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(54) **Titre : PEPTIDES INDUISANT CHEZ LES POISSONS UNE REPONSE IMMUNITAIRE CONTRE LES COPEPODES ET/OU LA FORMATION D'UN BOUCLIER MUQUEUX, VACCINS, UTILISATIONS ET METHODES POUR MODULER LA REPONSE IMMUNITAIRE D'UN POISSON ET/OU INDUIRE LA PRODUCTION D'UN BOUCLIER MUQUEUX**

(54) **Title: PEPTIDES INDUCING AN IMMUNE RESPONSE AGAINST COPEPODS AND/OR THE DEVELOPMENT OF A MUCOUS SHIELD IN FISH; VACCINES, USES AND METHODS FOR MODULATING THE FISH IMMUNE RESPONSE AND/OR FOR INDUCING DEVELOPMENT OF A MUCOUS SHIELD IN FISH**

(57) **Abrégé/Abstract:**
Publish without an abstract.

ABSTRACT

Isolated peptides comprising the sequences SEQ ID NO. 17, SEQ ID NO. 18, SEQ ID NO. 19, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25, SEQ ID NO. 26, SEQ ID NO. 27 or SEQ ID NO. 28, wherein one or more peptides induce an immune response against copepods and/or the formation of a mucous shield protector in fish. The peptide may be conjugated to an antigenic protein; for example, it may be covalently conjugated to KLH. Additionally, vaccines comprising the peptides alone or combined are provided.

PEPTIDES INDUCING AN IMMUNE RESPONSE AGAINST COPEPODS AND/OR THE DEVELOPMENT OF A MUCOUS SHIELD IN FISH; VACCINES, USES AND METHODS FOR MODULATING THE FISH IMMUNE RESPONSE AND/OR FOR INDUCING DEVELOPMENT OF A MUCOUS SHIELD IN FISH

BACKGROUND OF THE INVENTION

Copepods of the Caligidae family, commonly known as sea lice, are the most extensively reported ectoparasites in wild-type and cultured salmon species.

The dominant species that affects salmon farming in Chile is *Caligus rogercresseyi*, which is present in 99% of salmon farms, infesting both salmonids and native fish and thereby generating high mortality rates.

Global growth of intensive salmonids farming over the last decade has made the control of sea lice one of the main concerns in the industry due to important economic losses and environmental effects generated by these parasites.

Copepodes feed from fish skin, mucus and blood, and there is well-documented literature concerning their taxonomy, life cycles, and parasite-host relationship.

Infestation by sea lice in salmonids produces erosion, epidermis and scale loss, host tissue hemorrhage and osmoregulatory stress which may cause the death of the affected specimens due to their inability to preserve homeostasis. Stress conditions increase salmon susceptibility to infections because of weakness produced by the attack of sea lice.

As production levels increase in farming centers, the resulting large populations of confined salmons lead to an increasing incidence of ectoparasite pests and associated diseases, a reduction of growth rate of farming fish and lower quality standards resulting from muscle damage. This leads to loss of

commercial value, higher production costs necessary to afford treatments for combating the pest and related diseases in salmonids. Further problems are parasite resistance to therapeutic chemicals and toxicity of these products on marine life.

Vaccines using vitelogenin 1 as antigen (EP 2 405 003 and WO2007/039599), antigens comprising proteins fused to a promiscuous T cell epitope (US 2010/00221271) have been described. However, there remains a need for highly efficient vaccines which are more easily prepared for immunizing fish capable of being infested with copepods. Furthermore, compositions or vaccines promoting the formation in fish of a mucous protective shield against infestations by copepods are also needed.

DESCRIPTION OF THE DRAWINGS

Figure 1:

Figure 1 shows proteins extracted from *C. rogercresseyi* separated on 8% sodium dodecyl sulfate polyacrylamide gel under reducing conditions. Lane 1: Molecular weight marker (Fermentas #SM1811). Lane 2: Soluble protein concentrates from *C. rogercresseyi;*

Figure 2:

Figure 2 shows the amino acid sequences of SEQ ID NO. 1, SEQ ID NO. 2, and SEQ ID NO. 3 and the peptides used for identification; SEQ ID N°1 Vitellogenin 1 [*Lepeophtheirus salmonis*], SEQ ID NO. 2 Vitellogenin 2 [*Lepeophtheirus salmonis*], and SEQ ID N°3 Vitellogenin-like protein [*Lepeophtheirus salmonis*];

Figure 3:

Figure 3 is a graph showing the efficacy of vaccine A of the invention expressed as percent reduction of the number of post-challenge parasitic stages in

immunized trouts as compared to controls in three stages of challenge: fixation, development of juvenile Chalimus stages (I through IV) and adults (male and female).

Figure 4:

Figure 4 is a graph showing the mean number of male and female adult parasites found per fish and the standard deviation corresponding to each group (vaccine A and controls);

Figure 5:

Figure 5 is a graph showing induced immune response in fish vaccinated with vaccine A and controls; log serum titers of specific antibodies from 4 vaccinated groups (n=5) at various post-vaccination times are represented. Specific antibody titers were determined by means of an Elisa;

Figure 6:

Figure 6 shows the amino acid sequence of SEQ ID NO. 1 and identifies the peptides of the invention;

Figure 7:

Figure 7 is a graph showing the efficacy of the vaccines of the invention expressed as percent reduction of the number of parasitic stages post-challenge in immunized Atlantic salmons as compared to controls, in three stages of challenge: fixation, development of juvenile Chalimus stages (I through IV) and adults (male and female).

Figure 8:

Figure 8 is a graph showing the mean number of male and female adult parasites found per fish and the standard deviation of each group (vaccines 1-7 of the invention and controls);

Figure 9:

Figure 9 is a graph showing induction kinetics of specific antibodies in Atlantic salmon vaccinated with the different vaccines of the invention and their controls, challenged with infective stages of C. *rogercresseyi*.

Figure 10:

Figure 10 is a graph showing induction kinetics of specific antibodies in mucus of Atlantic salmon vaccinated with the different vaccines of the invention and their controls, challenged with infective stages of C. *rogercresseyi*.

Figure 11:

Figure 11 is a graph showing correlation between reduction percentage of PRI infestation (%) and serology is expressed as inverse log of the titer of specific antibodies for vaccines 1 and A.

Figure 12:

Figure 12 shows the results of a histological analysis of the epidermis of fish vaccinated with the different vaccines and their controls performed at time points 0, 10, 20, and 30 days post-vaccination. Skin slices stained with PAS-Alcian Blue for identifying mucus-secreting cells are shown.

Figure 13:

Figure 13 shows a histological analysis of the epidermis of fish vaccinated with the different vaccines and their controls performed on days 40-50-80 and 120 during immunization and challenge (below). Skin slices stained with PAS for identifying mucus-secreting cells are identified.

Figure 14:

Figure 14 is an application scheme timeline showing fish acclimation, immunization, challenge with the parasite and sampling.

SUMMARY OF THE INVENTION

An object of the present invention is to provide an isolated peptide

comprising an amino acid sequence having at least 90%, for example, 91, 92, 93, 94, 95, 96, 97, 98 and 99 % identity to the following sequences: SEQ ID NO. 17, SEQ ID NO. 18, SEQ ID NO. 19, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25, SEQ ID NO. 26, SEQ ID NO. 27, or SEQ ID NO. 28, wherein said peptide induces an immune response against copepods in fish. Fish may comprise salmonids, such as Atlantic salmon (*Salmo Salar*), Rainbow trout (*Oncorhynchus mykiss*), Coho salmon (*Oncorhynchus kisutch*), brown trout (*Salmo trutta*), or Chinook salmon (*O. tshawytscha*); and the copepods may comprise *Caligus rogercresseyi*, *Caligus absens, Caligus acanthopagri, Caligus aduncus, Caligus aesopus, Caligus affinis, Caligus furcatus, Caligus alaihi, Caligus alatus, Caligus amblygenitalis, Caligus angustatus, Caligus antennatus, Caligus arii , Caligus ariicolus, Caligus asperimanus , Caligus asymmetricus, Caligus atromaculatus, Caligus balistae , Caligus belones, Caligus bennetti, Caligus berychis, Caligus biaculeatus, Caligus bicycletus, Caligus bifurcatus, Caligus bifurcus , Caligus biseriodentatus, Caligus bocki , Caligus bonito, Caligus brevicaudatus, Caligus brevicaudus, Caligus brevis, Caligus buechlerae, Caligus callaoensis, Caligus callyodoni, Caligus calotomi, Caligus carangis, Caligus caudatus , Caligus centrodonti, Caligus chaenichthyis, Caligus cheilodactyli, Caligus chelifer, Caligus chiastos, Caligus chorinemi, Caligus chrysophrysi, Caligus clavatus, Caligus clemensi, Caligus confusus, Caligus constrictus, Caligus cookeoli, Caligus cordiventris, Caligus cordyla, Caligus cornutus , Caligus coryphaenae, Caligus cossacki, Caligus costatus, Caligus cresseyorum, Caligus cristatus, Caligus crusmae, Caligus cunicephalus, Caligus curtus , Caligus cybii, Caligus dactylopteni , Caligus dactylus, Caligus dakari, Caligus dampieri, Caligus dasyaticus, Caligus debueni, Caligus deformis, Caligus diaphanus, Caligus dicentrarchi, Caligus dieuzeidei, Caligus digitatus,*

Caligus djedabae, Caligus dubius, Caligus eleutheronemi, Caligus elevatus , Caligus elongatus, Caligus engraulidis, Caligus enormes, Caligus epidemicus , Caligus epinepheli, Caligus equulae, Caligus eventilis, Caligus fistulariae, Caligus flexispina, Caligus fortis, Caligus fronsuganinus , Caligus fugu, Caligus furcisetifer , Caligus gayi, Caligus germoi, Caligus glacialis, Caligus glandifer, Caligus gracilis, Caligus grandiabdominalis, Caligus guerini, Caligus gurnardi, Caligus haemulonis, Caligus hamatus, Caligus hamruri, Caligus hemiconiati, Caligus hobsoni, Caligus hoplognathi, Caligus hottentotus, Caligus hyalinae, Caligus hyalinus, Caligus ignotus, Caligus inanis, Caligus infestans, Caligus inopinatus, Caligus irritans, Caligus isonyx, Caligus itacurussensis, Caligus jawahari , Caligus kabatae, Caligus kahawai, Caligus kala, Caligus kalumai, Caligus kanagurta, Caligus kapuhili, Caligus kirti, Caligus kirtiodes, Caligus klawei, Caligus kurochkini, Caligus kuwaitensis, Caligus labracis, Caligus lacustris, Caligus lalandei, Caligus laticaudus, Caligus latigenitalis, Caligus latus, Caligus lepidopi, Caligus lessonius, Caligus lethrinicola, Caligus lichiae, Caligus ligatus, Caligus ligusticus, Caligus littoralis, Caligus lobodes, Caligus lolligunculae, Caligus longiabdominis, Caligus longicaudatus, Caligus longicaudus, Caligus longicervicis, Caligus longipedis, Caligus longipennatus, Caligus longirostris, Caligus longispinosus, Caligus lunatus, Caligus lutjani, Caligus macarovi, Caligus macrurus, Caligus malabaricus, Caligus mercatorus, Caligus minimus, Caligus mordax, Caligus mortis, Caligus mugilis, Caligus multispinosus, Caligus murrayanus, Caligus musaicus, Caligus mutabilis, Caligus nanhaiensis, Caligus nengai, Caligus nibeae, Caligus nolani, Caligus novocaledonicus, Caligus nuenonnae, Caligus obscurus, Caligus oculicola, Caligus ocyurus, Caligus oligoplitisi, Caligus olsoni, Caligus omissus, Caligus orientalis, Caligus oviceps, Caligus pagelli, Caligus pageti, Caligus pagri, Caligus pagrosomi, Caligus pampi, Caligus parvilatus, Caligus patulus, Caligus

pauliani, Caligus pectinatus, Caligus pelagicus, Caligus pelamydis, Caligus penrithi, Caligus phipsoni, Caligus piscinus, Caligus placidus, Caligus platurus, Caligus platytarsis, Caligus polycanthi, Caligus pomacentrus, Caligus pomadasi, Caligus praetextus, Caligus priacanthi, Caligus productos, Caligus pseudokalumai, Caligus pseudoproductus, Caligus pterois, Caligus punctatus, Caligus quadratus, Caligus randalli, Caligus raniceps, Caligus rapax, Caligus rectus, Caligus regalis, Caligus remorae, Caligus reniformis, Caligus robustus, Caligus rotundigenitalis, Caligus rufimaculatus, Caligus russellii, Caligus salmoneus, Caligus saucius, Caligus savala, Caligus schelegeli, Caligus schistonyx, Caligus sciaenops, Caligus sclerotinosus, Caligus scribae, Caligus sensilis, Caligus sensorius, Caligus sepetibensis, Caligus seriolae, Caligus serratus, Caligus sibogae, Caligus sicarius, Caligus similis, Caligus spinosurculus, Caligus spinosus, Caligus stokes, Caligus stromatei, Caligus suffuscus, Caligus tanago, Caligus temnodontis, Caligus tenax, Caligus tenuicaudatus, Caligus tenuifurcatus, Caligus tenuis, Caligus teres, Caligus tetrodontis, Caligus thyrsitae, Caligus torpedinis, Caligus trachynoti, Caligus triabdominalis , Caligus triangularis, Caligus trichiuri, Caligus tripedalis, Caligus truttae, Caligus tylosuri, Caligus undulatus, Caligus unguidentatus, Caligus uranoscopi, Caligus validus, Caligus ventrosetosus, Caligus vexator, Caligus willungae, Caligus wilsoni, Caligus xystercus, Caligus zei, Caligus zylanica, Lepeophtheirus europaensis, Lepeophtheirus grohmanni ,Lepeophtheirus nordmannii, Lepeophtheirus pectorales, Lepeophtheirus salmonis, Lepeophtheirus Thompson, or Tigriopus japonicus.

The peptide may be conjugated to an antigenic protein, for example the peptide may be covalently conjugated to hemocyanin (KLH - keyhole limpet hemocyanin) of *Megathura crenulata*, or others.

A vaccine against infestation of fish with copepods comprising at least one

peptide is provided, wherein said peptide has at least 90%, for example 91, 92, 93, 94, 95, 96, 97, 98 and 99 % identity to the sequences of SEQ ID NO. 17, SEQ ID NO. 18, SEQ ID NO. 19, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25, SEQ ID NO. 26, SEQ ID NO. 27, or SEQ ID NO. 28; and excipients and adjuvants. The adjuvant may be any known adjuvant, for example *Megathura crenulata* hemocyanin (keyhole limpet) (H8283-Sigma), a purified saponine, yeast β(1-3) D-glucanes, synthetic or natural microbial derivatives such as monophosphoryl lipid A (MPL), virosomes, polylactic-glycolic acid microparticles, *Mycobacterium phlei* cell wall backbone, amino alkyl glucosaminide phosphate, synthetic acetilated monosaccharides, lipid A derivatives, flagelin, oligodeoxynucleotides containing CpG motifs, genetically bacterial modified toxins, cholera toxin from *Vibrio colerae*, heat labile enterotoxin from *Escherichia coli*, human endogenous immunomodulators, cytokine, chemokines, immunopotentiator, double-stranded RNA, small immunopotentiator molecules such as imiquimod, resiquimod.

Preferably, the vaccine is an emulsion and the excipient is a non-mineral oil Montanide ISA 763 Seppic. Fish to be treated may be salmonids, such as Atlantic salmon (*Salmo Salar*), Rainbow trout (*Oncorhynchus mykiss*), Coho salmon (*Oncorhynchus kisutch*), brown trout (*Salmo trutta*), or Chinook salmon (*O. tshawytscha*); and the copepods may be, without limitation, C*aligus absens, Caligus acanthopagri, Caligus aduncus, Caligus aesopus, Caligus affinis, Caligus furcatus , Caligus alaihi, Caligus alatus, Caligus amblygenitalis, Caligus angustatus, Caligus antennatus, Caligus arii , Caligus ariicolus, Caligus asperimanus , Caligus asymmetricus, Caligus atromaculatus, Caligus balistae , Caligus belones, Caligus bennetti, Caligus berychis, Caligus biaculeatus, Caligus bicycletus, Caligus bifurcatus, Caligus bifurcus, Caligus biseriodentatus, Caligus*

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A vaccine against fish infestation by copepods comprising at least one peptide is provided, said peptide having at least 90%, for example 91, 92, 93, 94, 95, 96, 97, 98 and 99 % identity to the sequences of SEQ ID NO. 17, SEQ ID NO. 18, SEQ ID NO. 19, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25, SEQ ID NO. 26, SEQ ID NO. 27, or SEQ ID NO. 28; and excipients and adjuvants. The adjuvant may be any known adjuvant, for example *Megathura crenulata* hemocyanin (keyhole limpet) (H8283-Sigma), a purified saponine, yeast β(1-3) D-glucan, synthetic or natural microbial derivatives such as monophosphoryl lipid A (MPL), virosomes, polylactic-glycolic acid microparticles, *Mycobacterium phlei* cell wall backbone, amino alkyl glucosaminide phosphate, synthetic acetylated monosaccharides, lipid A derivatives, flagellin, oligodeoxynucleotides containing CpG motifs, genetically bacterial modified toxins, cholera toxin from *Vibrio colerae*, heat labile enterotoxin from *Escherichia coli*, human endogenous immunomodulators, cytokines, chemokines, immunopotentiator double-stranded RNA, small immunopotentiator molecules such as imiquimod, resiquimod.

