVMQRI
 851 RRDARTFVGD ASVGRMLEEI ENAFDFNTEE NNEQLRAVVF GNVLGNEFYL
 901 PVDKQFVTKV AEKVGEEMIK ILREEGSEKT LRYVRVLLPR TYVQVAPAVN
 951 GLPVLMINRH PIVVSLALKD LKIRLGAEKE ELTLNPLTFA ASGLIQPTVY
1001 FTAFHTAMTI NPLETSHVGY GLRTIEQTYM SLPIDASIQY THQTKTLAFT
1051 FRPRFEKIFF HKTRAMTFKT EVLLISDVER PILDQYTVIK TQRKPYAIDR
1101 VFGEKLGMAL RVQGLTVNEN YADNLIYKIF TGQHTAVTSI LKGIANEWVL
1151 PRAWTVRIEQ NKQTPIDKVK VVLRLGDYLK DRMLTEQTEQ K
```

Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E value	Links
BAH02666.2	vitellogenin [Ornithodoros moubata]	686	686	97%	0.0	
ABW82681.2	vitellogenin-2 precursor [Dermacentor variabilis]	657	657	95%	0.0	
XP_002403966.1	vitellogenin, putative [Ixodes scapularis] >gb|EEC14774.1| vitellogenin, putative [Ixodes scapularis]	418	521	73%	3e-114	U G
XP_002415224.1	vitellogenin, putative [Ixodes scapularis] >gb|EEC18889.1| vitellogenin, putative [Ixodes scapularis]	246	246	87%	1e-62	G
AAW78557.2	vitellogenin [Dermacentor variabilis]	208	208	77%	5e-51	
XP_002741413.1	PREDICTED: VITellogenin structural genes (yolk protein genes) family member (vit-1)-like [Saccoglossus kowalevskii]	128	128	83%	5e-27	G
AAA92143.1	GP80 precursor [Rhipicephalus microplus]					

NB GP80 has been tested as a vaccine candidate against the tick *Rhipicephalus (Boophilus) microplus* (Tellam et al., Vet Parasitol. 2002 Jan 3;103(1-2):141-56).

Appendices:

Appendix 1: Mite counts of vaccinated hens (Group 2) versus control hens (Group 1) for each individual cage.

25/11/2010				10/12/2010				11/01/2011				28/01/2011			
Group 1		Group 2		Group 1		Group 2		Group 1		Group 2		Group 1		Group 2	
Cage No	Mite No	Cage No	Mite No	Cage No	Mite No	Cage No	Mite No	Cage No	Mite No	Cage No	Mite No	Cage No	Mite No	Cage No	Mite No
3	1	1	0	3	0	1	0	3	2	1	1	3	19	1	143
4	1	2	2	4	0	2	0	4	6	2	0	4	144	2	7
7	0	5	2	7	0	5	0	7	4	5	2	7	90	5	183
8	3	6	4	8	0	6	0	8	1	6	70	8	5	6	134
11	0	9	1	11	0	9	0	11	0	9	0	11	0	9	1
12	1	10	1	12	0	10	0	12	0	10	4	12	0	10	1
15	3	13	2	15	0	13	1	15	0	13	218	15	3	13	130
16	0	14	0	16	0	14	0	16	3	14	1	16	1	14	6
17	1	19	0	17	0	19	0	17	1	19	0	17	11	19	0
18	0	20	4	18	0	20	0	18	1	20	0	18	3	20	121
21	2	23	3	21	0	23	0	21	0	23	0	21	4	23	110
22	1	24	0	22	0	24	0	22	9	24	0	22	1	24	3
25	1	27	1	25	0	27	0	25	1	27	0	25	1	27	1
26	0	28	5	26	0	28	0	26	0	28	23	26	0	28	411
29	0	31	2	29	0	31	0	29	0	31	0	29	198	31	244
30	0	32	0	30	0	32	0	30	1	32	1	30	2	32	26
35	0	33	0	35	0	33	0	35	0	33	1	35	1	33	2
36	0	34	1	36	0	34	0	36	0	34	0	36	1	34	1
39	0	37	5	39	0	37	0	39	0	37	1	39	0	37	7
40	1	38	0	40	0	38	0	40	0	38	0	40	0	38	0
43	0	41	0	43	0	41	0	43	0	41	0	43	0	41	3
44	1	42	1	44	0	42	0	44	0	42	0	44	0	42	11
47	0	45	1	47	0	45	0	47	0	45	25	47	4	45	35
48	1	46	1	48	0	46	0	48	0	46	1	48	1	46	2
49	0	51	0	49	0	51	0	49	6	51	16	49	12	51	5
50	0	52	1	50	0	52	0	50	6	52	38	50	6	52	46
53	0	55	0	53	0	55	0	53	0	55	11	53	12	55	18
54	0	56	2	54	0	56	0	54	0	56	3	54	56	56	2
57	2	59	1	57	0	59	0	57	10	59	32	57	27	59	148
