current *Eimeria*-based vaccines produces substantial vaccine-reaction morbidity and economic losses in vaccinated flocks. Thus an effective low-virulence vaccine against *Eimeria* is needed. An effective vaccine for *Eimeria* based on conserved immunogenic targets may also prove useful as a vaccine against other apicomplexan parasites.

SUMMARY

A vaccine vector comprising a first polynucleotide sequence encoding an Apicomplexan Rhomboid polypeptide and methods of using the same are provided herein.

In one aspect, there is provided a vaccine vector comprising a first polynucleotide sequence encoding an Apicomplexan Rhomboid polypeptide optionally expressed on the surface of the vaccine vector, wherein the Rhomboid polypeptide consists of a polypeptide having greater than 90% sequence identity to a polypeptide selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 37, SEQ ID NO: 38, an immunogenic fragment of SEQ ID NO: 1, an immunogenic fragment of SEQ ID NO: 2, an immunogenic fragment of SEQ ID NO: 3, an immunogenic fragment of SEQ ID NO: 4, an immunogenic fragment of SEQ ID NO: 37, an immunogenic fragment of SEQ ID NO: 38 and combinations thereof.

In one aspect, there is provided a pharmaceutical composition comprising the vaccine vector of as disclosed herein and a pharmaceutically acceptable carrier.

In one aspect, there is provided a use of the vaccine vector as disclosed herein or the pharmaceutical composition as disclosed herein in the manufacture of a medicament for enhancing the immune response of a subject against an Apicomplexan parasite.

In one aspect, there is provided a use of the vaccine vector of as disclosed herein or the pharmaceutical composition as disclosed herein in the manufacture of a medicament for reducing morbidity associated with infection with an Apicomplexan parasite in a subject as compared to a control subject not administered the vaccine vector.

In one ~~aspect~~embodiment, a vaccine vector comprising a first polynucleotide encoding an Apicomlexan Rhomboid polypeptide or an immunogenic fragment thereof and a second polypeptide sequence encoding an immunostimulatory polypeptide is disclosed. The Apicomplexan Rhomboid polypeptide and the immunostimulatory polypeptide are suitably expressed on the surface of the vaccine vector. The Apicomplexan Rhomboid polypeptide may comprise SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 37, SEQ ID NO: 38, an immunogenic fragment of at least one of SEQ ID NO: 1-4, 37-38 or

combinations of SEQ ID NO: 1-4 and 37-38. The immunostimulatory polypeptide may be a CD154 polypeptide capable of binding CD40 or an HMGB1 polypeptide. The CD154 polypeptides include fewer than 50 amino acids and comprise amino acids 140-149 of CD154 or a homolog thereof.

In another embodiment~~aspect~~, a vaccine vector comprising a first polynucleotide encoding an Apicomlexan Rhomboid polypeptide of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 37, SEQ ID NO: 38, an immunogenic fragment of at least one of SEQ ID NO: 1-4 or 37-38 or combinations of SEQ ID NO: 1-4 or 37-38. The Apicomplexan Rhomboid polypeptide may be expressed on the surface of the vaccine vector.

Vaccine vectors according to the present ~~invention~~ disclosure may be a virus, yeast, bacterium, or liposome vector. Pharmaceutical compositions may be comprised of the vaccine vectors described herein and a pharmaceutically acceptable carrier.

In still another embodiment~~aspect~~, methods of enhancing the immune response against an Apicomplexan parasite in a subject by administering a vaccine vector described herein to the subject are provided. The enhanced immune response may be an enhanced antibody response, an enhanced T cell response or a combination thereof.

In a still further embodiment~~aspect~~, methods of reducing morbidity and mortality associated with infection with an apicomplexan parasite in a subject by administering a vaccine vector as

CLAIMS

We claim:

1. A vaccine vector comprising a first polynucleotide sequence encoding an Apicomplexan Rhomboid polypeptide optionally expressed on the surface of the vaccine vector, wherein the Rhomboid polypeptide consists of a polypeptide~~is or~~ having~~s~~ greater than 90% sequence identity to a polypeptide selected from the group consisting~~at least one~~ of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 37, SEQ ID NO: 38, an immunogenic fragment of SEQ ID NO: 1, an immunogenic fragment of SEQ ID NO: 2, an immunogenic fragment of SEQ ID NO: 3, an immunogenic fragment of SEQ ID NO: 4, an immunogenic fragment of SEQ ID NO: 37, an immunogenic fragment of SEQ ID NO: 38 and~~or~~ combinations thereof.

2. The vaccine vector of claim 1 further comprising a second polynucleotide sequence encoding an immunostimulatory polypeptide, wherein the immunostimulatory polypeptide is expressed on the surface of the vaccine vector.

3. The vaccine vector of claim 2, wherein the immunostimulatory polypeptide comprises~~is~~ an HMGB1 polypeptide.

4. The vaccine vector of claim 3, wherein the HMGB1 polypeptide comprises a polypeptide selected from the group consisting~~at least one~~ of SEQ ID NOs: 15-23, a fragment of at least one of SEQ ID NOs: 15-23, a polypeptide having at least 95% sequence identity to SEQ ID NOs: 15-23 and combinations thereof.