The peptides may be conjugated to an antigenic protein, for example KLH or to any other known antigenic protein.

Further provided is the use of the peptide for preparing a vaccine that induces an immune response in fish or for preparing a composition generating the formation of a mucous shield protector in fish.

Further provided is a vaccine against fish infestation by copepods comprising the proteins of SEQ ID NO. 1, SEQ ID NO. 2, and SEQ ID NO. 3; excipients and adjuvants.

A method for modulating an immune response in fish comprising administering to said fish a necessary amount of a vaccine comprising at least one peptide selected from the group consisting of SEQ ID NO. 17, SEQ ID NO. 18, SEQ ID NO. 19, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25, SEQ ID NO. 26, SEQ ID NO. 27, SEQ ID NO. 28; and excipients. The method comprises administering from 1 to 500 µg peptide. When the vaccine comprises 4 peptides, 1 to 500 µg of each peptide is administered. The peptide may be conjugated to an antigenic protein, for example KLH.

A method for generating the formation of a mucous shield in fish comprising administering to said fish a necessary amount of a vaccine comprising at least one peptide having at least 90 % identity to a sequence selected from the group consisting of SEQ ID NO. 17, SEQ ID NO. 18, SEQ ID NO. 19, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25, SEQ ID NO. 26, SEQ ID NO. 27, SEQ ID NO. 28 and combinations thereof; and excipients. For example, administering from 1 to 500 µg of the peptide. The fish may be Atlantic salmon (*Salmo Salar*), Rainbow trout (*Oncorhynchus mykiss*) and Coho salmon (*Oncorhynchus kisutch*), brown trout (*Salmo trutta*) and Chinook salmon (*O. tshawytscha*) and the copepods may belong to the Caligidae family. The peptide may be conjugated to an antigenic protein, for example keyhole limpet

hemocyanin from *Megathura crenulata*.

DETAILED DESCRIPTION OF THE INVENTION

For the purpose of the present invention, the term "vaccine" refers to a composition that induces an immune response in an animal, for example in fish, and it also refers to a composition that induces formation of a mucous shield, wherein said shield is a biologic protection against infestation by copepods in fish.

Efficacy assays were carried out with multimeric proteins of the vitellogenin family as candidate immunogens for developinga new vaccine against *C. rogercresseyi* and other copepods.

Candidate soluble proteins as immunogens were separated by electrophoresis on 8% sodium dodecyl sulfate polyacrylamide gels from a suspension of homogenized *Caligus rogercresseyi* adult parasites.

When subjected to electrophoresis on sodium dodecyl sulfate polyacrylamide gels under non-reducing conditions, 3 prominent bands of 220 kDa (SEQ ID NO. 1), 212 kDa (SEQ ID NO. 2) and 173 kDa (SEQ ID NO. 3) the soluble protein concentrates from the homogenized adult parasites suspension were observed. Under reducing conditions, 4 bands corresponding to proteins of 220 kDa, 173 kDa, 116 kDa, and 97 kDa were observed (Figure 1) .

Tryptic digestion analysis and Matrix-Assisted Laser Desorption/Ionization (Maldi-tof) of bands extracted from the gel showed that the different peptidic fractions were highly homologous (>90%) to amino acid sequences of multimeric phospholipoglyco proteins of the vitellogenin family of copepods, such as vitellogenin 1, vitellogenin 2, and vitellogenin-like of *Lepeophtheirus salmonis, Trigiopus japonicus,* and *Paracyclopina nana*, respectively.

Sequences of the peptidic fractions were analyzed using the information available in GenBank. The results showed that there was a correlation between

the isolated proteins of the invention and known proteins of the vitellogenin family (Figure 2).

Table 1 shows that the amino acids sequences of Vitellogenin 1 [*Lepeophtheirus salmonis*], Vitellogenin 2 [*Lepeophtheirus salmonis*] and Vitellogenin-like proteins [*Lepeophtheirus salmonis*] specifying the homology of the identified peptides to the isolated proteins from *Caligus rogercresseyi*.

Table 1

REFERENCE	MASS	IDENTIFIED PEPTIDE
Vitellogenin 1 [*Lepeophtheirus salmonis*] (SEQ ID NO. 1)	220-kDa	SLAVYALK (SEQ ID NO. 4) FYMETIQKV (SEQ ID NO. 5) KVETTMGVISPFTKQ (SEQ ID NO. 6)
Vitellogenin 2 [*Lepeophtheirus salmonis*] (SEQ ID NO. 2)	212-kDa	KALVALFQTKM (SEQ ID NO. 7) RYYACGPRS (SEQ ID NO. 8)
Vitellogenin-like protein [*Lepeophtheirus salmonis*] (SEQ ID NO. 3)	173-kDa	PLIYGETEIK (SEQ ID NO. 9) QYSHFETDYGLGVSK (SEQ ID NO. 10 VKNSVVAFR (SEQ ID NO. 11) IYGSHFPRNFVIGVNPLKK (SEQ ID NO. 12) IILGHEFTPGYIENR (SEQ ID NO. 13) NAIVSQFQSVM (SEQ ID NO. 14) SAGSHLDAK (SEQ ID NO. 15) WGSSYNVYSFLK (SEQ ID NO. 16)

Peptides obtained from the digestion of isolated proteins of *Caligus rogercresseyi* of the invention correlated with known proteins belonging to the vitellogenin family. Figure 2 shows the sequences of Vitellogenin 1 [*Lepeophtheirus salmonis*], Vitellogenin 2 [*Lepeophtheirus salmonis*], and Vitellogenin-like proteins [*Lepeophtheirus salmonis*]. The peptides which were

found to be part of the amino acid sequences of the proteins of the invention are identified.

The isolated proteins of *C. rogercresseyi* were used to prepare different vaccines for immunizing fish (trouts), said vaccines comprising:

Vaccine A: comprises 1 µg of each of the following proteins: 220kDa (SEQ ID NO. 1), 212kDa (SEQ ID NO. 2), and 173kDa (SEQ ID NO. 3) in an oil emulsion formulated with Montanide ISA 763 Seppic non-mineral oil (70 % v/v oil – 3 0% v/v water), and 10 µg of *Megathura crenulata* hemocyanin (keyhole limpet) (H8283-Sigma).

Non-specific control composition: Each dose contained 3 µg of BmSS (BmSS recombinant protein from *Boophilus microplus* gut) in an oil emulsion formulated with Montanide ISA 763 Seppic non-mineral oil (70 % v/v oil – 30 % v/v water) and 10 µg of *Megathura crenulata* hemocyanin (keyhole limpet) (H8283-Sigma).

Adjuvant control compositions: Each dose contained 30 µg of PBS in an oil emulsion formulated with Montanide ISA 763 Seppic non-mineral oil (70 % v/v oil – 30 % v/v water) and 10 µg of *Megathura crenulata* hemocyanin (keyhole limpet) (H8283-Sigma).

PBS control composition: Each dose contained only 100 µl of PBS.

After inoculating fish with the vaccine or their controls, the fish were challenged with an infection with *C. rogercresseyi*

During fish immunization, challenge and monitoring periods it was observed that the vaccines did not cause local, systemic, inflammatory, and/or granulomatous reactions at the site of administration. The vaccines resulted innocuous and safe. None of the compositions or vaccines modified the behavior or appetite of the fish.

To verify the fixation degree of the copepods a count of parasite specimens was carried out 72hs post-challenge in all the challenged fish. Copepodites were fixed at the expected rate. The degree of protection of the vaccines was expressed as the reduction percent of post-challenge parasitic stages count in trouts immunized with each of the vaccines as compared to controls.

A significant and gradual increase of developing juvenile stages and adults of copepod parasites was observed in control groups. Only vaccine A of the invention resulted highly efficacious at reducing the number of juvenile and adult specimens. This vaccine showed a protection of from 70 to 75% depending on the stage. In ponds with fish treated with a non-specific control composition, protection only reached a 25 and 32 %, whereas in ponds with fish treated with adjuvant control composition the protection only reached 19 to 20%, in both cases with respect to PBS control (Fig 3).

Mean abundance was calculated as the average of adult male and female parasites per fish, considering the total number of fish of each group (Fig 4). The average number of male and female adult parasites per fish in the PBS control group was from 4.1 to 4.6 times higher than the number detected in fish treated with vaccine A of the invention. Fish immunized with vaccine A showed less difference in abundance between male and female.

A comparison between the average number of male and female adult parasites found in the PBS control group and in the groups treated with non-specific control compositions and adjuvant control provided no significant differences.

It was observed that immunization with vaccine A and subsequent challenge of the vaccinated fish caused a 75% decrease in the infestation with *C. rogercresseyi*, and furthermore that this composition or vaccine was found to be

safe, innocuous and efficacious.

Figure 5 shows specific antibody titers detected by ELISA in serum from animals treated with the different vaccines. Only vaccine A turned out to be immunogenic, showing a significant difference with respect to antibody titers of fish inoculated with non-specific, adjuvant and PBS controls.

In order to determine the subsistence of antibody titers in the groups of fish treated according to figure 5, fish were bled at different times, and sera were analyzed by ELISA as described in the examples. As can be seen in figure 5, only vaccine A of the invention induced a specific immune response which lasted for at least 120 days and was significantly higher than that induced by the control compositions.

The present invention also relates to peptides and combinations thereof, conjugated or not, in an oily composition or vaccine for preventing and/or activating a humoral immune response in salmonids. The peptides, or a combination thereof, increase mucus density, the number and diameter of secretory cells and epithelial thickness, thereby generating a biologic shield or mucous against infestation by pathogens, for example by sea lice *C. rogercresseyi*, thus reducing by 80% (with respect to the control) the number of lice in juvenile stages and adults after challenge with the parasite in the treated salmonids.

As mentioned previously, the proteins of the invention identified by mass spectroscopy having molecular weights of 220 kDa (SEQ ID NO. 1), 212kDa (SEQ ID NO. 2), and 173kDa (SEQ ID NO. 3) belong to multimers of the vitellogenin family.

To find immunogenic peptides within the protein sequence, an analysis for predicting linear B epitopes with BepiPred 1.0b (Technical University of Denmark)

in the 220 kDa protein (Vitellogenin 1 [*Lepeophtheirus salmonis*] (SEQ ID NO. 1) was carried out. Twelve peptides were selected. Their sequence is that shown in figure 6. The selected peptides were also conjugated to a hemocyanin extracted from the mollusk designated keyhole limpet (KLH - Keyhole limpet hemocyanin [*Lapa californiana*]).

Peptide sequences used for manufacturing the vaccines were as follows:

Peptide 1: GYSPSYYGWAPSKEYVYEFE (SEQ ID NO. 17). MW: 2525.75

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Cystein was added to COOHt, and it was conjugated to KLH.

Peptide 2: ESLFVEKDEPVVVTNWKKALL (SEQ ID NO. 18). MW: 2548.01

Cystein was added to COOHt, and it was conjugated to KLH.

Peptide 3: SQKEIHEVMEESGRACTGKQ (SEQ ID NO. 19) MW: 2282.70

It was conjugated to KLH at the cysteine of the sequence.

Peptide 4: STVSHQIPKPKTPKTVGNLF (SEQ ID NO. 20) MW: 2282.70.

Cystein was added to COOHt, and it was conjugated to KLH.

Peptide 5: KTLKAKSPQLYYVSTVSFSD (SEQ ID NO. 21) MW: 2282,70

Cystein was added to COOHt, and it was conjugated to KLH.

Peptide 6: QKITQKLQITPRTLQEPELS (SEQ ID NO. 22) MW: 2282.70

Cystein was added to COOHt, and it was conjugated to KLH.

Peptide 7: HGLPFKYTKTRNFVDVQSVAPTASGFPVRIQ (SEQ ID NO. 23) MW: 2282.70

Cystein was added to COOHt, and it was conjugated to KLH.

Peptide 8: CSQSSTNTVNPNTCEEKERS (SEQ ID NO. 24) MW: 2282.70

It was conjugated to KLH at the cysteine of the sequence.

Peptide 9: PVNESSGSSTPPSSTPGPLL (SEQ ID NO. 25) MW: 2282.70

Cystein was added to COOHt, and it was conjugated to KLH.

Peptide 10: SCQGIPTPEEKTKFEKESHE (SEQ ID NO. 26) MW: 2282.70

It was conjugated to KLH at the cysteine of the sequence.

Peptide 11: PTTYNRMIEEASNCQSSSSSGSGMGGGS (SEQ ID NO. 27) MW: 2282.70 It was conjugated to KLH at the cysteine of the sequence.

Peptide 12: SSPSSSDSSSHHAQPSTGRFQ (SEQ ID NO. 28) MW: 2282.70

Cystein was added to COOHt, and it was conjugated to KLH.

Peptides 1 to 4 correspond to the amino terminal sequence, peptides 5 to 8, correspond to the medium region and peptides 9 to 12 correspond to the carboxyl terminal region of the vitellogenin 1 protein (SEQ ID NO. 1) of *Lepeophtheirus salmonis*.

During the immunization, challenge and monitoring periods of the fish it was observed that the vaccines did not cause local, systemic, inflammatory, and/or granulomatous reactions at the site of administration.

Vaccine 1 of the invention comprises 50 µg of each of conjugated peptides 1-4 (peptides of SEQ ID NO. 17, SEQ ID NO. 18, SEQ ID NO. 19, and SEQ ID NO. 20);

Vaccine 2 of the invention comprises 50 µg of each of conjugated peptides 5-8 (peptides of SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, and SEQ ID NO. 24);

Vaccine 3 of the invention comprises 50 µg of each of conjugated peptides 9-12 (peptides of SEQ ID NO. 25, SEQ ID NO. 26, SEQ ID NO. 27, and SEQ ID NO. 28);

Each of the vaccines was prepared as an emulsion in Montanide ISA 763 Seppic non-mineral oil (70 % v/v oil – 30 % v/v water) and 10 µg of hemocyanin keyhole limpet from *Megathura crenulata* (H8283-Sigma), to a final volume of

0.05ml.

Vaccine 5 of the invention comprises 50 µg of each of non-conjugated peptides 1-4 (peptides of SEQ ID NO. 17, SEQ ID NO. 18, SEQ ID NO. 19, and SEQ ID NO. 20);

Vaccine 6 of the invention comprises 50 µg of each of non-conjugated peptides 5-8 (peptides of SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, and SEQ ID NO. 24);

Vaccine 7 of the invention comprises 50 µg of each of non-conjugated peptides 9-12 (peptides of SEQ ID NO. 25, SEQ ID NO. 26, SEQ ID NO. 27, and SEQ ID NO. 28).

Each of the vaccines was prepared as an emulsion in Montanide ISA 763 Seppic non-mineral oil (70 % v/v oil – 30 % v/v water) and 10 µg of hemocyanin keyhole limpet from *Megathura crenulata* (H8283-Sigma), to a final volume of 0.05ml.

Composition 8 is the adjuvant control composition: Each dose contained 30 µg of PBS in an oil emulsion formulated with Montanide ISA 763 Seppic non-mineral oil (70 % v/v oil – 30 % v/v water) and plus 10 µg of *Megathura crenulata* hemocyanin (keyhole limpet) (H8283-Sigma).

Composition 9 corresponds to the PBS control composition: Each dose of the PBS control vaccine contained only 0.05 µl of PBS.

The vaccines were all innocuous and safe. None of the compositions or vaccines modified the behavior or the appetite of the fish.

A count of parasite specimens was carried out 72hs post-challenge in all the fish challenged to measure fixation. Copepodites were fixed at the expected rate. The degree of protection of the vaccines was expressed as percent reduction of parasitic stages post-challenge in salmons immunized with each of the vaccines

as compared to controls.