58	1	60	0	58	0	60	0	58	5	60	68	58	8	60	291
61	0	63	0	61	0	63	0	61	25	63	19	61	401	63	7
62	3	64	0	62	0	64	1	62	10	64	5	62	37	64	12
67	3	65	1	67	0	65	0	67	4	65	0	67	44	65	2
68	5	66	0	68	0	66	0	68	2	66	2	68	4	66	27
71	0	69	4	71	0	69	0	71	0	69	156	71	34	69	318
72	1	70	3	72	0	70	0	72	0	70	25	72	2	70	82
75	5	73	3	75	0	73	1	75	3	73	2	75	25	73	30
76	2	74	0	76	0	74	0	76	0	74	1	76	2	74	1

79	0	77	1	79	0	77	0	79	1	77	20	79	11	77	91
80		78	0	80	2	78	0	80		78	16	80	31	78	8
81		83	2	81	0	83	0	81	0	83	0	81	1	83	7
82	0	84	2	82	2	84	0	82	0	84	4	82	2	84	96
85	0	87	0	85	0	87	0	85	0	87	0	85	0	87	1
86	0	88	3	86	0	88	0	86	2	88	24	86	3	88	54
89	2	91	1	89	0	91	0	89	0	91	2	89	0	91	2
90	0	92	0	90	0	92	0	90	4	92	7	90	1	92	197
93	1	95	0	93	0	95	0	93	0	95	0	93	1	95	1
94	1	96	0	94	0	96	0	94	0	96	0	94	1	96	8
99	3	97	0	99	0	97	0	99	160	97	5	99	181	97	10
100	0	98	0	100	0	98	0	100	0	98	6	100	72	98	1
103	0	101	3	103	0	101	0	103	0	101	11	103	10	101	81
104	0	102	1	104	0	102	0	104	1	102	25	104	59	102	75
107	0	105	0	107	0	105	0	107	7	105	0	107	19	105	16
108	0	106	0	108	0	106	0	108	6	106	0	108	55	106	10
111	2	109	0	111	0	109	0	111	82	109	184	111	43	109	121
112	2	110	1	112	0	110	0	112	224	110	58	112	141	110	55
114	1	115	0	114	0	115	0	114	2	115	0	114	10	115	87
117	2	116	0	117	0	116	0	117	0	116	0	117	5	116	23
118	0	119	1	118	0	119	0	118	3	119	1	118	31	119	0
118	1	120	1	118	0	120	0	118	0	120	1	118	53	120	80
121	0	123	1	121	0	123	0	121	3	123	0	121	18	123	23
122	0	124	1	122	0	124	0	122	0	124	137	122	83	124	260
125	0	127	0	125	0	127	0	125	1	127	0	125	7	127	3
126	0	128	0	126	0	128	0	126	0	128	0	126	47	128	1
131	0	129	0	131	0	129	0	131	0	129	0	131	1	129	0
132	1	130	0	132	0	130	0	132	1	130	1	132	0	130	1
135	0	133	0	135	0	133	0	135	0	133	0	135	1	133	0
136	1	134	4	136	0	134	0	136	0	134	0	136	20	134	0
139	0	137	0	139	0	137	0	139	0	137	0	139	20	137	1
140	0	138	1	140	0	138	0	140	0	138	0	140	1	138	0
143	0	141	1	143	0	141	0	143	0	141	6	143	0	141	135
144	0	142	1	144	0	142	0	144	0	142	0	144	0	142	59
145	0	147	1	145	0	147	0	145	0	147	6	145	10	147	68
146	0	148	1	146	0	148	1	146	0	148	127	146	3	148	162
149	1	151	1	149	0	151	0	149	1	151	0	149	310	151	19
150	0	152	0	150	0	152	0	150	1	152	0	150	124	152	1
153	0	155	3	153	0	155	0	153	0	155	51	153	51	155	14
154	0	156	1	154	0	156	0	154	0	156	7	154	35	156	221
157	1	159	0	157	0	159	0	157	0	159	0	157	43	159	11
158	1	160	0	158	0	160	0	158	4	160	7	158	141	160	17
163	0	161	2	163	0	161	0	163	0	161	0	163	3	161	9
164	0	162	0	164	0	162	0	164	0	162	0	164	0	162	0
167	1	165	2	167	2	165	0	167	1	165	6	167	2	165	238
168	0	166	2	168	0	166	0	168	0	166	0	168	0	166	0
171	0	169	0	171	0	169	0	171	1	169	0	171	45	169	8
172	3	170	0	172	0	170	0	172	2	170	0	172	5	170	5

175	5	173	1	175	1	173	0	175	1	173	191	175	16	173	225
176	1	174	2	176	0	174	0	176	0	174	1	176	1	174	184
177	1	179	6	177	0	179	0	177	0	179	0	177	30	179	32
178	0	180	0	178	0	180	0	178	0	180	0	178	10	180	60
181	0	183	0	181	0	183	0	181	0	183	0	181	91	183	1