5. The vaccine vector of any one of claims 2-4, wherein the immunostimulatory polypeptide comprises~~is~~ a CD154 polypeptide capable of binding CD40, the CD154 polypeptide having fewer than 50 amino acids and comprising amino acids 140-149 of a polypeptide selected from the group consisting of SEQ ID NO: 24, SEQ ID NO: 25 and~~or~~ a homolog thereof, or is a polypeptide selected from the group consisting of SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30 and polypeptides having at least 90% sequence identity to at least one of SEQ ID NOs: 26-30.

~~6. The vaccine vector of claim 5, wherein the CD154 polypeptide is SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30 or has at least 90% sequence identity to at least one of SEQ ID NO: 26-30.~~

67. The vaccine vector of any one of claims 21-56, wherein the vector comprises more than one copy of the first polynucleotide and/or more than one copy of the second polynucleotide sequence.

78. The vaccine vector of any one of claims 2-67, wherein the first polynucleotide sequence is linked in the same reading frame to the second polynucleotide sequence.

89. The vaccine vector of claim 78, wherein the first polynucleotide and the second polynucleotide are linked via a spacer nucleotide sequence.

910. The vaccine vector of any one of claims 1-89, wherein the vaccine vector is selected from the group consisting of a virus, a bacterium, a yeast and a liposome.

101. The vaccine vector of claim 910, wherein the vaccine vector is a *Bacillus* spp.

112. The vaccine vector of any one of claims 1-101, further comprising a third polynucleotide encoding a TRAP polypeptide.

123. The vaccine vector of claim 112, wherein the TRAP polypeptide is selected from the group consisting of polypeptides having at least 95% sequence identityeal to SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 40, an immunogenic fragment of SEQ ID NO: 5, an immunogenic fragment of SEQ ID NO: 6, an immunogenic fragment of SEQ ID NO: 7 andor an immunogenic fragment of SEQ ID NO: 40.

134. The vaccine vector of claim 21, wherein the first polynucleotide and the second polynucleotide encode a polypeptide selected from the group consistingvaccine vector comprises the polypeptide of SEQ ID NO: 32, SEQ ID NO: 34 andor a polypeptide having 95% sequence identity to SEQ ID NO: 32 or SEQ ID NO: 34.

145. A pharmaceutical composition comprising the vaccine vector of any one of claims 1-134 and a pharmaceutically acceptable carrier.

156. A method of enhancing the immune response against an apicomplexan parasite in a subject comprising administering to the subject A use of the vaccine vector of any one of claims 1-134 or the pharmaceutical composition of claim 145 in the manufacture of a medicament for enhancing the immune response of a subject against an Apicomplexan parasite in an amount effective to enhance the immune response of the subject to the apicomplexan parasite.

167. The usemethod of claim 156, wherein the enhanced immune response comprises an enhanced antibody response, an enhanced T cell response or both.

17~~8~~. ~~A method of reducing morbidity associated with infection with an apicomplexan parasite in a subject comprising administering to the subject~~ A use of the vaccine vector of any one of claims 1-13~~4~~ or the pharmaceutical composition of claim 14~~5~~ in the manufacture of a medicament for reducing morbidity associated with infection with an Apicomplexan parasite in a subject ~~in an amount effective to reduce the morbidity associated with subsequent infection of the subject with an apicomplexan parasite~~ as compared to a control subject not administered the vaccine vector.

18~~9~~. The use~~method~~ of any one of claims 15-17~~16-18~~, wherein the vaccine vector is administered by a route selected from the group consisting of oral, mucosal, parenteral, subcutaneous, intramuscular, intraocular and *in ovo*.

19~~20~~. The use~~method~~ of any one of claims 15-18~~16-19~~, wherein the subject is ~~member of~~ a poultry species or a mammal.

~~21. The method of claim 18, wherein the poultry species is a chicken or turkey.~~

~~22. The method of any one of claims 16-21, wherein the subject is a mammal.~~

20~~3~~. The use~~method~~ of claim 19~~22~~, wherein the subject is selected from the group consisting of a human, swine, chicken, turkey and~~or~~ cow.

21~~4~~. The use~~method~~ of any one of claims 15-20~~16-23~~, wherein ~~from~~ about 10^4 to about 10^9 vector copies of the vaccine are administered to the subject.

~~25. The method of any one of claims 16-23, wherein from about 10^5 to about 10^7 vector copies of the vaccine are administered to the subject.~~

22~~6~~. The use~~method~~ of any one of claims 15-21~~16-25~~, wherein the vaccine vector is killed prior to administration to the subject or is not capable of replicating in the subject.

23~~7~~. The use~~method~~ of any one of claims 15-22~~16-26~~, wherein the Aapicomplexan parasite is selected from the group consisting of *Eimeria, Plasmodium, Toxoplasma*, *Neospora* and *Cryptosporidium*.