Vaccines 1 and A resulted to be the most effective. Vaccine 1 showed a reduction of 81% and 77.7% and vaccine A of 72 and 68.5 % in the number of juvenile and adult specimens, respectively. Vaccines 2, 3, 4, 5, 6, and 7 showed less reduction in the number of specimens (Fig 7).

Counts carried out in ponds with fish immunized with vaccines 2 and 3 showed 11- 24.2 % and 25 – 35.4% efficacy as compared to the control with PBS (Fig 7). A protection of 18-28%, 22- 27% and 28-24 %, respectively, was observed in controls formulated with non-conjugated peptides (vaccines 5-6-7), whereas the adjuvant control composition showed 18-20% less than the PBS control (Fig 7).

In addition, mean abundance per fish was determined. Mean abundance corresponds to the amount of male and female adult parasites found in each fish as compared to the total number in all fish of each group. The number of male and female adult parasites found per fish in the PBS control group was 5.5 and 3.88 times more than that detected in fish treated with vaccine 1 that comprised peptides 1-4 conjugated with KLH, the latter vaccine showing the least difference in mean abundance between male and female parasites (Fig 8).

A comparison of mean abundance in the PBS control group with that of vaccine A, shows an abundance 3.6 and 2.84 times higher than the latter. The differences observed were statistically significant.

No significant differences were observed between the number of male and female adult parasites in the PBS control group and the adjuvant control groups and vaccines 5, 6 and 7. However, there was a relatively lower abundance in the group treated with vaccines 2 and 3 as compared to the PBS control.

It was observed that through immunization with peptides 1-4 of the amino-terminal portion of the *C rogercresseyi* protein of 220 kDa (SEQ ID NO. 1)

conjugated with KLH a 78% reduction of infestation induced through challenge with infestation of *C rogercresseyi* was achieved, and in addition this vaccine is safe, innocuous and efficacious.

It was further observed that the efficacy of vaccine 1 is somewhat superior to that seen in fish immunized with vaccine A comprising complete proteins. Moreover, the synthesis of such small peptides, for example by solid phase techniques, is an automated process and is readily carried out. Conjugation with transporter proteins, for example KLH, induces an improved immunological response and an adequate protection of salmons infested with *C rogercresseyi*.

Sera were titered in 2-fold serial dilutions from the 1:4 dilutions. The variation coefficient of positive and negative sera was calculated in 12 determinations as an intra-assay repeatability rate, resulting in values from 4 to 19% .

The results show that immunization of Atlantic salmon with a dose of 200ug of peptides 1-4 (vaccine 1) induces high titers of specific serum antibodies, detected by ELISA (Fig 9). A single immunization in vaccinated fish was enough to induce serum titers higher than 1.5 and 2 log between 20 and 40 dpv, which increased to 3 log during the immunization course. Serum titers obtained from fish treated with vaccine 1 were correlated with an increase of specific antibody levels found in the group of fish vaccinated with vaccine A. Vaccines 2-3-5-6, and 7 induced similar and lower serum titers than those observed with vaccines 1 and A. Controls with PBS and adjuvant did not induce a specific antibody response in vaccinated fish (Fig.9).

The level of specific antibodies was determined by measuring absorbance at 405 nm in mucus extracted from vaccinated fish (Fig 10). Samples were assayed without dilution. The results show, for example, that a single

immunization of Atlantic salmon with 200ug of peptides 1-4 formulated with hemocyanin in non-mineral oil (vaccine 1) induced production of specific antibodies in mucus which increased during the immunization period. These levels of antibodies obtained by immunization of fish vaccine 1 were correlated with the increase in specific antibody levels found in mucus of the group of fish vaccinated with vaccine A. Vaccines 2-3-5-6, and 7 induced lower levels of antibodies. Controls with PBS and adjuvant did not induce specific antibody response in mucus from untreated control fish (Fig 10).

It will be obvious for a person skilled in the art that the peptides and vaccines of the invention may be used for immunization against any type of copepod, given that the peptides used therefor are comprised in vitellogenin 1 of, for example, *Lepeophtheirus salmonis* or other known copepods.

The presence of specific antibodies in serum and mucus of fish vaccinated with vaccines 1 and A is correlated with a significant reduction in post-challenge infestation percentage of fish with *C. rogercresseyi*. The vaccine formulated with peptides 1-4 showed the best results. These results strongly suggest that detecting serum-mucous antibodies is an essential tool for demonstrating potency of a vaccine, and that there is a correlation between effective protection (as percent reduction of infestation) and immune response (Fig 11).

Histological studies evaluating the number of mucus-secreting cells, their diameter, and thickness of epithelium were also carried out analyzing three fields per slice . A statistical treatment of the results was performed according to the Kruskal Wallis test for differences in the medians. Similarly, correlations were made between reduction of infestation by challenge with *C rogercresseyi* and the SHIELD (shield) effect produced by variation in the number and diameter of mucus producing cells, as well variation of wall thickness. (Tables 2 to 9)

Table 2. Thickness, number and diameter of mucus-secreting cells at time 0 (pre-vaccination) for different vaccines and control groups.

Vaccine	Thickness	S.D.	E.E.	Vaccine	No. of cells	S.D.	E.E.	Vaccine	Diameter	S.D.	E.E.
Vaccine 1	27.92	7.02	1.81	Vaccine 1	13.47	3.66	0.95	Vaccine 1	12.93	1.69	0.44
Vaccine 2	29.06	6.92	1.79	Vaccine 2	12.93	5.18	1.34	Vaccine 2	14.34	1.47	0.38
Vaccine 3	27.64	6.25	1.61	Vaccine 3	13.33	6.59	1.7	Vaccine 3	13.64	1.69	0.44
Vaccine 4	29.29	11.11	2.87	Vaccine 4	11.73	3.01	0.78	Vaccine 4	12.8	2.27	0.59
Vaccine 5	31.72	7.09	1.83	Vaccine 5	12.53	3.18	0.82	Vaccine 5	13.44	1.75	0.45
Vaccine 6	25.17	8.44	2.18	Vaccine 6	13.8	5.31	1.37	Vaccine 6	14.73	1.83	0.47
Vaccine 7	29.15	11.3	2.92	Vaccine 7	13.8	5.31	1.37	Vaccine 7	12.96	1.9	0.49
Adjuvant control	33.42	9.38	2.42	Adjuvant control	12.6	2.26	0.58	Adjuvant control	13.44	1.75	0.45
Control PB	31.73	8.42	2.17	Control PB	12.93	3.51	0.91	Control PB	12.94	1.31	0.34

Table 3. Thickness, number and diameter of mucus-secreting cells 10 days post-vaccination for different vaccines and control groups.

Vaccine	Thickness	S.D.	E.E.	Vaccine	No. of cells	S.D.	E.E.	Vaccine	Diameter	S.D.	E.E.
Vaccine 1	28.14	9.02	2.33	Vaccine 1	13.8	5.31	1.37	Vaccine 1	15.16	1.9	0.49
Vaccine 2	27.67	13.78	3.56	Vaccine 2	12.8	2.24	0.58	Vaccine 2	14.45	1.56	0.28
Vaccine 3	32.29	7.18	1.85	Vaccine 3	11.2	2.51	0.65	Vaccine 3	13.62	1.72	0.31
Vaccine 4	28.83	7.05	1.82	Vaccine 4	11.4	2.41	0.62	Vaccine 4	15.57	1.4	0.36
Vaccine 5	30.76	5.77	1.49	Vaccine 5	12.6	2.26	0.58	Vaccine 5	13.74	2.23	0.34
Vaccine 6	28.35	6.93	1.79	Vaccine 6	12.93	3.51	0.91	Vaccine 6	14.68	2.01	0.26
Vaccine 7	28.84	7.38	1.91	Vaccine 7	10.67	3.29	0.85	Vaccine 7	12.52	1.43	0.37
Adjuvant control	28.44	5.82	1.5	Adjuvant control	11.47	3.36	0.87	Adjuvant control	13.28	1.47	0.38
Control PB	32.21	7.02	1.81	Control PB	11.68	4.38	1.57	Control PB	13.03	1.31	0.34

Table 4. Thickness, number and diameter of mucus-secreting cells 20 days post-vaccination for different vaccines and control groups.

Vaccine	Thickness	S.D.	E.E.	Vaccine	No. of cells	S.D.	E.E.	Vaccine	Diameter	S.D.	E.E.
Vaccine 1	28.14	9.02	2.33	Vaccine 1	13.8	5.31	1.37	Vaccine 1	15.16	1.9	0.49
Vaccine 2	27.67	13.78	3.56	Vaccine 2	12.8	2.24	0.58	Vaccine 2	14.45	1.56	0.28
Vaccine 3	32.29	7.18	1.85	Vaccine 3	11.2	2.51	0.65	Vaccine 3	13.62	1.72	0.31
Vaccine 4	28.83	7.05	1.82	Vaccine 4	11.4	2.41	0.62	Vaccine 4	15.57	1.4	0.36
Vaccine 5	30.76	5.77	1.49	Vaccine 5	12.6	2.26	0.58	Vaccine 5	13.74	2.23	0.34
Vaccine 6	28.35	6.93	1.79	Vaccine 6	12.93	3.51	0.91	Vaccine 6	14.68	2.01	0.26
Vaccine 7	28.84	7.38	1.91	Vaccine 7	10.67	3.29	0.85	Vaccine 7	12.52	1.43	0.37
Adjuvant control	28.44	5.82	1.5	Adjuvant control	11.47	3.36	0.87	Adjuvant control	13.28	1.47	0.38
Control PB	32.21	7.02	1.81	Control PB	11.68	4.38	1.57	Control PB	13.03	1.31	0.34

Table 5. Thickness, number and diameterof mucus-secreting cells 30 days post-vaccination for different vaccines and control groups.

Vaccine	Thickness	S.D.	E.E.	Vaccine	No. of cells	S.D.	E.E.	Vaccine	Diameter	S.D.	E.E.
Vaccine 1	37.37 *	5.21	1.86	Vaccine 1	15.4 *	3.36	0.87	Vaccine 1	17.68 *	1.01	0.41
Vaccine 2	33.33	5.71	2.25	Vaccine 2	11.68	2.38	1.57	Vaccine 2	12.19	1.33	0.37
Vaccine 3	32.4	6.5	2.19	Vaccine 3	13.13	2.18	0.82	Vaccine 3	13.67	1.28	0.44
Vaccine 4	38.65 *	5.7	1.99	Vaccine 4	15.47 *	4.79	1.5	Vaccine 4	16.61 *	1.33	0.36
Vaccine 5	34.32	6.46	1.48	Vaccine 5	10.67	3.29	0.85	Vaccine 5	14.26	1.39	0.28
Vaccine 6	33.41	7.84	3.57	Vaccine 6	11.47	3.36	0.87	Vaccine 6	14.53	1.28	0.31
Vaccine 7	34.88	4.64	2.52	Vaccine 7	12.01	3.88	1.49	Vaccine 7	13.41	1.62	0.34
Adjuvant control	32.8	5.21	1.89	Adjuvant control	11.89	3.58	0.79	Adjuvant control	13.65	1.19	0.3
Control PB	31.19	6.02	2.33	Control PB	11.27	2.46	0.89	Control PB	14.34	1.28	0.39

Table 6. Thickness, number and diameter of mucus-secreting cells 40 days post-vaccination for different vaccines and control groups.

Vaccine	Thickness	S.D.	E.E.	Vaccine	No. of cells	S.D.	E.E.	Vaccine	Diameter	S.D.	E.E.
Vaccine 1	39.05 *	5.65	2.19	Vaccine 1	16.83 *	1.67	0.89	Vaccine 1	18.45 *	1.47	0.38
Vaccine 2	38.11	9.15	1.99	Vaccine 2	13.01	3.88	1.49	Vaccine 2	13.53	1.13	0.29
Vaccine 3	35.02	7.13	3.48	Vaccine 3	13.2	2.89	0.93	Vaccine 3	14.17	1.16	0.3
Vaccine 4	40.18 *	5.7	3.57	Vaccine 4	15.21 *	2.47	1.56	Vaccine 4	17.82 *	1.4	0.36
Vaccine 5	33.28	7.41	3.52	Vaccine 5	12.89	3.58	0.79	Vaccine 5	13.44	1.9	0.49
Vaccine 6	34.79	7.83	2.89	Vaccine 6	11.62	2.46	0.89	Vaccine 6	14.69	1.07	0.28
Vaccine 7	31.32	5.07	2.33	Vaccine 7	12.27	3.14	1.12	Vaccine 7	13.53	1.19	0.34
Adjuvant control	36.58	6.35	3.56	Adjuvant control	12.23	3.03	1.23	Adjuvant control	15.65	1.23	0.29
Control PB	34.94	7.12	1.85	Control PB	11.45	2.84	0.92	Control PB	14.77	1.04	0.33

Table 7. Thickness, number and diameter of mucus-secreting cells 50 days post-vaccination for different vaccines and control groups.

Vaccine	Thickness	S.D.	E.E.	Vaccine	No. of cells	S.D.	E.E.	Vaccine	Diameter	S.D.	E.E.
Vaccine 1	46.2 *	8.42	2.17	Vaccine 1	17.23 *	2.46	0.75	Vaccine 1	19.45 *	1.69	1.44
Vaccine 2	39.75	7.74	3.55	Vaccine 2	13.67	4.31	1.33	Vaccine 2	14.41	1.47	1.38
Vaccine 3	36.89	6.51	1.68	Vaccine 3	13.2	2.89	0.93	Vaccine 3	13.44	1.69	0.44
Vaccine 4	48.77 *	6.8	1.76	Vaccine 4	16.76 *	2.86	1.44	Vaccine 4	17.29 *	1.9	0.49
Vaccine 5	39.59	8.77	1.23	Vaccine 5	11.89	3.58	0.79	Vaccine 5	13.58	1.4	0.36
Vaccine 6	37.32	9.52	2.2	Vaccine 6	11.27	2.46	0.89	Vaccine 6	15.47	1.9	0.49
Vaccine 7	36.26	11.67	4.05	Vaccine 7	12.01	3.14	1.12	Vaccine 7	43.31	1.9	0.49
Adjuvant control	34.16	8.17	4.43	Adjuvant control	12.84	3.82	0.82	Adjuvant control	15.23	2.27	0.59
Control PB	36.96	9.03	2.85	Control PB	12.04	3.62	1.06	Control PB	15.57	1.75	0.45

Table 8. Thickness, number and diameter of mucus-secreting cells 80 days post-vaccination for different vaccines and control groups.

Vaccine	Thickness	S.D.	E.E.	Vaccine	No. of cells	S.D.	E.E.	Vaccine	Diameter	S.D.	E.E.
Vaccine 1	49.62 *	9.13	3.84	Vaccine 1	17.68 *	3.28	1.79	Vaccine 1	19.72 *	2.13	0.13
Vaccine 2	39.21	6.18	2.16	Vaccine 2	13.22	3.05	0.94	Vaccine 2	14.7	1.68	0.68
Vaccine 3	37.5	6.3	1.59	Vaccine 3	13.81	2.65	0.72	Vaccine 3	13.38	1.15	0.15
Vaccine 4	52.25 *	11.23	3.69	Vaccine 4	16.85 *	3.83	1.39	Vaccine 4	18.68 *	1.31	0.31
Vaccine 5	38.89	8.16	1.31	Vaccine 5	11.89	1.78	0.65	Vaccine 5	13.51	1.33	0.25
Vaccine 6	38.88	7.63	2.1	Vaccine 6	11.27	2.67	0.97	Vaccine 6	14.73	1.3	0.65
Vaccine 7	34.76	8.96	1.18	Vaccine 7	12.92	1.32	0.88	Vaccine 7	13.65	1.23	0.74
Adjuvant control	35.8	7.31	2.24	Adjuvant control	13.14	2.18	0.91	Adjuvant control	15.44	1.62	0.68
Control PB	33.19	5.22	1.4	Control PB	12.04	3.62	1.06	Control PB	14.84	1.38	0.46

Table 9. Thickness, number and diameter of mucus-secreting cells 120 days post-vaccination for different vaccines and control groups.