182	1	184	0	182	0	184	0	182	7	184	0	182	0	184	0
185	0	187	0	185	0	187	0	185	0	187	0	185	0	187	39
186	0	188	0	186	0	188	0	186	0	188	0	186	1	188	10
189	0	191	0	189	0	191	0	189	0	191	0	189	0	191	0
190	0	192	0	190	0	192	0	190	4	192	0	190	0	192	0
	0.76		1.02		0.073		0.04		6.516		17		31.3		56.27
	71.8		99		7.073		4.04		625.5		1647		3039		5458

15/02/2011				01/03/2011				15/03/2011			
Group 1		Group 2		Group 1		Group 2		Group 1		Group 2	
Cage No	Mite No	Cage No	Mite No	Cage No	Mite No	Cage No	Mite No	Cage No	Mite No	Cage No	Mite No
3	1210	1	217	3	2541	1	388	3	1063	1	101
4	607	2	180	4	2854	2	398	4	992	2	81
7	26	5	964	7	156	5	1100	7	40	5	560
8	182	6	575	8	920	6	572	8	1005	6	204
11	839	9	138	11	1160	9	223	11	1033	9	63
12	331	10	830	12	1031	10	613	12	1060	10	240
15	88	13	1362	15	173	13	1060	15	130	13	320
16	500	14	174	16	768	14	42	16	682	14	75
17	75	19	148	17	444	19	522	17	235	19	404
18	76	20	586	18	580	20	1923	18	140	20	520
21	212	23	361	21	2235	23	472	21	1042	23	440
22	350	24	62	22	1383	24	782	22	960	24	208
25	11	27	440	25	540	27	362	25	350	27	240
26	91	28	871	26	1528	28	1020	26	600	28	1060
29	513	31	526	29	2842	31	748	29	5362	31	224
30	172	32	121	30	872	32	1050	30	920	32	280
35	1	33	18	35	92	33	464	35	520	33	322
36	1	34	48	36	1184	34	1149	36	780	34	336
39	1	37	224	39	6	37	652	39	280	37	1032
40	0	38	4	40	276	38	10	40	2804	38	44
43	300	41	75	43	691	41	73	43	880	41	284
44	34	42	381	44	530	42	112	44	2086	42	272
47	112	45	803	47	206	45	1200	47	63	45	408
48	2	46	5	48	708	46	88	48	995	46	140
49	863	51	21	49	361	51	196	49	776	51	40
50	215	52	40	50	628	52	784	50	744	52	130
53	41	55	60	53	2190	55	264	53	2628	55	93
54	490	56	38	54	2448	56	416	54	2024	56	276
57	431	59	119	57	2568	59	740	57	2040	59	144
58	211	60	78	58	226	60	1592	58	952	60	664

61	665	63	63	61	2000	63	146	61	2890	63	131
62	484	64	150	62	1184	64	123	62	1030	64	172
67	579	65	3	67	1728	65	336	67	940	65	296
68	95	66	61	68	2834	66	1301	68	1432	66	224
71	340	69	27	71	1368	69	1776	71	1820	69	895
72	16	70	29	72	296	70	676	72	800	70	406
75	102	73	23	75	960	73	984	75	1808	73	452
76	196	74	32	76	2652	74	796	76	3192	74	310
79	146	77	51	79	1808	77	853	79	1596	77	286
80	591	78	26	80	944	78	1116	80	3024	78	151
81	40	83	23	81	1576	83	610	81	982	83	110
82	14	84	53	82	684	84	1842	82	1400	84	468
85	8	87	8	85	2992	87	596	85	3064	87	896
86	71	88	32	86	1360	88	368	86	1640	88	200
89	17	91	5	89	1416	91	130	89	1600	91	96
90	211	92	89	90	1280	92	450	90	2040	92	788
93	407	95	1	93	700	95	568	93	1010	95	212
94	4	96	3	94	121	96	576	94	688	96	69
99	432	97	95	99	720	97	1178	99	1862	97	431
100	870	98	314	100	880	98	473	100	3400	98	300
103	252	101	113	103	264	101	400	103	1020	101	903
104	275	102	124	104	1338	102	183	104	960	102	660
107	583	105	44	107	2112	105	900	107	3065	105	220
111	882	106	12	111	948	106	621	111	1744	106	180
112	653	109	143	112	936	109	1036	112	784	109	310
113	330	110	93	113	1084	110	928	113	2456	110	276