Vaccine	Thickness	S.D.	E.E.	Vaccine	No. of cells	S.D.	E.E.	Vaccine	Diameter	S.D.	E.E.
Vaccine 1	52.28 *	9.09	3.61	Vaccine 1	16.49 *	3.28	1.79	Vaccine 1	20.17 *	1.3	0.28
Vaccine 2	42.14	5.96	3.45	Vaccine 2	12.07	2.51	1.17	Vaccine 2	16.62	1.44	0.24
Vaccine 3	4136	7.19	3.65	Vaccine 3	13.32	1.32	0.88	Vaccine 3	15.49	1.26	0.3
Vaccine 4	53.72 *	9.92	2.42	Vaccine 4	17.51 *	3.83	1.39	Vaccine 4	19.16 *	1.22	0.3
Vaccine 5	39.45	7.96	2.15	Vaccine 5	12.23	2.52	1.05	Vaccine 5	14.42	1.34	0.41
Vaccine 6	36.99	5.78	3.71	Vaccine 6	13.68	2.23	1.12	Vaccine 6	15.22	1.32	0.35
Vaccine 7	39.06	6.32	2.16	Vaccine 7	13.3	3.68	0.92	Vaccine 7	14.65	1.27	0.33
Adjuvant control	37.46	5.66	2.26	Adjuvant control	13.58	3.47	1.48	Adjuvant control	14.37	1.38	0.47
Control PB	34.89	7.12	1.76	Control PB	12.94	2.66	0.86	Control PB	13.87	1.43	0.27

At time 0, histological samples taken from the abdominal and lateral zone of the fish were stained with the same intensity both for PAS as for PAS-Alcian Blue

staining, with prevalence of neutral mucopolysaccharides. Between days 10 and 50 post-vaccination the mucus of the fish immunized with Vaccine 1 and Vaccine A turned more dense and more acidic (Figures 12 and 13)

The thickness of the epithelium and the number of PAS+ cells did not show significant differences between day 0 and day 20. These observations are described in tables 2 through 9 and are also shown in figures 12 and 13.

By day 30 after vaccination, a significant thickening of the epithelium was observed, as well as an increase in the number and diameter of mucus-secreting cells PAS+ in the groups treated with vaccines 1 and A as compared to day 0 and as compared with the controls (PBS and adjuvant). In the case of vaccines 2-3-5-6, and 7, the values were lower than those observed for vaccines 1 and A. Immunogenic stimulation also caused hyperplasia of goblet cells .

At day 40 post-vaccination, activation of macrophages and an increase of lymphocytic infiltration were observed, and they were accompanied by an increase of specific serum antibody titers. At day 50 post-vaccination, the immune response was potentiated, further increasing epithelia thickness, the number of PAS+ cells and the diameter thereof, mainly, but not exclusively, in fish treated with vaccines 1 and A.

From day 80 to day 120 post-vaccination, a significant increase in the number and diameter of cells was observed, keeping the same values observed during challenge. A relatively significant increase of epithelium thickness was recorded as compared to data from day 50.

There were no statistically significant differences in epithelium thickness, number of PAS+ cells and their diameter in control fish. There were however minor variations in fish treated with vaccines 2-3-5-6, and 7.

It would be obvious for a person of skill in the art that the peptides and

vaccines of the invention may be used as compositions useful for generating a mucous shield, and, as has been shown, the mucous shield decreases infestation by copepods in treated fish.

This invention is better illustrated in the following examples, which should not be construed as limiting its scope. On the contrary, it should be clearly understood that other embodiments, modifications and equivalents thereof may be possible after reading the present description, which may be suggested by a person of skill without departing from the spirit of the present invention and/or the scope of the appended claims.

Examples

Example 1: Isolation, processing, analysis and identification of proteins and peptides

Proteins were isolated from a suspension of 0.5g of adult specimens of *C. rogercresseyi* in PBS-Tween 0.05%. Samples were frozen and homogenized using a Precellys 24 tissue homogenizer (Bertin Technologies-France using 2mL tubes containing ceramic and glass beads (prefilled bead- tubes Cat. No. 03119.200.RD000 Precellys-France). Two cycles of 50 sec at 6000 rpm were performed, and then at 5000 rpm for 15 min in a centrifuge (Eppendorf Refrigerated Microcentrifuge Model 5417 R-USA). The supernatant was collected and then concentrated with CentriPlus YM50 (cut off>50 kDa) (Millipore-Fisher Sci) at 2500 rpm in a Sorvall centrifuge with SS34 rotor.

The collected soluble proteins were separated by electrophoresis on 8% sodium dodecyl sulfate polyacrylamide gels under reducing and non-reducing conditions and stained with Coomassie Brilliant Blue G-250 as described by Laemmli et al 1970.

Tryptic digestion followed by mass spectrometry and Maldi-tof (Matrix-

Assisted Laser Desorption/Ionization- time of flight).

Bands extracted from a 8% sodium dodecyl sulfate polyacrylamide gel were destained using methanol-acetic (20:7), diluted in 0.1M of ammonium carbonate pH 8.0 and then reduced with 10 mM Dithiotreitol for 30 minutes at room temperature, followed by alkylation with 50mM iodoacetamide. Tryptic digestion and bidimentional separation were performed according to Cordwell et al 1999. A Thermo Electron LTQ-FT spectrometer with a Protana nanospray system as ion source was used for mass spectrometry. Phenomenex Jupiter 10/C18 reverse phase columns were used as interphase. The samples subjected to tryptic digestion were injected in the column and peptides were eluted with 0.1M acetic acid - 100% acetonitrile with a gradient flow of 0.4µl/min during 2 hs. The nanospray source was operated at 2.5 kV. Analysis by tryptic digestion and mass spectrometry Maldi-tof of the gel-extracted bands revealed that different peptidic fractions had been obtained which were analyzed using the Sequest algorithm and the NCBI NR -2006 data base.

Example 2: Preparation of conjugated peptides

Conjugated peptides were obtained using solid phase chemical synthesis (SPPS) techniques. SPPS follows a general pattern of repetitive cycles of coupling-washing-unprotection-washing. The free amino terminal end of a peptide bound to a solid phase was coupled to a single N-protected amino acid unit. This unit was then unprotected, thereby showing a new amino terminal end which could bind to another amino acid.

The conjugation to KLH was performed through the free cysteine group or by addition using the MBS method (m-maleimidobenzoyl-N- hydroxysuccinimide Ester or activated maileimide) which is preferred for coupling amino acids according to Hermanson. The peptides were sequenced using the method

described by Merrifield

Example 3: Preparation of the vaccines

Vaccine A: Three soluble proteins of high molecular weight were selected from the parasite homogenate . Protein quantification was carried out by densitometry in a densitometer UV-P System using BSA (V-Sigma fraction) as reference. Each dose of vaccine A contained 1 µg of each of the proteins of 220465.60 Da (SEQ ID NO. 1), 212947.00 Da (SEQ ID NO. 2), and 173132.50 Da (SEQ ID NO. 3) in an oil emulsion formulated with Montanide ISA 763 Seppic non-mineral oil (70 % v/v oil -30 % v/v water), and 10 µg of hemocyanin keyhole limpet from *Megathura crenulata* (H8283-Sigma).

Non-specific control composition: BmSS recombinant protein from *Boophilus microplus* intestine was used, which is highly immunogenic and protective in bovine infestation with native ticks. Each dose of vaccine contained 3 µg of BmSS in an oil emulsion formulated with Montanide ISA 763 Seppic non-mineral oil (70 % v/v oil – 30 % v/v water) and 10 µg of *Megathura crenulata* hemocyanin (keyhole limpet) (H8283-Sigma).

Adjuvant control compositions: Each dose of vaccine adjuvant controls contained 30 µg of PBS in an oil emulsion formulated with Montanide ISA 763 Seppic non-mineral oil (70 % v/v oil – 30 % v/v water) and 10 µg of *Megathura crenulata* hemocyanin (keyhole limpet) (H8283-Sigma).

PBS control: Each dose of PBS control vaccine contained only 100 µl of PBS.

Once the peptides were obtained, part of the same were conjugated with the antigenic protein KLH using a liquid phase conjugation method such as that described in the examples. The following vaccines were prepared:

Vaccine 1 comprised 50 µg of each of conjugated peptides 1-4 (peptides of

SEQ ID NO. 17, SEQ ID NO. 18, SEQ ID NO. 19, and SEQ ID NO. 20);

Vaccine 2 comprised 50 µg of each of conjugated peptides 5-8 (peptides of SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, and SEQ ID NO. 24);

Vaccine 3 comprised 50 µg of each of conjugated peptides 9-12 (peptides of SEQ ID NO. 25, SEQ ID NO. 26, SEQ ID NO. 27, and SEQ ID NO. 28);

Each vaccine was prepared as an emulsion in Montanide ISA 763 Seppic non-mineral oil (70 % v/v oil – 30 % v/v water) and 10 µg of hemocyanin keyhole limpet from *Megathura crenulata* (H8283-Sigma), to a final volume of 0.05ml.

Vaccine 5 comprised 50 µg of each of non-conjugated peptides 1-4;

Vaccine 6 comprised 50 µg of each of non-conjugated peptides 5-8;

Vaccine 7 comprised 50 µg of each of non-conjugated peptides 9-12.

Each of them was prepared as an emulsion in Montanide ISA 763 Seppic non-mineral oil (70 % v/v oil – 30 % v/v water) and 10 µg of hemocyanin keyhole limpet from *Megathura crenulata* (H8283-Sigma), to a final volume of 0.05ml.

Composition 8, adjuvant controls: Each dose of vaccine adjuvant controls contained 30 µg of PBS in an oil emulsion formulated with Montanide ISA 763 Seppic non-mineral oil (70 % v/v oil – 30 % v/v water) and plus 10 µg of *Megathura crenulata* hemocyanin (keyhole limpet) (H8283-Sigma).

Composition 9, PBS control: Each dose of PBS control vaccine contained only 0,05 ml of PBS.

Example 4: Assays in fish and challenge:

The assay included the most sensitive species to *C. rogercresseyi*: rainbow trouts (Oncorhynchus mykiss) of 30g free from infestation (n = 50/group) without previous vaccination and without antibiotic treatment before commencement of the study.

Group 1 was vaccinated with vaccine A, group 2 with the non-specific

control composition, Group 3 with the adjuvant control composition, and Group 4 only with PBS.

The assays were carried out at the Aquaculture Unit of the city of Mercedes [*Unidad de Acuicultura de la ciudad de Mercedes*](Province of Buenos Aires, Argentina). The animals were acclimated during 4 days in 200 L fresh water ponds at a temperature of 17-20 ºC, with 5 mg/l oxygen (minimum), with a turnover rate of 1L /hour and with a density of up to 20 Kg/m3.

Fish were anesthetized with 20% Benzocaine at a dose of 50 ppm. A single immunization was performed (0.1 mL/fish) intraperitoneally in the ventral midline using a 1 mL syringe and 25G x 5/8" needle. The control group was vaccinated with 0.1 ml/fish of sterile PBS and marked with a cut made in the adipose fin for its later identification. No reactions at the injection site /tissue damage/ survival were observed. After the vaccination, the fish were placed in identified ponds where they remained without stressing conditions until their immunization period was completed. At 450-500 UTA, fish were moved to ponds with sea water (25ppt), before the challenge with infecting stages of *C. rogercresseyi*. Temperature was monitored daily throughout this period,.

During the days of adaptation and during the immunization period, the fish were fed with a commercial diet at 3.5% body weight every day. Immunization schedule and sampling are shown in figure 14.

Serum and mucus samples were taken prior to vaccination, at the time of the challenge and every 10 days after the same to determine the immuno response to the vaccine. Average parasitic load of challenged fish was determined.

<u>Challenge using *C. rogercresseyi*</u>

For the culture of *C rogercresseyi*, specifically the small copepod stage,

ovigerous females were collected weekly from a filter at a processing farm for Atlantic salmon using tipped tweezers. Samples were sent to the laboratory transported in plastic containers containing sea water with constant aeration system. After spawning, naupliar stages were withdrawn and placed in beakers containing 600 ml of filtered and sterilized sea water under constant aeration. They were kept in a Hotcold-S culture chamber at an average temperature of 13ºC, until copepodites emerged. Once infective stages were obtained, counting was performed in a Neubauer chamber, and concentrating the specimens at 4000 copepodites/600 mL of filtered water.

Challenge took place when the fish reached 600UTA, then introducing 4000 copepodites (in the specified quantity of filtered sea water) into each 50 fish/ pond, and expecting a 50%.fixation rate. The amount of water was reduced to 50%, oxygen bubbling and water flow were stopped for 6 hours after infection (static flow), after which they were resumed and water flow rate was kept at 0.5 liters/ hour so as to avoid affecting fixation of *C rogercresseyi*.

Post-challenge parasitic load and reduction efficacy of *C rogercresseyi* loads were determined in vaccinated versus control groups at the time of fixation, at the time of development of juvenile stages (Chalimus I, II, III) and upon development of chalimus IV, female and male adults.

Treatment with peptide vaccines

The treatment was carried out in the most commercialized species, Atlantic salmon (*Salmo salar*), using 30g specimens free from infestation (n = 50/group) without prior vaccination, without a history of recent condition and without antibiotic treatments previous to commencement of the assay. The assays were carried out at the Unit of Aquaculture of the city of Mercedes [*Unidad de Acuicultura de la ciudad de Mercedes*](Province of Buenos Aires, Argentina). Fish

were anesthetized with 20% Benzocaine at a dose of 50 ppm. A single immunization was performed (0.1 mL/fish) intraperitoneally in the ventral midline using a 1 mL syringe and 25G x 5/8" needle. The control group was vaccinated with 0.1 ml/fish of sterile PBS and marked with a cut practiced in the adipose fin for its later identification. Any reactions at the injection site /tissue damage/ survival were recorded. Challenge was performed when the Atlantic salmons reached a weight of 80g, at 600UTA.

Example 5: Serum and Histological Assays

Serum and mucus samples were collected to test specific antibody titers using an ELISA from day 0 or pre-immune, up to 10, 20, 30, 40 days, at the time of challenge (50 days) and every 10 days post challenge until 120 days post-vaccination (dpv).

Proteins of 220 kDa (SEQ ID NO. 1), 212 kDa (SEQ ID NO. 2), and 173 kDa (SEQ ID NO. 3) were used as capture antigens at a concentration of 50µg/mL. As a secondary antibody, an anti IgM of coho salmon (*Oncorhyncus kisutch*) (IgG1 monoclonal fraction) (Grupo Bios-Bios, Chile) was employed and as a conjugated antibody a mouse anti-IgG marked with peroxidase (goat anti-mouse, Dako, Denmark) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)as a substrate.

For the histological analysis samples of fish epidermis were taken using a scalpel, making cuts on the abdominal and lateral zones of the fish on days 0-10-20-30-40-50, 80, and 120. Cuts were embedded in 4% formol buffer and they were stained using PAS (periodic acid- Shiff) and PAS–Alcian Blue dyes.

Mucus was obtained by scraping the surface of the fish with a scalpel. The extracted material was placed in 15 mL tubes with 2 mL of PBS+ protease inhibitor coctail (Promega G6521 50X) thereby achieving a dense suspension. It was

centrifuged at 3000g for 10 minutes and the supernatant was collected and kept at -20ºC. Samples were assayed non-diluted and in duplicate. Five 5 serum and mucus samples were per group and per sampling time for the serum analysis, and 3 samples were used for the histological analysis.

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CLAIMS

Having thus specifically described and determined the nature and the best mode for carrying out the present invention, the inventors claim ownership and of exclusive right on:

1. An isolated peptide, characterized by comprising an amino acid sequence having at least 90 % identity to a sequence selected from the group consisting of SEQ ID NO. 17, SEQ ID NO. 18, SEQ ID NO. 19, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25, SEQ ID NO. 26, SEQ ID NO. 27, SEQ ID NO. 28 and combinations thereof, said peptide inducing an immune response against copepods and/or generating a mucous shield in fish.

2. The peptide according to claim 1, characterized in that the fish are selected from the group consisting of Atlantic salmon (*Salmo Salar*), Rainbow trout (*Oncorhynchus mykiss*), Coho salmon (*Oncorhynchus kisutch*), brown trout (Salmo trutta), and Chinook salmon (O. tshawytscha) and the copepods belong to the Caligidae family.

3. The peptide according to claim 1, characterized by comprising an antigenic protein conjugated to said peptide.

4. The peptide according to claim 3, characterized in that the antigenic protein is hemocyanin (KLH - keyhole limpet hemocyanin) from *Megathura crenulata*.

5. A vaccine which induces an immune response against copepods and/or a mucous shield in fish, characterized by comprising at least one peptide, wherein said peptide has an amino acid sequence showing at least 90 % identity to a sequence selected from the group consisting of SEQ ID NO. 17, SEQ ID NO. 18, SEQ ID NO. 19, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID

NO. 23, SEQ ID NO. 24, SEQ ID NO. 25, SEQ ID NO. 26, SEQ ID NO. 27, SEQ ID NO. 28 and combinations thereof; excipients and adjuvants.

6. The vaccine according to claim 5, characterized in that the fish are selected from the group consisting of Atlantic salmon (*Salmo Salar*), Rainbow trout (*Oncorhynchus mykiss*), Coho salmon (*Oncorhynchus kisutch*), brown trout (Salmo trutta), and Chinook salmon (O. tshawytscha) and the copepods belong to the Caligidae family.

7. The vaccine according to claim 5, characterized in that it is in the form of an emulsion.

8. The vaccine according to claim 5, characterized in that the excipient is a non-mineral oil.

9. The vaccine according to claim 5, characterized in that the peptide comprises an antigenic protein conjugated to said peptide.

10. The vaccine according to claim 9, characterized in that the antigenic protein is hemocyanin from the keyhole limpet *Megathura crenulata*.

11. A vaccine that induces an immune response against copepods and/or the development of a mucous shield in fish, characterized by comprising peptides as set forth in SEQ ID NO. 17, SEQ ID NO. 18, SEQ ID NO. 19, and SEQ ID NO. 20; and excipients.

12. The vaccine according to claim 11, characterized in that at least one of the peptides is conjugated to an antigenic protein.

13. The vaccine according to claim 11, characterized in that the four peptides are conjugated to an antigenic protein.

14. A vaccine that induces an immune response against copepods and/or a mucous shield in fish, characterized by comprising peptides as set forth in SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, and SEQ ID NO. 24; and

excipients.

15. The vaccine according to claim 14, characterized in that at least one of the peptides is conjugated to an antigenic protein.

16. The vaccine according to claim 14, characterized in that the four peptides are conjugated to an antigenic protein.

17. A vaccine that induces an immune response against copepods and/or the development of a mucous shield in fish, characterized by comprising peptides as set forth in SEQ ID NO. 25, SEQ ID NO. 26, SEQ ID NO. 27, and SEQ ID NO. 28; and excipients.

18. The vaccine according to claim 17, characterized in that at least one of the peptides is conjugated to an antigenic protein.

19. The vaccine according to claim 17, characterized in that the four peptides are conjugated to an antigenic protein.

20. The vaccine according to any of claims 11, 14, and 17, characterized in that the copepod belong to the Caligidae family.

21. The vaccine according to any of claims 11, 14 and 17, characterized in that the excipient is a non-mineral oil.

22. The vaccine according to any of claims 11, 14, and 17, characterized in that the fish are selected from the group consisting of Atlantic salmon (*Salmo Salar*), Rainbow trout (*Oncorhynchus mykiss*), Coho salmon (*Oncorhynchus kisutch*), brown trout (Salmo trutta), and Chinook salmon (O. tshawytscha) and the copepods belong to the Caligidae family.

23. The vaccine according to any of claims 11, 14, and 17, characterized in that it is in the form of an emulsion.

24. Use of the peptides of claim 1 for preparing a vaccine.

25. Use of the peptides of la claim 1 for preparing a composition that

induces development of a mucous shield in fish.

26. A vaccine against copepods infesting fish, characterized by comprising the proteins of SEQ ID NO. 1, SEQ ID NO. 2, and SEQ ID NO. 3; excipients and adjuvants.

27. The vaccine according to claim 26, characterized in that the excipient is a non-mineral oil.

28. A method for modulating an immune response in fish, characterized by comprising administering to said fish the necessary amount of a vaccine comprising at least one peptide having at least 90 % identity to a sequence selected from the group consisting of SEQ ID NO. 17, SEQ ID NO. 18, SEQ ID NO. 19, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25, SEQ ID NO. 26, SEQ ID NO. 27, SEQ ID NO. 28 and combinations thereof; and excipients.

29. The method according to claim 28, characterized in that the peptide is administered in an amount of from 1 and 500 µg.

30. The method according to claim 28, characterized in that the fish are selected from the group comprising Atlantic salmon (*Salmo Salar*), Rainbow trout (*Oncorhynchus mykiss*) and Coho salmon (*Oncorhynchus kisutch*), brown trout (*Salmo trutta*) and Chinook salmon (*O. tshawytscha*) and the copepods belong to the Caligidae family.

31. The method according to claim 28, characterized in that the peptide comprises an antigenic protein conjugated to said peptide.

32. The method according to claim 31, characterized in that the antigenic protein is hemocyanin from the keyhole limpet *Megathura crenulata*.

33. A method for modulating immune response in fish, characterized by comprising administering to said fish a necessary amount of a vaccine comprising

the proteins set forth in SEQ ID NO. 1, SEQ ID NO. 2, and SEQ ID NO. 3; excipients and adjuvants.

34. The method according to claim 33, characterized in that each protein is administered in an amount of from 1 and 10 µg.

35. The method according to claim 33, characterized in that the fish are selected from the group comprising Atlantic salmon (*Salmo Salar*), Rainbow trout (*Oncorhynchus mykiss*) and Coho salmon (*Oncorhynchus kisutch*), brown trout (*Salmo trutta*) and Chinook salmon (*O. tshawytscha*) and the copepods belong to the Caligidae family.

36. A method for generating the development of a mucous shield in fish, characterized by comprising administering to said fish a necessary amount of a vaccine comprising at least one peptide having at least 90 % identity to a sequence selected from the group consisting of SEQ ID NO. 17, SEQ ID NO. 18, SEQ ID NO. 19, SEQ ID NO. 20, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID NO. 23, SEQ ID NO. 24, SEQ ID NO. 25, SEQ ID NO. 26, SEQ ID NO. 27 and SEQ ID NO. 28; and excipients.

37. The method according to claim 36, characterized in that the peptide is administered an amount of from 1 and 500 µg.

38. The method according to claim 36, characterized in that the fish are selected from the group comprising Atlantic salmon (*Salmo Salar*), Rainbow trout (*Oncorhynchus mykiss*) and Coho salmon (*Oncorhynchus kisutch*), brown trout (*Salmo trutta*) and Chinook salmon (*O. tshawytscha*) and the copepods belong to the Caligidae family.

39. The method according to claim 36, characterized in that the peptide comprises an antigenic protein conjugated to said peptide.

40. The method according to claim 39, characterized in that the antigenic

protein is hemocyanin from the keyhole limpet *Megathura crenulata*.

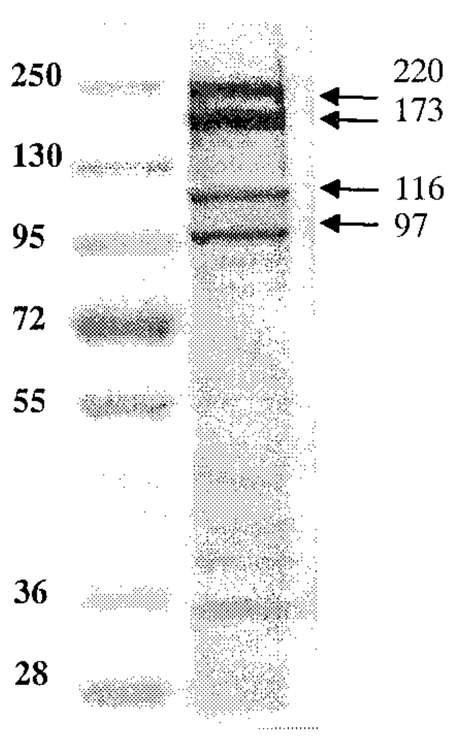

Figure 1

SEQ ID NO. Vitellogenin 1 [Lepeophtheirus salmonis]
MKLVLSLLLFAGVALGYSPSYYGWAPSKEYVYEFETQMLTGIPEIRSQYSGLKLSSKVRIQSFP
DYSLRVQFVEPKFVTVNQEIPSIDGRLYVPSTHSEELPKAIYGPLVTPFEVHFKRGVIESLFVEKD
EPVVVTNWKKALLSQIQTDLSGSREGHVQKLNHEVFPLVAKDSQEMKNVSFFHTMERTLHGD
CETTYTLHPLPLYQAHELEEQWRNHKIKVFETVPEYFQSIRSQKEIHEVMEESGRACTGKQYFQ
VTKTHNFDNCRERPVFSAWSGIKSNCDVTRSGCDDAFNSIVSTRYIICGTPDLFVIRKATTENIIS
LSPTGFNTPEKLTSFSTVTLELRTILSTVSHQIPKPKTPKTVGNLFMEYPEHESFNSESISEQWTK
GSIISPTNISGFTGFKSLSGFYPIHPMPTMDTAPTLLYPISLSKPELIRDVQEMMSKIVRETYEVPE
SCSSSSDLAGYIVSIAEALRPLSLTELKELDTEVHRFMEVRDKEAILTSQYLFYDILAMVGTNPSI
SYIKQLIGSDKIPIQYAPDVLESALRNIKTPTPELFNLVFTMVKTLKAKSPQLYYVSTVSFSDLLH
RACINPSSMVAQFPVHVYGNFCNPETPFIKDQYITFLESQIQGGSQGSKSEKVVLINALGKLGHY
KAVSTLVKFIQGKVSQEPMIR**SLAVYALK**RTAMQYPAKVKPILMSIINNPGEHPEVRIAAVSVL
PFSSPSTTELQKIALRTWFEPSKQVTSFIYSTLKSLRTTQVPELMQFRNKVKSVIPMVRRTHSGIQ
FSHNIHISTFLDYLKIVANNKLEIVNTPESMLPAKISFSEDWINRSMRIKGLSFSLYSQGMDYVFE
KMMTHLGLKESPSPIVTSELQKITQKLQITPRTLQEPELSLKIKFMGLERIFSLDSK**FYMETIQKV**
TEKLRSSPQILSHGLPFKYTKTRNFVDVQSVAPTASGFPVRIQSVTPMVYSVKGYTSGNFSSNVP
SVQHEVSFNHSARVKITPILHA**KVETTMGVISPFTKQ**YIGSGFEMGLHSSTPLDVKVSVNSLGQ
LKLTMKSPEEVQNEVELAHVYTKPFTFKKSYETIVPASRSPGNKMILSGTILKKFTFNPTKSTVG
IDAPLKISTDYPVMDLAVAYKKFSNAPNPMAILKAMTIPSTLRYVSFNLKFNPTTSTTKELRTRF
SLASGYKPAPSEFIRYMLPRGYTQETEIMKMCREHRPHDITGCIQSQTKSLHAASEVSNEARSLC
LEHVRINMFPIKSQSIFDQEMQNCIKSVKICESVRFVCSQSSTNTVNPNTCEEKERSCIYRQINLIK
LNTVLHNLQTGTGVSVTVDASLNSPYEMRTYSTILALGASTSQHHEIRGYVNAEIKSHELPAHV
LIADSKSRLPSISSRWNLEQMINDEITMEHDMVIYYGKRTSPRDGMHKIILEAFATKSNGLRKSI
VESPEYILCNKEIGEGRTLAPVCEKLRHLSASVDTFKIRTKFPMRQTSSTYAKSYIRNVLETVFFP
YLTERYFDATSAVEGIKKDETLLEAFVSREGDLAQIKYKEHGFEWDVSNIRLPKTLTQHILPLSF
RNHQLTTGSRFIQKFTSQQSPASCTVEPNFVTTFDNKTYPYTLNDCEHLIVKDCSGLWPMAVTA
RKAGSQMEVKMIVGKHVVVMSPLQSSVINNITVNGIHIPLVEGGLYKYIEPVNESSGSSTPPSST
PGPLLSSLGPISTEGRTVIKFWSYLDGTVVVKHIKTGLIVIFDGERIEVNPPAFLSSKACGICGDM
NGESSADLASPKMCIFEQPRMAAYSYMIKESCQGIPTPEEKTKFEKESHECVLKKISVTPLEDLIT
RLIKVRSGPLGIISKHLIEYRANGNEICFSQRVLDICGGRSVPVIGHMASTPFTCLQSYSHLAKHL
KERVVANEEIPELMKYPTTYNRMIEEASNCQSSSSSGSGMGGGSLPSSPSSSDSSSHHAQPSTGR
FQPQIYNY

Figure 2

SEQ ID NO. Vitellogenin 2 [Lepeophtheirus salmonis]

MKLFAFLCFIGLATATSPSWQWQAGKEHIFHYTGRLLSGIPGLRPHFSGIGIETEVHLQVKSLED
IRLNLRQVNYTQVNGPLSPGLPHVTSSYEGSNWRYVLLPQFTQAPIDIKKLLKVPITFAIHDGEI
KTITVSGTEQEWSLFK**KALVALFQTKM**ETSTLDLEMNTIVKDSDSTKNYWKVSEETIEGVCD
VIYQVNELPEYIVKERAHYFPHLECTSKKFYEITKTKEIDSCKKSAVYTFMKPAVKAESCNSFK
CLSNTFGSASSMT**RYYACGPRS**NWILQTIVNEGIVQRPVGVKSETITTGTRQVLKLRTIQPISSE
VPKPPQPRTTESIMYEYINAGQVSRQQIGIIPKIPQSELKSGEIYKYLPRHFNPAPSSTESKQHLSA
TKIKAELKSYIISIIDDLSSVEELAQKEIPLRLTTFIRGMTLLKVEDIKSLYTDLKSTVYSPAHSNQ
EKISMFHNIFFDAVMVSGTTPAVLFLKDMIKSKEIPTYQATYLLMLLPHHIITPTKEVFTSLLEIIQ
SEIVISNTILYNTAILSMSNLVEKTCLDKSRQVSYPTAVFGQFCDAQSEIVTEKWIPYLTKAVQT
APTADRRNAIIMALGALKHKDIIPALLPLVEGHGPIEQGSGVAFPNISRTLSIYAIGNVRVHHPEL
VLPIILSVYSNPAENTQLRIAAFNMLVNMNPPMNIIQKIAAMTWSEKNTEVLKKTYTGFYTLSR
SVDISNLEDTSPESTLAKKTQLVVPLMRKTSGVLQSTSTLYESSHLPLLEAGYMNIFSWISNYRS
VIPSDVFMKIINSIGEFNYSPFEGAFKLYGGNDFMEKLSNFKDMSESEQKAAFELFSKPGSPTEG
QVYGGRYTSESRRQSMMEHETQYLMKTGTPGLLESTIQSVKSQLHAEWMKVVQQLKIETRED
GPLATLVYLNFFDDATFFTSISEVTVTALREKILPYLKDAKSNNWTQTIQKVKSTICEKQLPVNG
QKMVNLGSAEFLIPSDMGFPIVIEQNMPGLVSIRGSMEMNCNVQTPTIKFEALPMLSVHHHVHV
GTYSPFTKKLVVTGLKQDLTVNIPTKTHVLYKVSTNEVKIILKPVEMKKPTDLLYFSTKPFTTA
QSYFSLMPRCHSSDLKYIKSEVPMRAVSIPSGGSVLGLSLSATIETETPFLDLPYVMQMAKMYN
YNPVNMIRFLFAPHSVTQNGQPSIRYHTFRVKYDALSSSTKEAEFTFLPGCAVKKMGQKEPLIM
SIAPKTSGQGGSTLWSWMPYGVQVHPISTKVLPVVQETLKSIMMEKTHMSHGFAAVLRLEAKF
NGGQRPRLFTYQASIGRGKDLANVTRWHGTWETEPNSPSMSQKICVDGHVTLPTVPTWDIGM
TRNMNMNLLIKNTIGFGRTCNESQIKTFVTSRVSEKQLQWSRESPVAKICEKFIERRVPGAMSTP
ECQETHWLARMYDEVDIKFESINVPTTIKNTVGKVTSSLQRLWWPYVTENHSFTHVTGHYQPK
RESSIHLSFCKSSETVSMTLKTPERHVKFSNIRIPYVFRPFLPFVAGESNLMKAVQITSGSRMLPT
CRIEKDWLRTFDNKSLPLHMDDCFHVIAGDCSTTMQFGILARVVPHTVAKEIKVYMQKTEVKL
IPTPSYSRGNRDVKIQINGSEFVIPREITKTFPVGSVTPVVEIYRSIDDVYHLKGITTGITIKTNGER
ISVSPSLSMKGRLCGICGDMNDQALADISGPTRCVYSSPSVEVAAYRVSTPQCSPMDTKIKQILD
LETNNCARFQEMPTEVIKTYASIAGKCTRQQHMILERGSETCFSTTPVTQCGAQCSPKPKQLAH
KKVGFHCMKSGRLTELYREKVIQGLVLPELQSRPITFSTAILVPQSCSSIVSGHPILSLISSSNSNGI
MNTIDMGGSSGSNGSIGSGSSRL

Figure 2 (continued)