114	468	115	107	114	2024	115	550	114	1050	115	584
117	225	116	196	117	3612	116	2432	117	4620	116	798
118	598	119	44	118	2576	119	868	118	1652	119	240
118	425	120	31	118	2075	120	1880	108	852	120	364
121	198	123	326	121	944	123	525	121	1160	123	224
122	193	124	77	122	1795	124	1680	122	2000	124	968
125	300	127	393	125	1340	127	328	125	320	127	194
126	388	128	16	126	2640	128	648	126	1022	128	336
131	1029	129	27	131	1324	129	608	131	2354	129	140
132	53	130	199	132	218	130	820	132	3025	130	320
135	5	133	10	135	1056	133	1127	135	712	133	736
136	110	134	46	136	880	134	113	136	844	134	184
139	713	137	15	139	1568	137	809	139	2513	137	630
140	8	138	57	140	3640	138	1048	140	2904	138	284
143	11	141	18	143	712	141	1868	143	1056	141	432
144	28	142	16	144	764	142		144	452	142	228
145	586	147	769	145	2106	147	1929	145	1088	147	233
146	467	148	450	146	2084	148	1682	146	1236	148	410
149	1299	151	800	149	2792	151	1288	149	2981	151	312
150	528	152	150	150	1952	152	608	150	3120	152	97
153	362	155	230	153	1384	155	512	153	1250	155	130
154	641	156	1232	154	920	156	1724	154	880	156	400

157	2622	**159**	386	**157**	3056	**159**	752	**157**	3520	**159**	110
158	810	**160**	500	**158**	1250	**160**	1180	**158**	2368	**160**	604
163	442	**161**	291	**163**	1835	**161**	660	**163**	3358	**161**	648
164	257	**162**	454	**164**	2115	**162**	1357	**164**	3872	**162**	700
167	253	**165**	1164	**167**	1776	**165**	1513	**167**	1018	**165**	631
168	324	**166**	50	**168**	1584	**166**	520	**168**	1792	**166**	140
171	608	**169**	155	**171**	3104	**169**	1340	**171**	4362	**169**	300
172	662	**170**	903	**172**	106	**170**	1231	**172**	5104	**170**	410
175	402	**173**	870	**175**	1888	**173**	1155	**175**	1542	**173**	1520
176	484	**174**	216	**176**	1104	**174**	1968	**176**	963	**174**	608
177	15	**179**	278	**177**	644	**179**	740	**177**	1020	**179**	135
178	76	**180**	312	**178**	1056	**180**	793	**178**	1360	**180**	556
181	543	**183**	14	**181**	1156	**183**	1200	**181**	5266	**183**	504
182	23	**184**	43	**182**	243	**184**	186	**182**	3800	**184**	280
185	1	**187**	280	**185**	588	**187**	484	**185**	680	**187**	452
186	2	**188**	126	**186**	624	**188**	524	**186**	624	**188**	424
189	5	**191**	6	**189**	912	**191**	564	**189**	2086	**191**	86
190	5	**192**	4	**190**	844	**192**	328	**190**	1190	**192**	160
	337.7		232.8		1340		816.03		1671		366.2
	32755		22580		1E+05		78339		2E+05		35525

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Claims

1. One or more *Dermanyssus gallinae* (*D. gallinae*) antigens, for use in raising an immune response in an avian species.

2. Use of one or more *Dermanyssus gallinae* (*D. gallinae*) antigens, in the manufacture of a medicament for raising an immune response in an avian species.

3. A method of raising an immune response in an avian species, said method comprising administering one or more *Dermanyssus gallinae* (*D. gallinae*) antigens to said avian species.