SEQ ID NO. Vitellogenin-like protein [Lepeophtheirus salmonis]

MSPLIKMRVIAILFCFVATSSGSIFEDGTEYTFETETSAVVGTMDHIPHSSGFSYKYMTQMQIHG
NSIKVKLSDFKLSQFNGKHEGGEYPFDHTNFIATNRDVPPFEVKLDSHGLFSSLKVSPKLTIFQR
NMIKGWVQLQLNMDKINHHEHEFHSQEQSIFGDCDTLYTVNDHKIVKSVTHTKDCKNRVHVL
IDDWRGHRCDLEDPDHPESRENPNGLYSASNTIYVVDKKGDHFHPKAIIGSSSVVAQFYESQGI
SFVAHSNSSSILKSSGAISQEITVVGVDLDSLHYEFEDSEYTWKSERDLKAREGYLATGQFFEDD
MPTISKYVKEKLAKTHDIMNKMSTEASTIEKAHMYGINSIYPAMLAMDYSALKQLSEELHSDK
SAEGVYRYNLFNELLGSLGTSASAILVRDMIAENKFDNFRDAVRTLTAVPFHIRHPSKQLLKGF
ETLYSYEGHQFIKDTIPIVLGHLARVTCERAGVMHSPASEECFHSVVDGYADKTIEKIMGSSDH
TEQIKLLGMSFNLRLGNVAEKLK**PLIYGETEIK**SGHLRTLAVPAAAFGAINSGKGAEHLLPIFV
ETENDHELRLTALSYLMDAHPTSTHFNTIVAVLYREKDYEVINYAFTLFEKYARNINPCKKSVS
VLAKYFLKYLK**QYSHFETDYGLGVSK**TYSRQFQQPKYGCGGEYSYWVIGSHRSTLPLSVAIS
MDSTMFGGYTANGMCVQLRIEGLSKALIRKFKTISPDIWKSEELKNILMGDMHIKERPDQPINV
EVLLF**VKNSVVAFR**QYDEDSIKEGGSLKEIFDELQGLGDTYSMNHQRAMRFGSLLYQQPLEIG
APVAYMNSFTGVFDIQATVKKGNARGLMFRDVKYTMNIFGQGSRIMMVQNLQTKNAYSVSQ
DR**IYGSHFPRNFVIGVNPLKK**EFKLSVERPPYEDPLMIMMHSQTNVVTRSQSINNKQDISANC
AECKTITPISYGPDAAKTRVIVDRECDNTGSYIHGEYFDCEMESNRGKVLYHLWRAMTPYHKN
PKTIGNSIRMGIRQIRAYFVFFPRAEKCGAMLRWSQSKQNPVKEIEISLRFNTSPNGERLYFRGR
KWALTGIVKAKGEPQDRVYK**IILGHEFTPGYIENR**LKFRMQRVAVPGLLSDYSICFNMENKY
PDFGEEFMTYDKSTQLKMSGNARLQYGAAADCDSTPGEMKLSFEHETTEEAREAMKHTWYY
EKCMEQKQHPEWASRGDRLPFTEACHMTTWDATTARKYTWKMNFVKMTDRM**NAIVSQFQS**
VMKTGLLPYWDIDPEIIPATSADPHMNIEATLKNHDKNVDIYMETSQGGQHFNDIPLSLNWRP
MLRNLKFTSNTRRLMQYKIIHGCTATIDHVYTLDNVTYPYTPTSCWTLASGHCSPHPTYAVFV
KK**SAGSHLDAKI**YLGGHSIEFQASGPKKINVLINGQAIEVGEKEHVHEQDGQEIFKVLK**WGSS**
YNVYSFLKIWVVYDGHAVSLIPAPSVTGQHCGLCGNFNRNQYDEFESKDAHQLKTSEELVED
YKWKC

Figure 2 (continued)

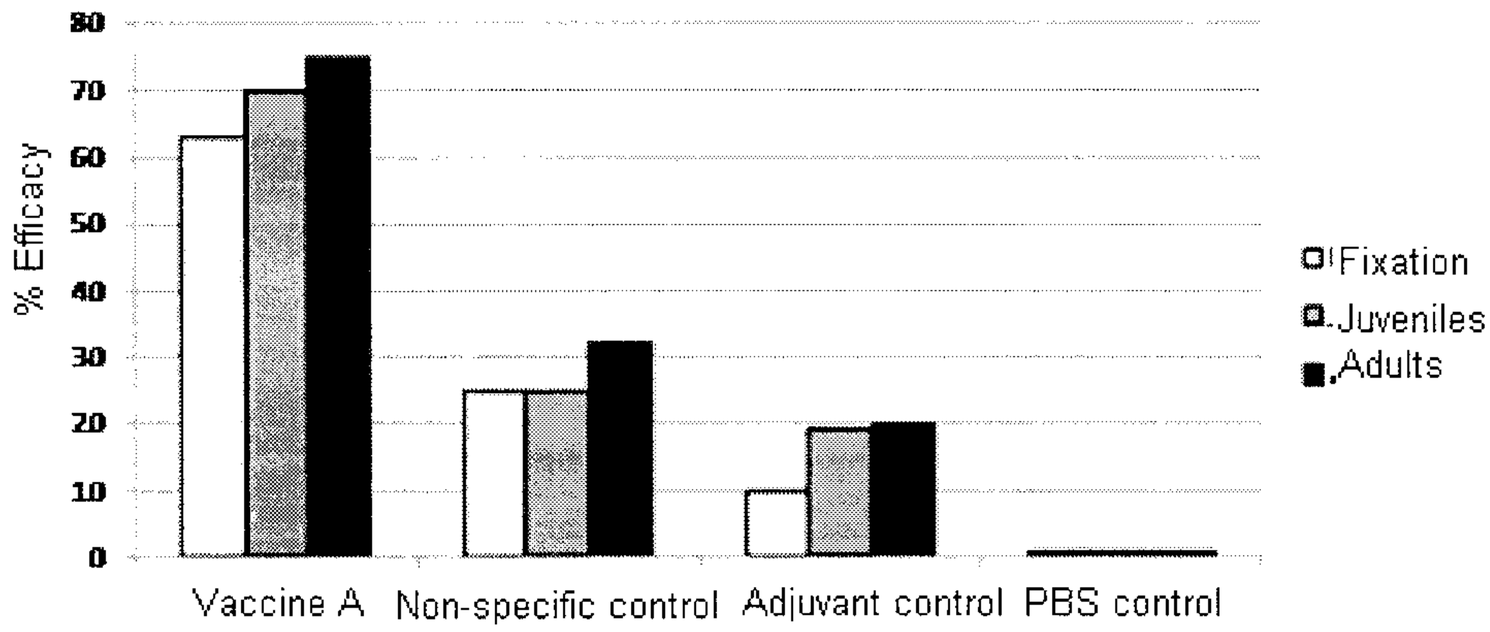

Figure 3

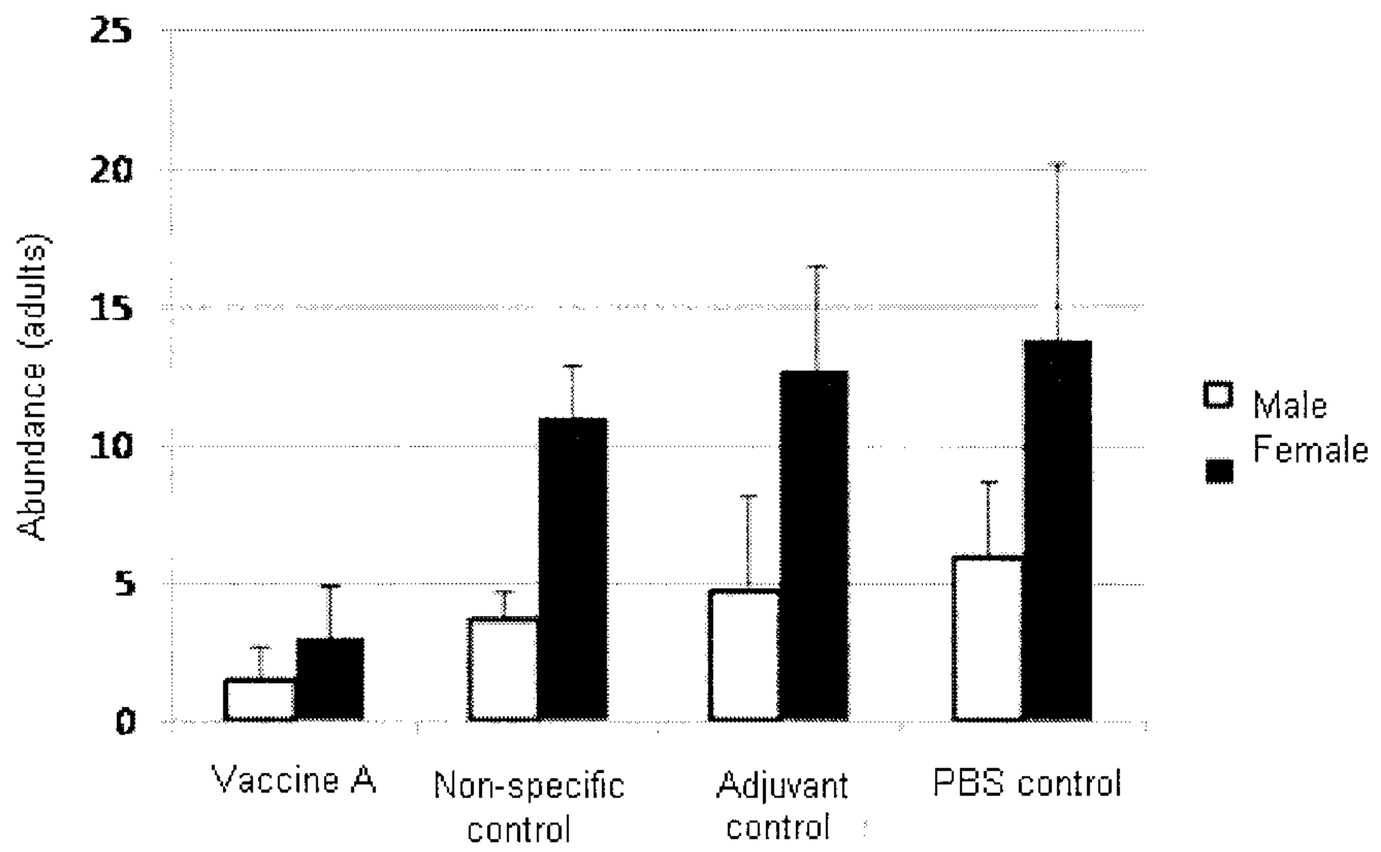

Figure 4

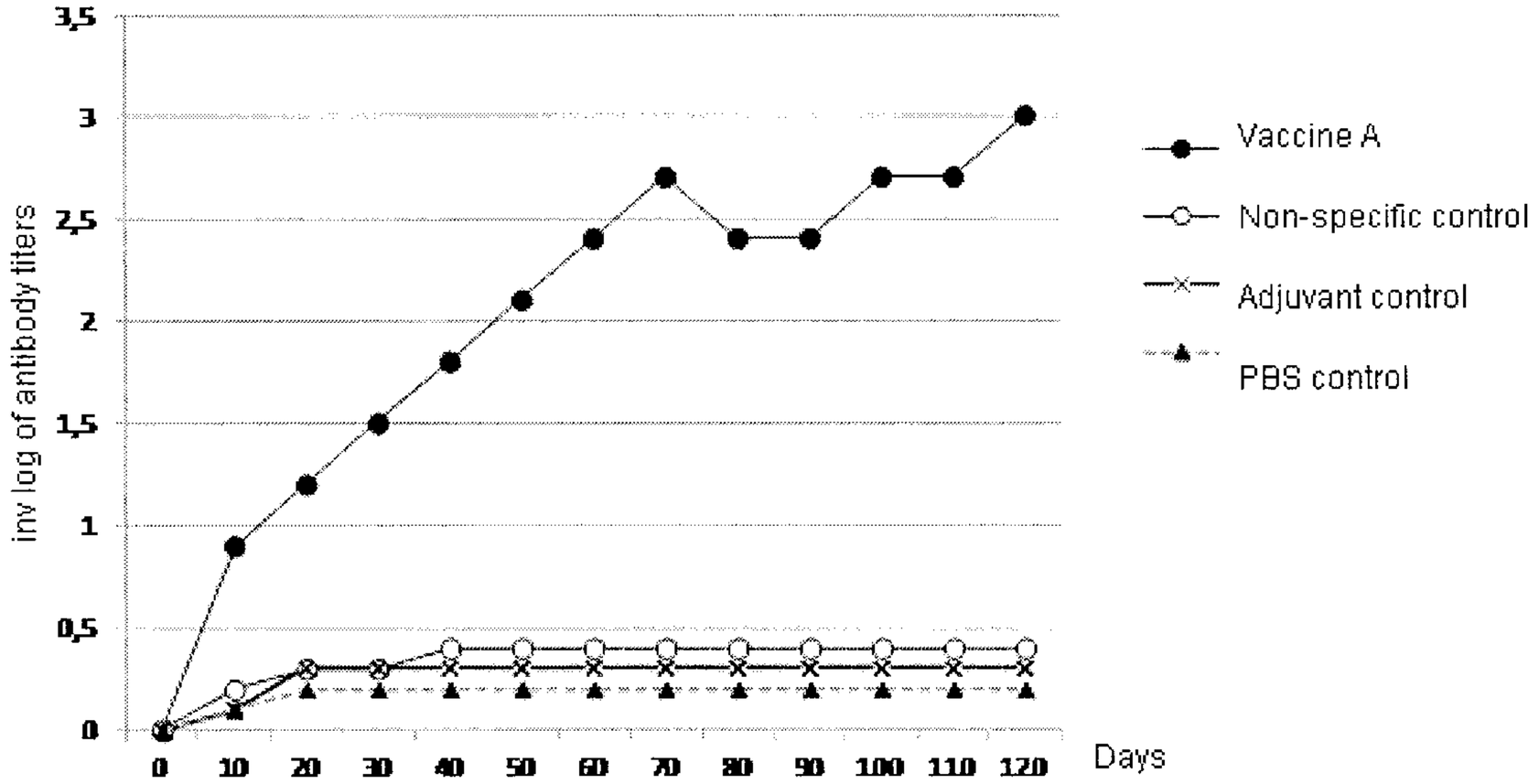
3,5
3
2,5
2
1,5
1
0,5
0
inv log of antibody titers
0
10
20
30
40
50
60
70
80
90
100
110
120
Days
Vaccine A
Non-specific control
Adjuvant control
PBS control