4. The one or more antigens, use, or method according to any preceding claim wherein the avian species is a poultry or fowl species. In other embodiments these terms extend to include domesticated or game bird species such as, for example, chicken, pheasant, grouse, turkey, guineafowl and/or duck species.

5. The one or more antigens, use, or method according to claim 4 wherein the avian species is a domesticated or game bird species such as, for example, chicken, pheasant, grouse, turkey, guineafowl and/or duck species.

6. A vaccine or vaccine compositions comprising one or more *D. gallinae* antigens for raising immune responses in avian species.

7. The one or more antigens, use or method or vaccine according to any preceding claim said one or more antigens are obtained by subjecting *D. gallinae* to a homogenisation protocol to yield a homogenised suspension of *D. gallinae* components comprising said one or more antigens and optionally subjecting the homogenate to one or more size/density separation techniques (such as centrifugation) so as to remove unwanted *D. gallinae* debris from useful antigen containing fractions of the homogenate.

8. The one or more antigens, use, method or vaccine according to claim 7 wherein the antigen containing fractions are subjected to one or more sterilisation and/or inactivation procedures to render them suitable for administration.

9. The one or more antigens, use or method according to claim 7 or 8 wherein said one or more antigens are further prepared for cold or freeze storage by the addition of one or more cryopreservative/cryoprotectant agents.

10. The one or more antigens, use or method according to claims 7-9 wherein said homogenate is be further processed in order to yield antigen fractions comprising fewer and more highly purified (or cleaner)/concentrated antigens or specific or select antigens.

11. The one or more antigens, use or method according to claim 10 wherein said one or more purified antigens are characterised by migration in SDS (i.e. reducing) PAGE techniques as having a size/weight of approximately 80KDa and/or approximately 120KDa.

12. The one or more antigens, use or method according to claim 11 wherein said one or more purified antigens are a GP80-like protein or an immunogenic or antigenic fragment thereof.

13. The one or more antigens, use or method according to any one of claims 1 or 10-12 wherein said one or more antigens comprises SEQ ID NO:1, SEQ ID NO:2 and/or SEQ ID NO:3 or is at least 30%, 40%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous or identical to any one of the sequences given as SEQ ID NOS: 1, 2 and 3 or an immunogenic fragment thereof.

14. A purified or recombinant antigen or composition comprising said purified or recombinant antigen for use, uses, medicaments and methods provided by this invention comprise one or more *D. gallinae* antigens comprising a sequence at selected from the group consisting of:

(f) SEQ ID NO: 1

(g) SEQ ID NO: 2

(h) SEQ ID NO: 3

(i) a sequence at least 30%, 40%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous or identical to any one of (a), (b) or (c); and

(j) a sequence at least 30%, 40%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous or identical to an immunogenic fragment of any one of (a), (b) or (c).

15. A vaccine or vaccine composition comprising one or more *D. gallinae* purified or recombinant antigens comprising a sequence selected from the group consisting of:

(a) SEQ ID NO: 1

(b) SEQ ID NO: 2

(c) SEQ ID NO: 3

(d) a sequence at least 30%, 40%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous or identical to any one of (a), (b) or (c); and

(e) a sequence at least 30%, 40%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous or identical to an immunogenic fragment of any one of (a), (b) or (c).

16. An isolated nucleic acid encoding a sequence selected from the group consisting of:

(a) SEQ ID NO: 1

(b) SEQ ID NO: 2

(c) SEQ ID NO: 3

(d) a sequence at least 30%, 40%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous or identical to any one of (a), (b) or (c); and

(e) a sequence at least 30%, 40%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous or identical to an immunogenic fragment of any one of (a), (b) or (c).

for use in raising an immune response in an avian.

17. A vector comprising the isolated nucleic acid according to claim 16.

18. A host cell transfected or transformed with a vector according to claim 17.

19. A process for the production of a *D. gallinae* antigen encoded by a sequence of SEQ ID NO: 1, 2 or 3 (or an immunogenic fragment thereof) and for use in raising an immune response in an avian, said method comprising the step of (a) transforming a host cell with a nucleic acid

sequence according to this invention or transfecting a host cell with a nucleic acid construct of the invention; (b) culturing the cells obtained in (a) under conditions in which expression of the nucleic acid (or rather a protein encoded thereby) takes place; and (c) isolating the expressed recombinant protein or peptide from the cell culture and/or the culture supernatant.

20. According to any one of claims a farmed population of chickens, treated, vaccinated or immunised with a vaccine or composition described herein, aid vaccine or composition comprising one or more of the *D. gallinae* antigens described herein.

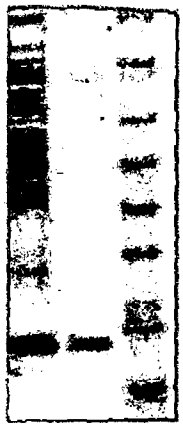

Figure 1

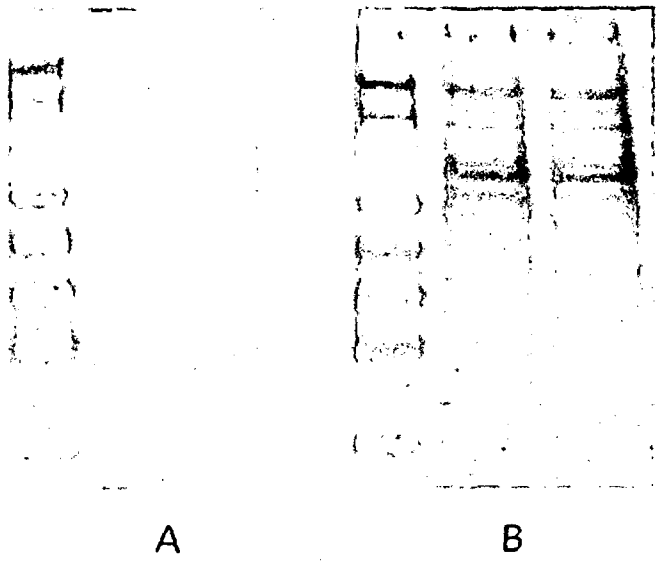

Figure 2

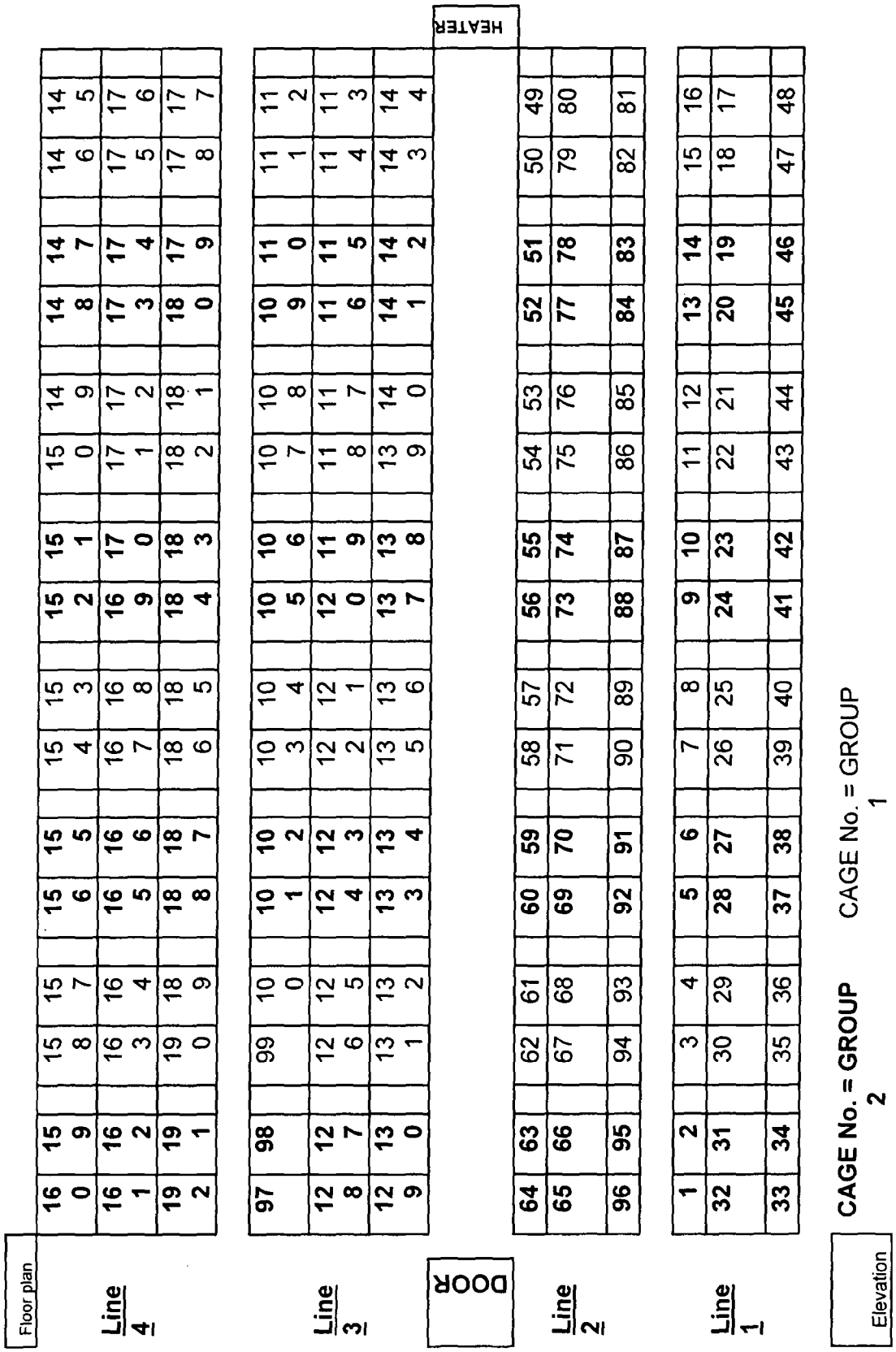
Floor plan
Line 4
Line 3
DOOR
Line 2
Line 1
HEATER
CAGE No. = GROUP 2
CAGE No. = GROUP 1