Figure 5

MKLVLSLLLFAGVAL**GYSPSYYGWAPSKEYVYEFE**TQMLTGIPEIRSQYSGLKLSSKV
RIQSFPDYSLRVQFVEPKFVTVNQEIPSIDGRLYVPSTHSEELPKAIYGPLVTPFEVH
FKRGVI**ESLFVEKDEPVVVTNWKKALL**SQIQTDLSGSREGHVQKLNHEVFPLVAKDSQ
EMKNVSFFHTMERTLHGDCETTYTLHPLPLYQAHELEEQWRNHKIKVFETVPEYFQSI
R**SQKEIHEVMEESGRACTGKQ**YFQVTKTHNFDNCRERPVFSAWSGIKSNCDVTRSGCD
DAFNSIVSTRYIICGTPDLFVIRKATTENIISLSPTGFNTPEKLTSFSTVTLELRTIL
STVSHQIPKPKTPKTVGNLFMEYPEHESFNSESISEQWTKGSIISPTNISGFTGFKSL
SGFYPIHPMPTMDTAPTLLYPISLSKPELIRDVQEMMSKIVRETYEVPESCSSSSDLA
GYIVSIAEALRPLSLTELKELDTEVHRFMEVRDKEAILTSQYLFYDILAMVGTNPSIS
YIKQLIGSDKIPIQYAPDVLESALRNIKTPTPELFNLVFTMV**KTLKAKSPQLYYVSTV**
SFSDLLHRACINPSSMVAQFPVHVYGNFCNPETPFIKDQYITFLESQIQGGSQGSKSE
KVVLINALGKLGHYKAVSTLVKFIQGKVSQEPMIRSLAVYALKRTAMQYPAKVKPILM
SIINNPGEHPEVRIAAVSVLPFSSPSTTELQKIALRTWFEPSKQVTSFIYSTLKSLRT
TQVPELMQFRNKVKSVIPMVRRTHSGIQFSHNIHISTFLDYLKIVANNKLEIVNTPES
MLPAKISFSEDWINRSMRIKGLSFSLYSQGMDYVFEKMMTHLGLKESPSPIVTSEL**QK**
ITQKLQITPRTLQEPELSLKIKFMGLERIFSLDSKFYMETIQKVTEKLRSSPQILS**HG**
LPFKYTKTRNFVDVQSVAPTASGFPVRIQSVTPMVYSVKGYTSGNFSSNVPSVQHEVS
FNHSARVKITPILHAKVETTMGVISPFTKQYIGSGFEMGLHSSTPLDVKVSVNSLGQL
KLTMKS
PEEVQNEVELAHVYTKPFTFKKSYETIVPASRSPGNKMILSGTILKKFTFNPTKSTVG
IDAPLKISTDYPVMDLAVAYKKFSNAPNPMAILKAMTIPSTLRYVSFNLKFNPTTSTT
KELRTRFSLASGYKPAPSEFIRYMLPRGYTQETEIMKMCREHRPHDITGCIQSQTKSL
HAASEVSNEARSLCLEHVRINMFPIKSQSIFDQEMQNCIKSVKICESVRFV**CSQSSTN**
TVNPNTCEEKERSCIYRQINLIKLNTVLHNLQTGTGVSVTVDASLNSPYEMRTYSTIL
ALGASTSQHHEIRGYVNAEIKSHELPAHVLIADSKSRLPSISSRWNLEQMINDEITME
HDMVIYYGKRTSPRDGMHKIILEAFATKSNGLRKSIVESPEYILCNKEIGEGRTLAPV
CEKLRHLSASVDTFKIRTKFPMRQTSSTYAKSYIRNVLETVFFPYLTERYFDATSAVE
GIKKDETLLEAFVSREGDLAQIKYKEHGFEWDVSNIRLPKTLTQHILPLSFRNHQLTT
GSRFIQKFTSQQSPASCTVEPNFVTTFDNKTYPYTLNDCEHLIVKDCSGLWPMAVTAR
KAGSQMEVKMIVGKHVVVMSPLQSSVINNITVNGIHIPLVEGGLYKYIE**PVNESSGSS**
TPPSSTPGPLLSSLGPISTEGRTVIKFWSYLDGTVVVKHIKTGLIVIFDGERIEVNPP
AFLSSKACGICGDMNGESSADLASPKMCIFEQPRMAAYSYMIKE**SCQGIPTPEEKTKF**
EKESHECVLKKISVTPLEDLITRLIKVRSGPLGIISKHLIEYRANGNEICFSQRVLDI
CGGRSVPVIGHMASTPFTCLQSYSHLAKHLKERVVANEEIPELMKY**PTTYNRMIEEAS**
NCQSSSSSGSGMGGGSLP**SSPSSSDSSSHHAQPSTGRFQ**PQIYNY

Figure 6

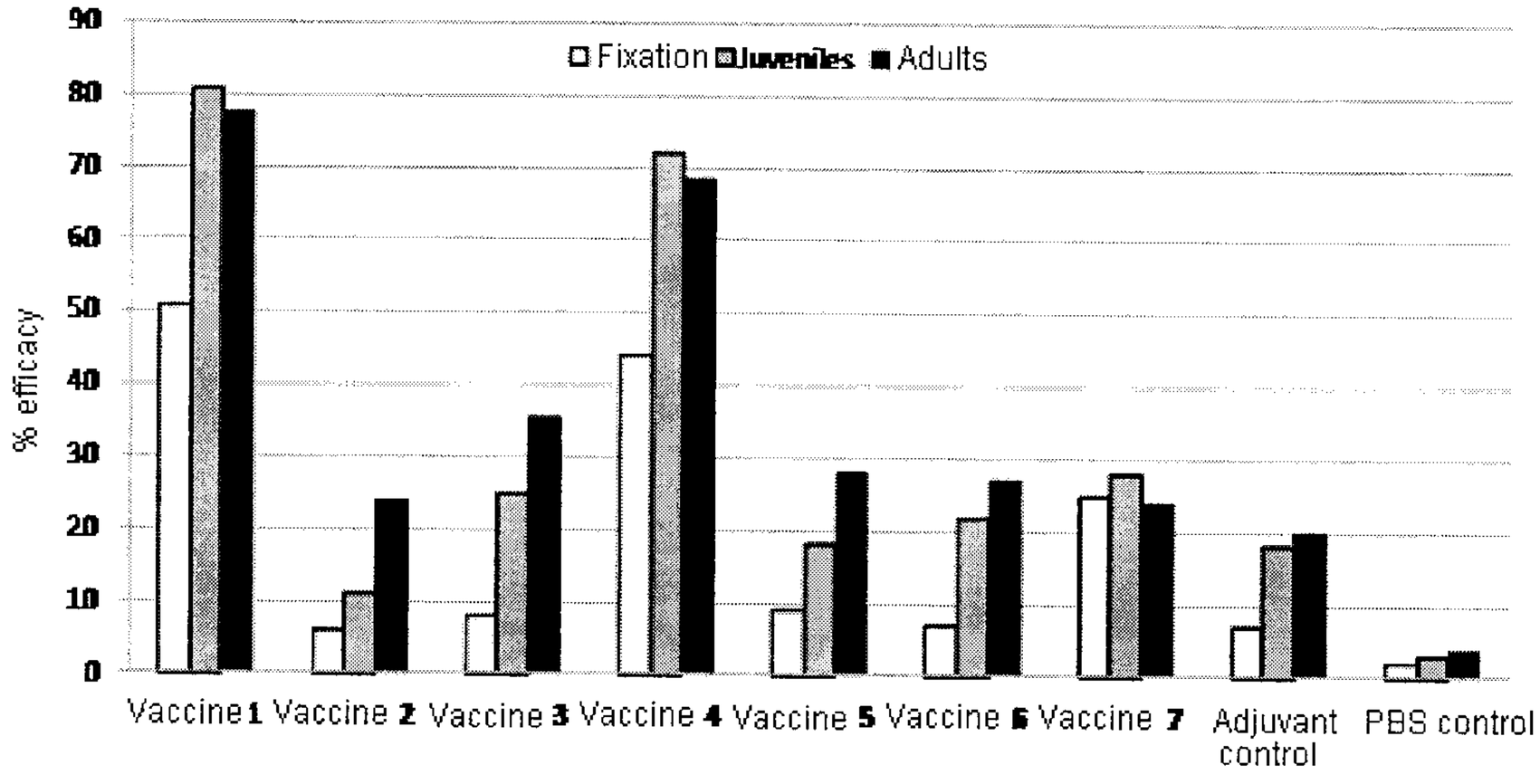
Fixation Juveniles Adults
% efficacy
90
80
70
60
50
40
30
20
10
0
Vaccine 1
Vaccine 2
Vaccine 3
Vaccine 4
Vaccine 5
Vaccine 6
Vaccine 7
Adjuvant control
PBS control