Elevation

Figure 3

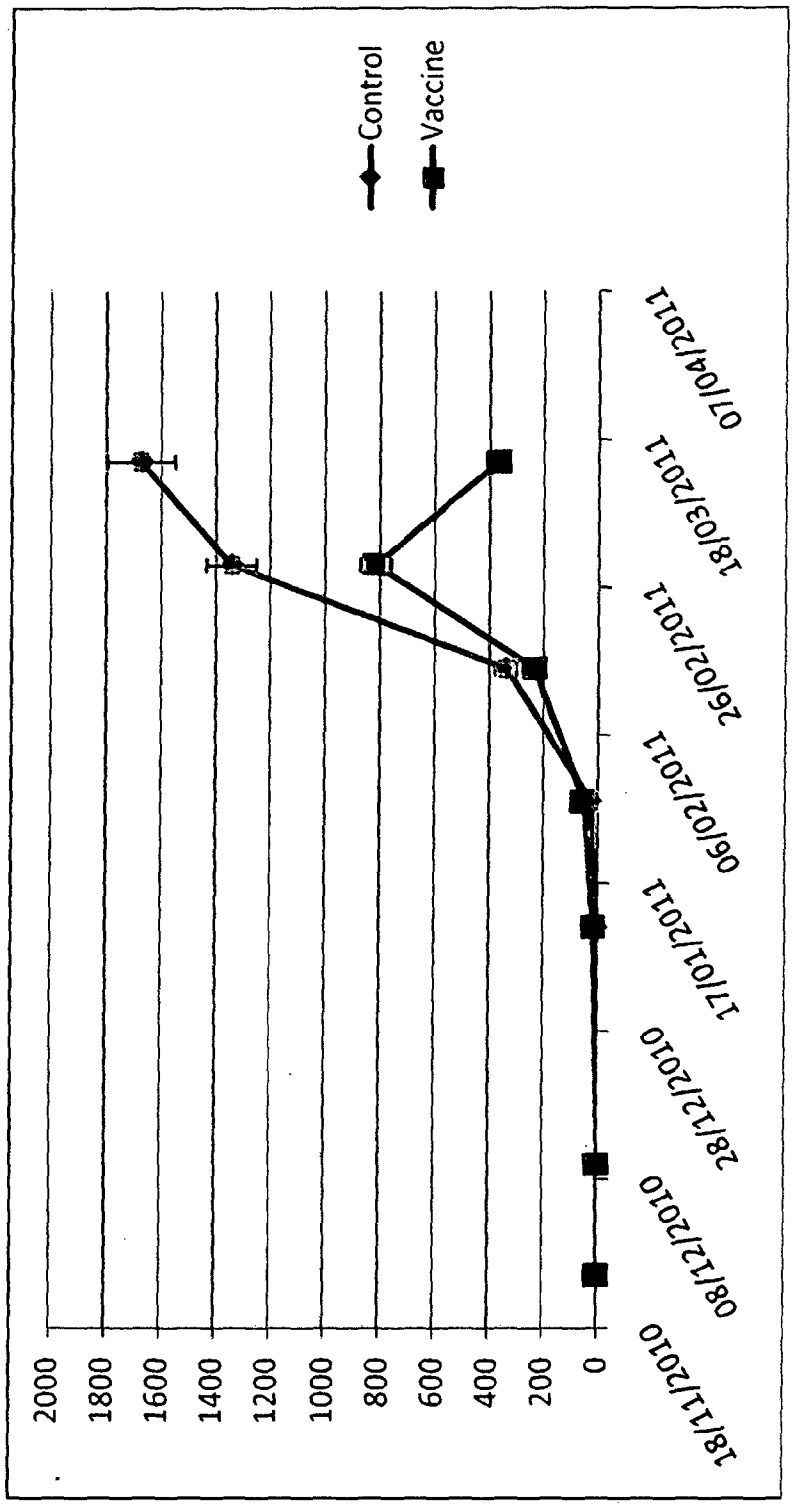
Control
Vaccine
2000
1800
1600
1400
1200
1000
800
600
400
200
0
18/11/2010
08/12/2010
28/12/2010
17/01/2011
06/02/2011
26/02/2011
18/03/2011
07/04/2011

Figure 4