Figure 7

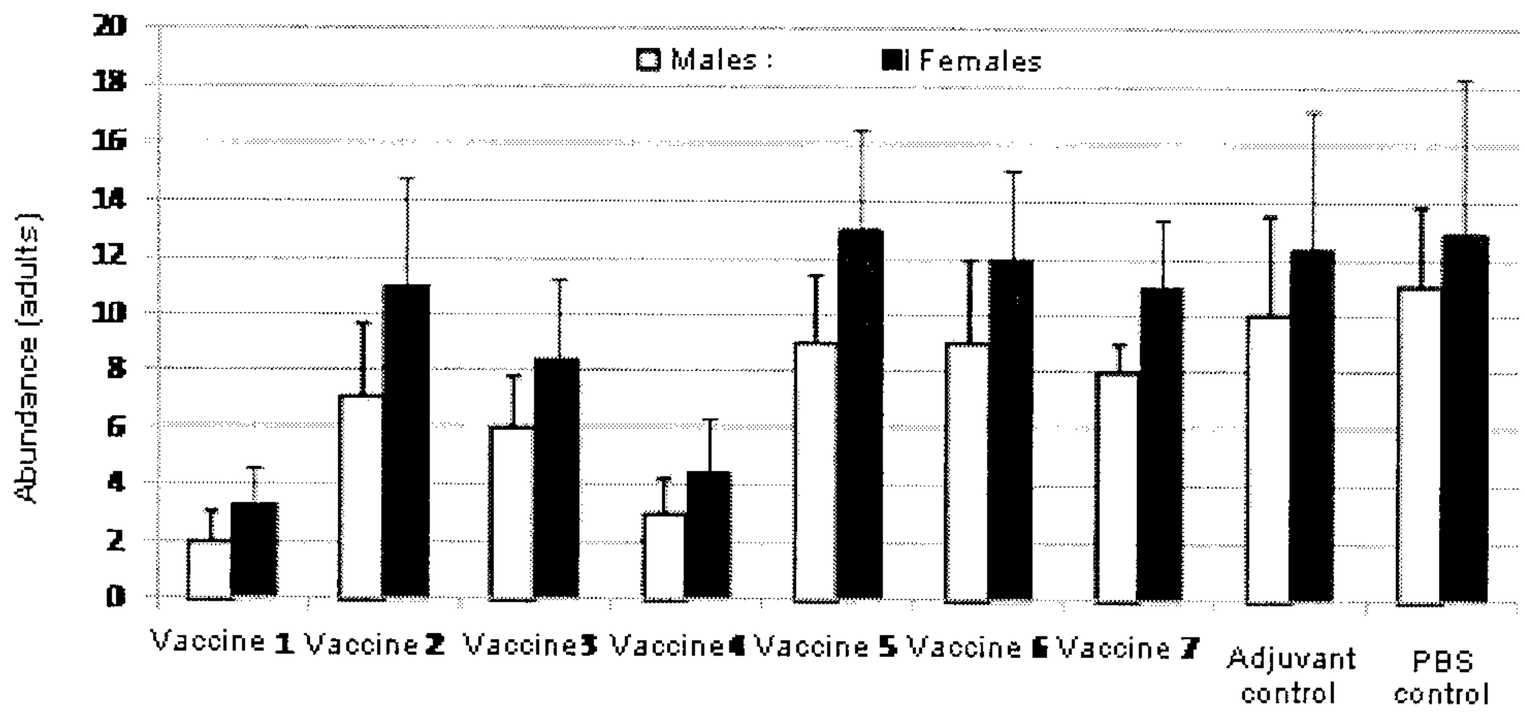

Figure 8

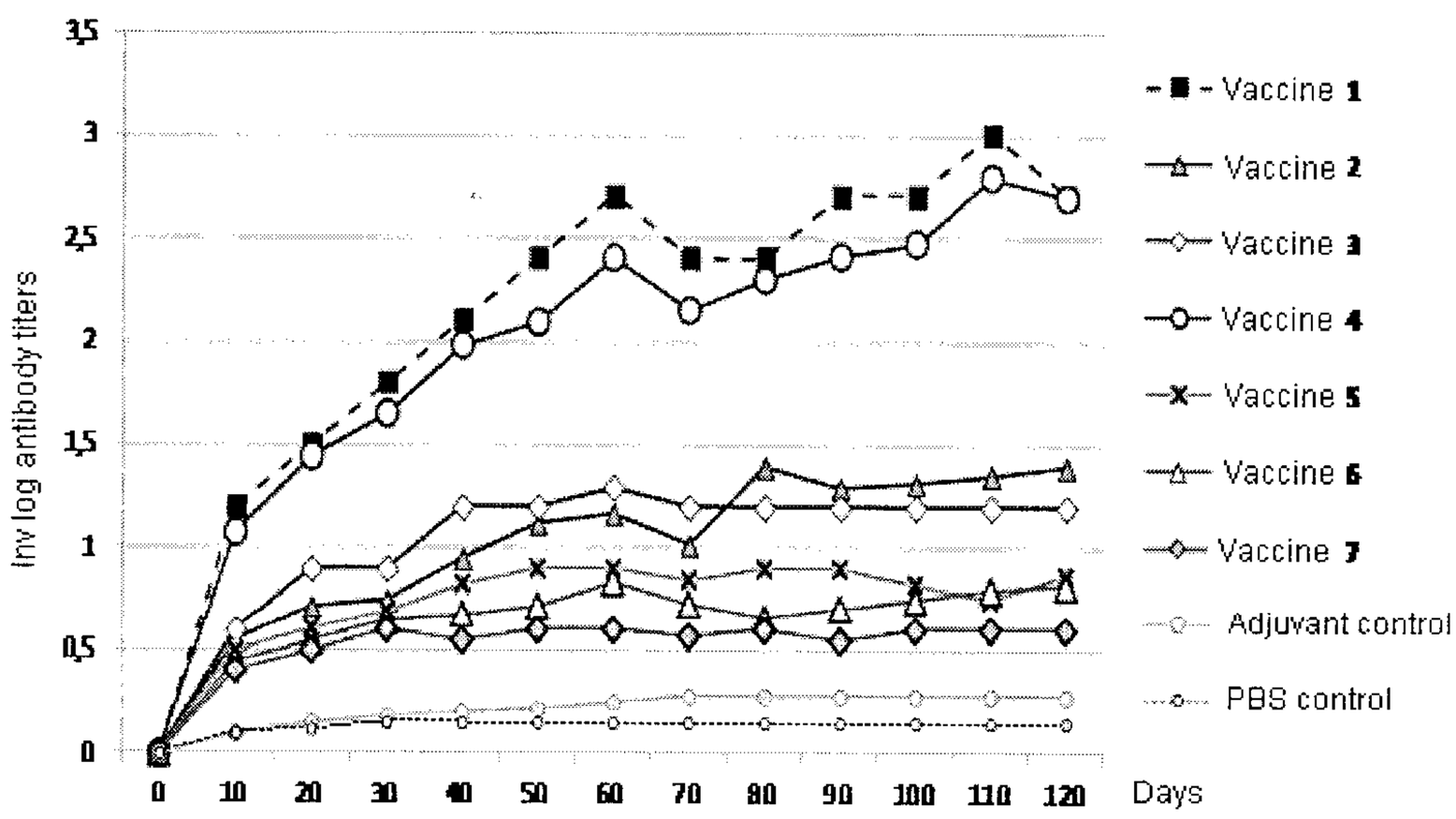

Figure 9

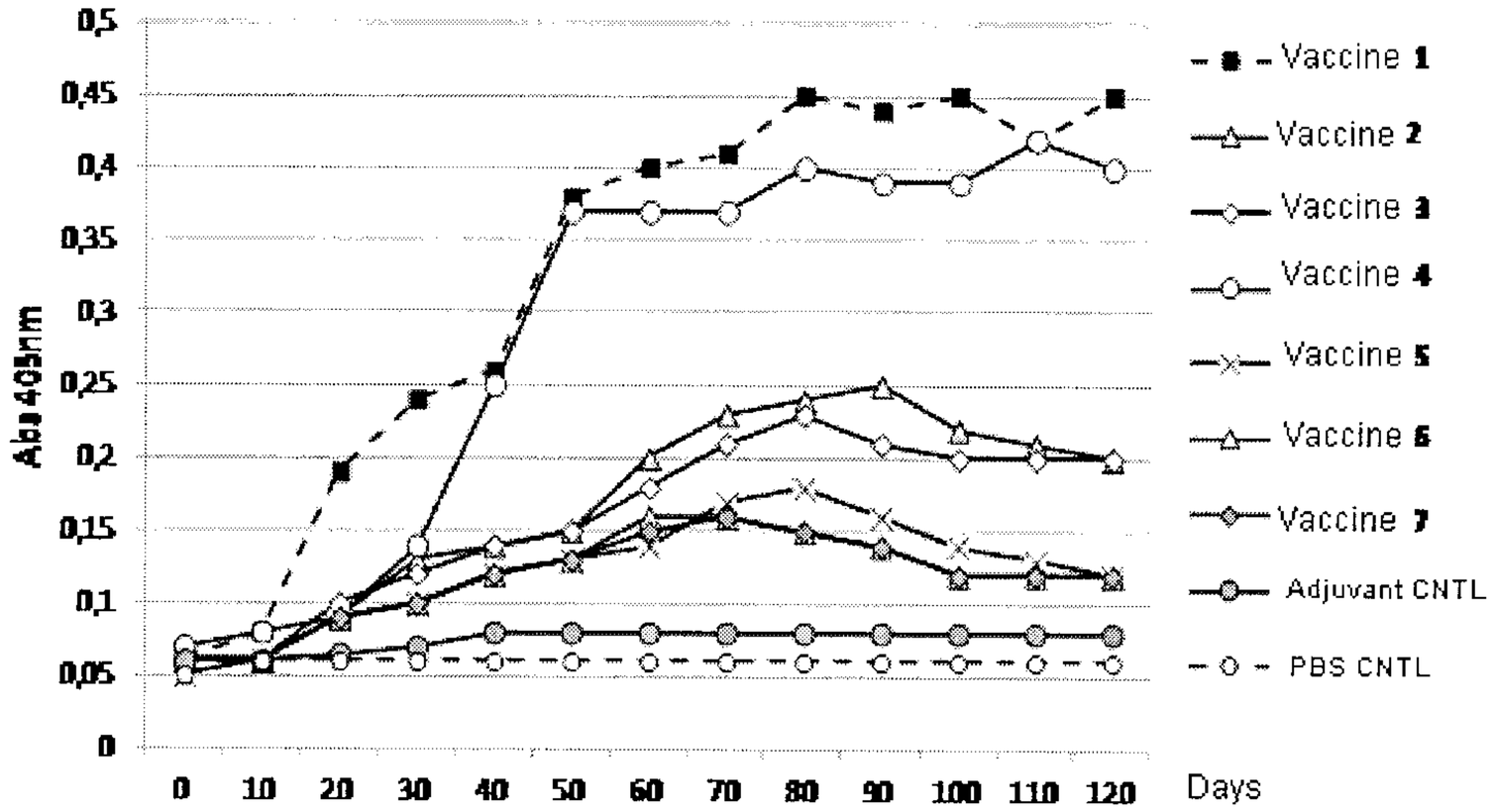

Figure 10

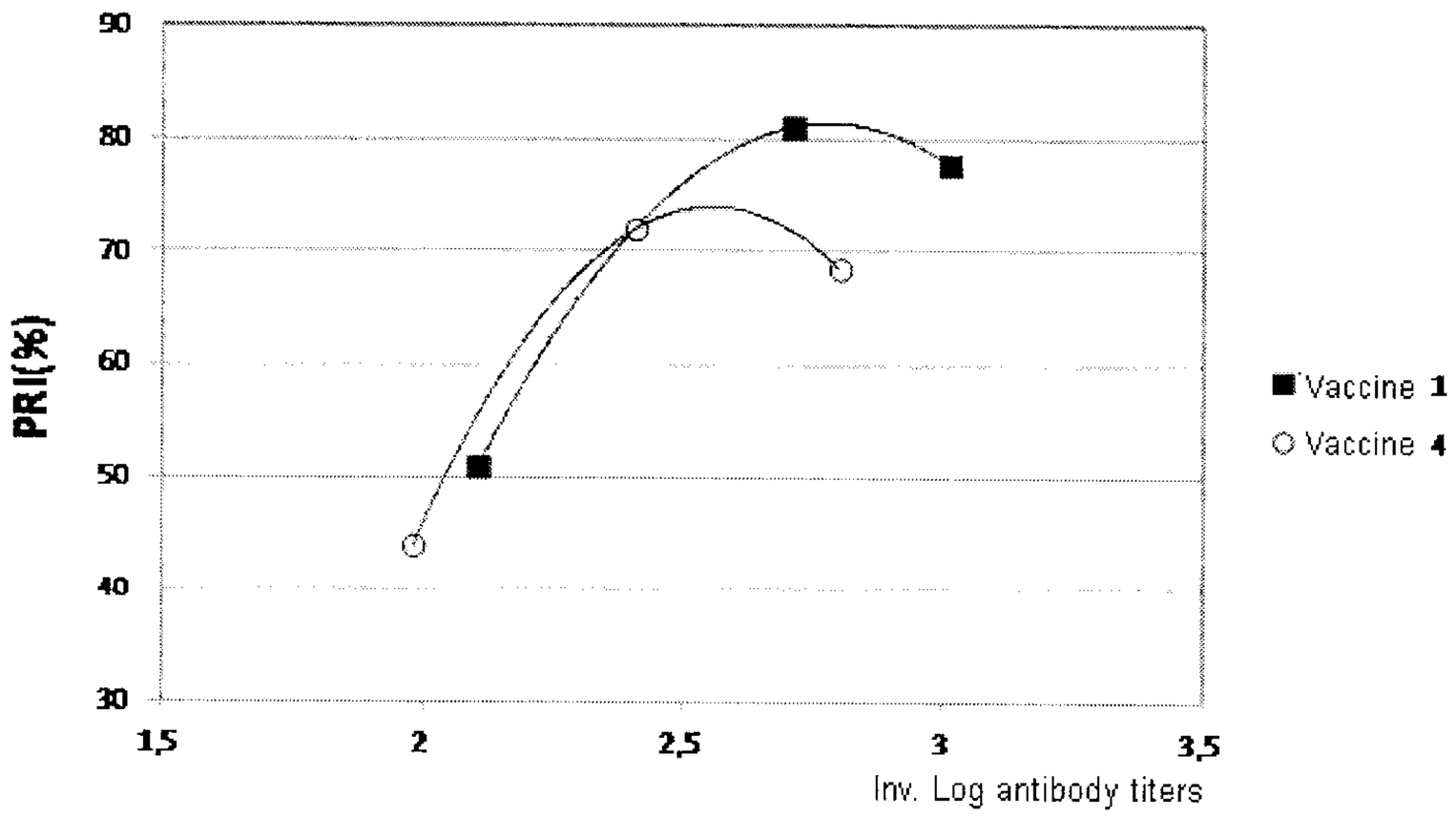

Figure 11

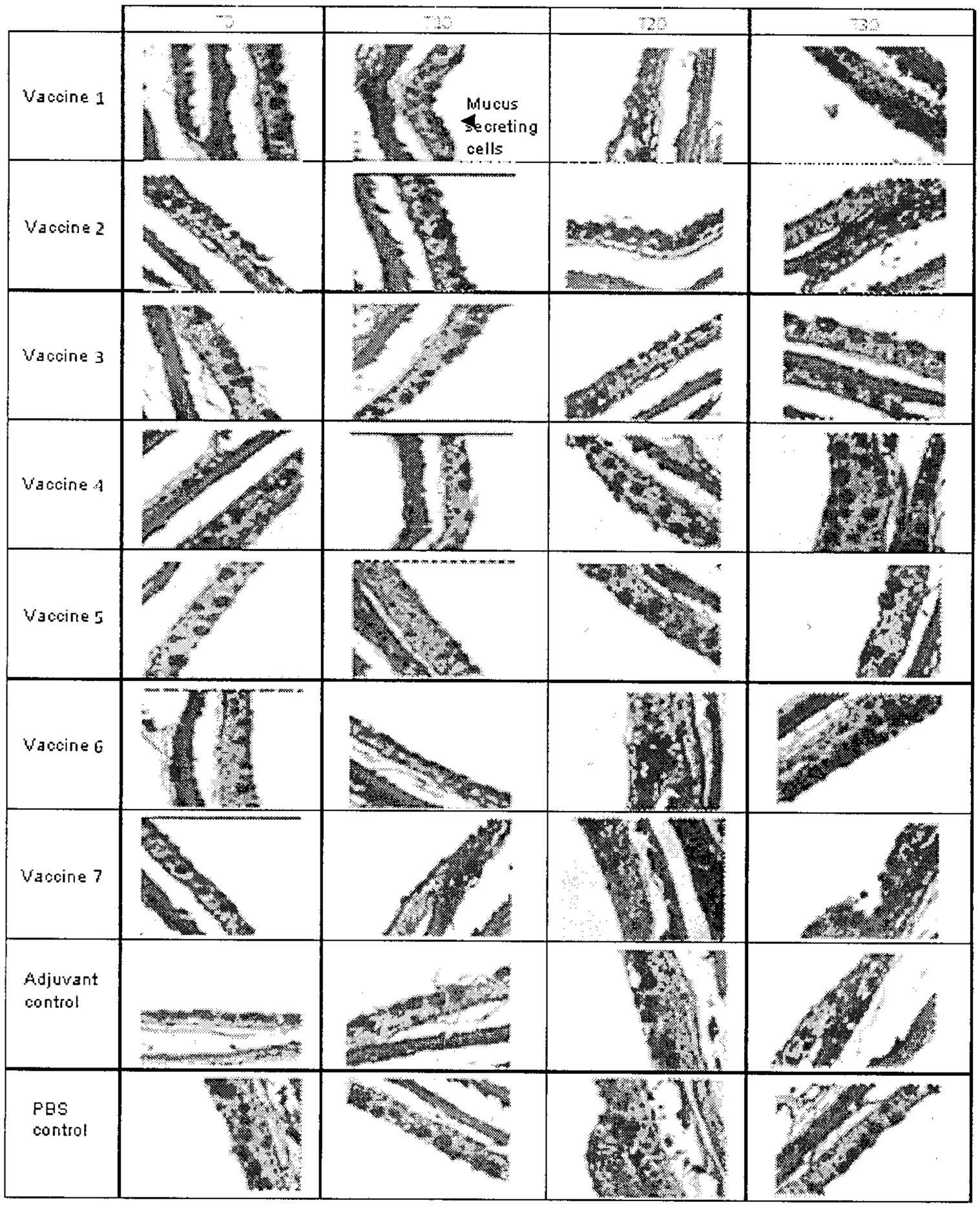
Day 5
Day 10
Day 20
Day 30
Vaccine 1
Mucus secreting cells
Vaccine 2
Vaccine 3
Vaccine 4
Vaccine 5
Vaccine 6
Vaccine 7
Adjuvant control
PBS control

Figure 12

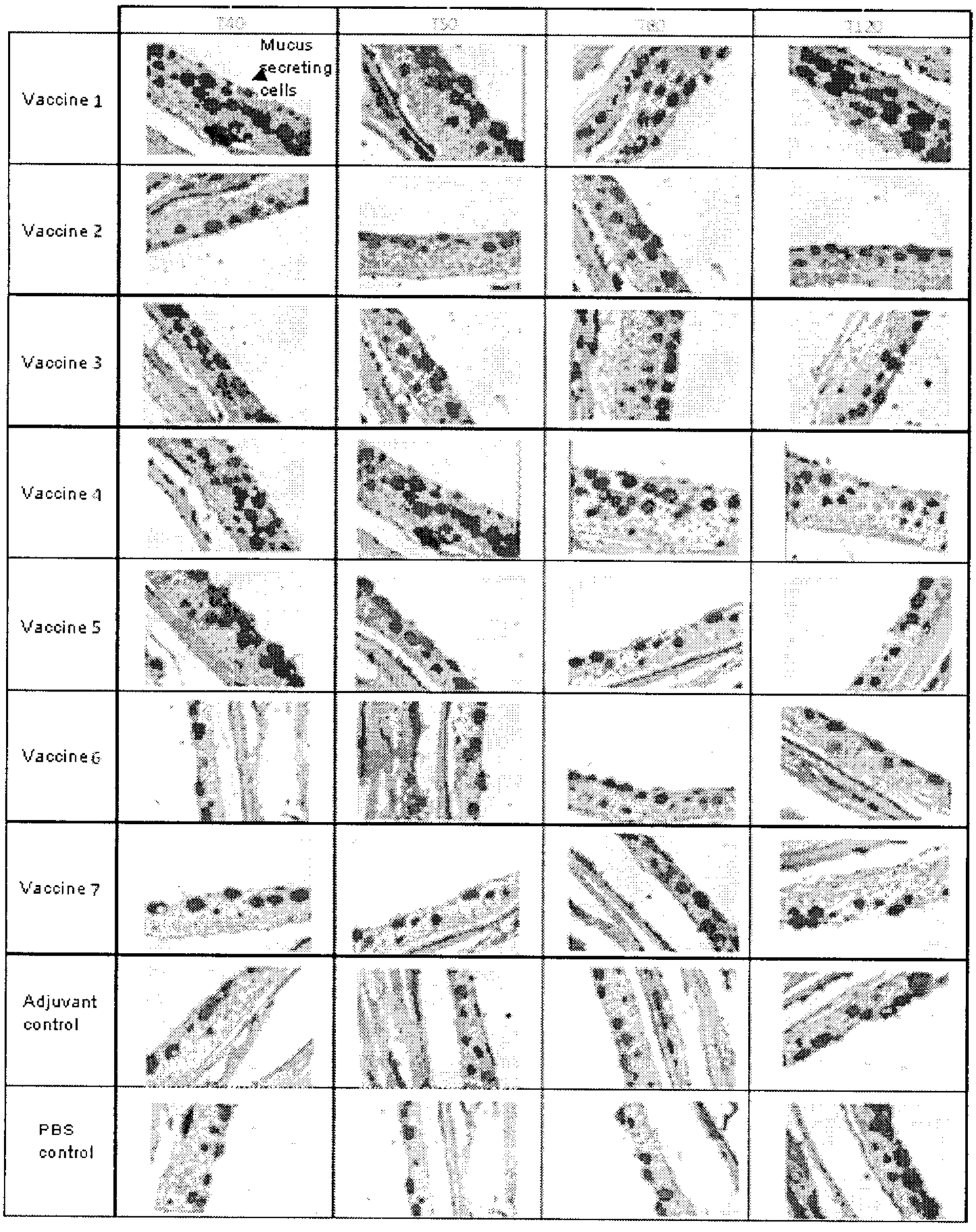
T40
T90
T80
T120
Mucus secreting cells
Vaccine 1
Vaccine 2
Vaccine 3
Vaccine 4
Vaccine 5
Vaccine 6
Vaccine 7
Adjuvant control
PBS control

Figure 13

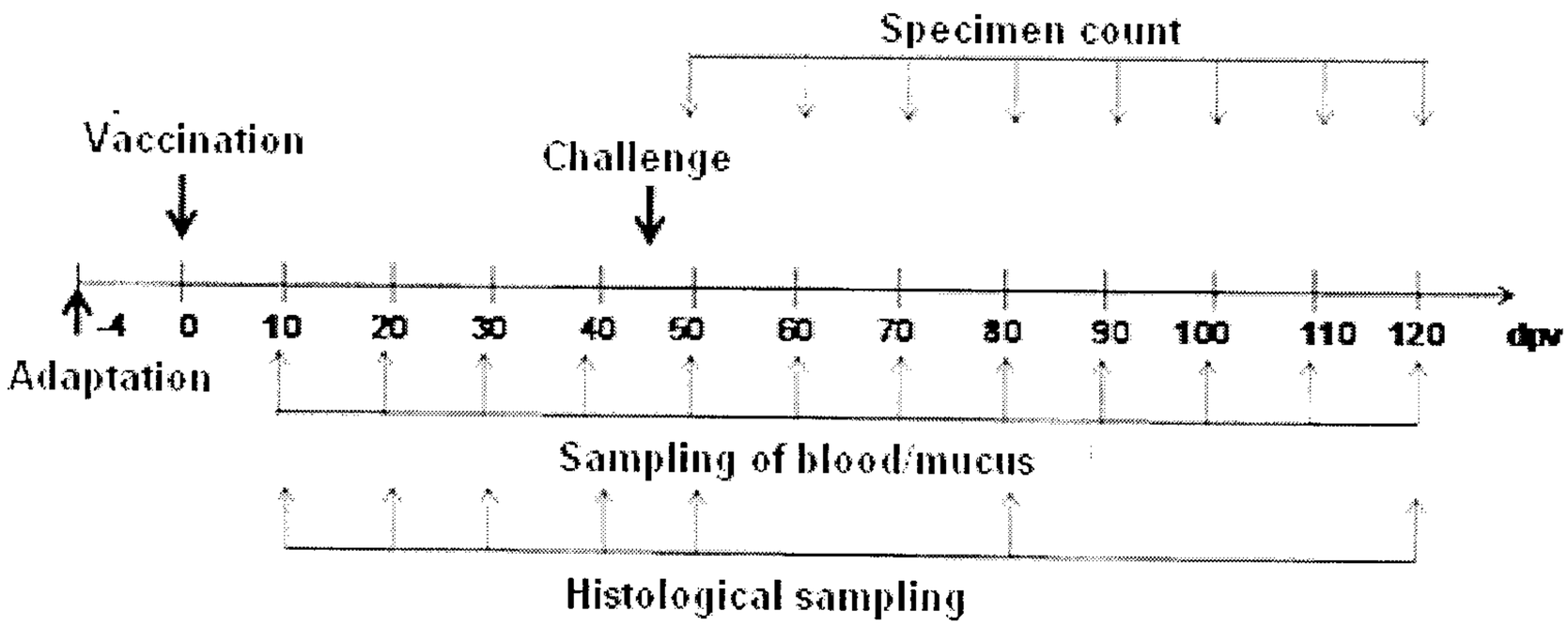
Specimen count
Vaccination
Challenge
-4
0
10
20
30
40
50
60
70
80
90
100
110
120
dpv
Adaptation
Sampling of blood/mucus
Histological sampling

Figure 14