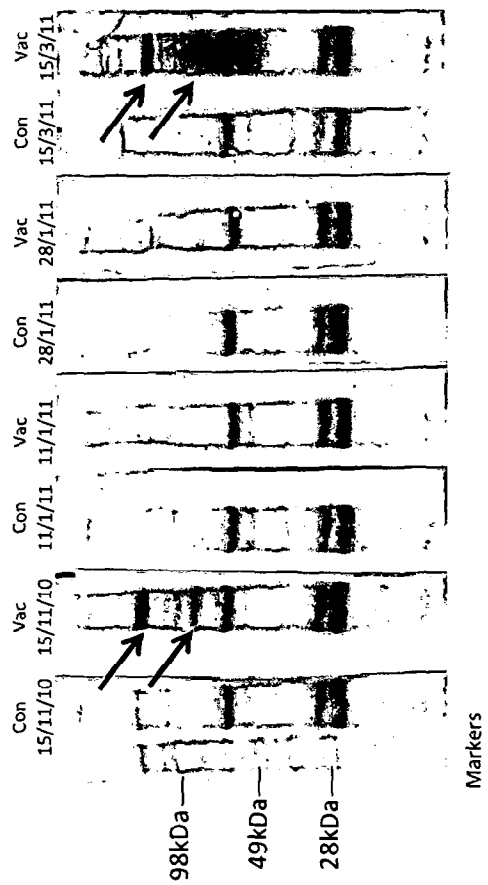
Vac 15/3/11
Con 15/3/11
Vac 28/1/11
Con 28/1/11
Vac 11/1/11
Con 11/1/11
Vac 15/11/10
Con 15/11/10
Markers
98kDa
49kDa
28kDa

Figure 5

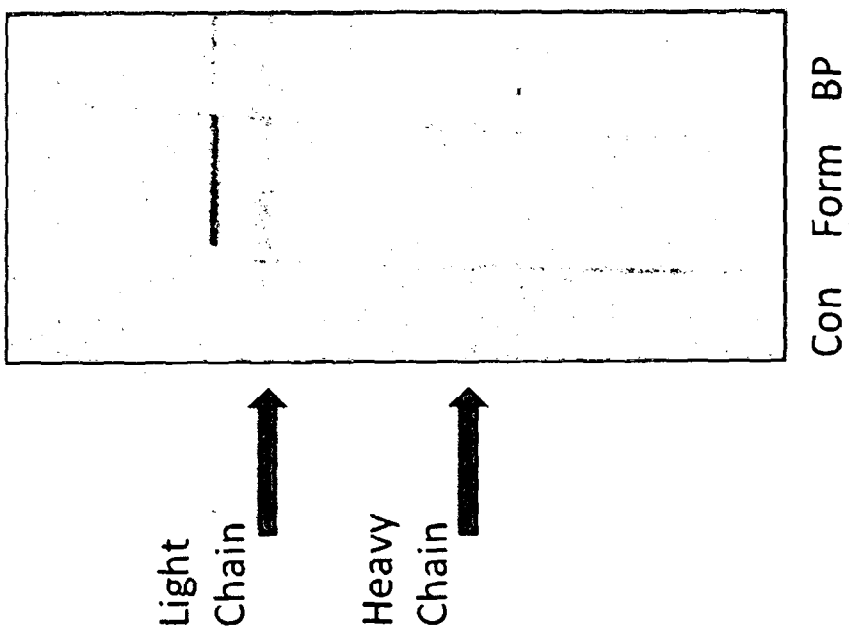

Figure 6