post challenge. At the time of necropsy, gross pathology and the percentage of lung pathology present in each lung lobe was recorded by the attending veterinarian. Samples of lung consisting of tissue from the margin of the lesion and good tissue were collected. Two samples were collected per calf and stored on ice until the time of delivery to the lab for testing. An additional sample from each calf was fixed in 10% formalin for histopathological examination.

Bacterial Recovery

[0157] Tissue was seared using a propane torch and then aseptically cut to expose an interior surface. A dry swab was inserted into the tissue and rotated to sample. The swab was dipped in to 5 ml of BHI broth supplemented with tris-buffer and thiamine monophosphate as suggested by Asmussen and Baugh (1981) . Broth tubes were incubated for 24-72 hours at 37°C w/ 130rpm shaking. The second set of tissue was stored at -70°C. Following incubation, broth cultures were scored as positive or negative for growth based on turbidity in comparison to positive and negative control tubes.

PCR

[0158] To verify the presence of *H. somni* within the cultures from infected lungs, a 50uL sample was taken from each broth culture and subjected to DNA extraction (Qiagen DNA kit) and tested for presence of *H. somni* DNA by q-PCR. The primers utilized were *H. somni* species specific for sequences within the 16S rDNA - Forward Primer (5'-GAAGGCGATTAGTTTAAGAG-3') (SEQ ID NO: 1) and Reverse Primer (5'-TTCGGGCACC AAGTRTTCA-3') (SEQ ID NO: 2) (Angen and others 1998). PCR was carried out in a 96-well format in 25uL volumes using a CFX-96 Thermocycler (Bio-Rad) with Bio-Rad Master Mix, 0.4ug/uL Bovine Serum Albumin (BSA), and a set of in-process control primers to verify the validity of the reaction in each well. A standard curve of *H. somni* DNA was run on each plate. An initial activation step at 95.0°C for five minutes was performed, followed by forty-five cycles of denaturing at 95.0°C for fifteen seconds and annealing/elongation step at 60.0°C for thirty seconds with a real-time capture of probe fluorescence at the end of each cycle. Data was captured and analyzed using the Bio-Rad CFX software. Samples were run in duplicate and mean threshold cycle (C_t) was compared to the standard to determine whether each sample was positive or negative. In the event that a sample had one positive and one negative well, the sample was re-run in triplicate with final

SDS Extracted OMPs from the LgD1-TN08 isolate has correlated to induction of IgG response to this protein.

[0265] The 38kDa protein was found to have high N-Terminal homology to the porin protein ompA which has been demonstrated to be highly conserved in many members of the *Pasteurellaceae* family. Again, efficacy of vaccines consisting of SDS Extracted OMPs from the LgD1-TN08 isolate has correlated with induction of IgG response to this protein.

[0266] The 23kDa protein was most closely linked to Hs_0779, a hypothetical protein characterized only by genomics data from *H. somni* isolate 129Pt. The function of this protein has not previously been described. IgG response to this protein has been observed from animals vaccinated with SDS Extracted OMPs from the LgD1-TN08 Isolate, however this response has occurred in all vaccinates regardless of vaccine efficacy.

[0267] The BLAST results are provided as follows:

Sample: **Hs_LGD1_061812-1**
Comments: ~41kDa immunoreactive OMP observed to correlate to efficacy in proof-of-concept OMP Vaccine studies.

Sequence: E L M I V V A I I G I L A G I A I P Q Y Q L G (SEQ ID NO: 3)
BLAST Results
E-value = $4e^{-11}$: **fimbral subunit** (fimA) of *H. somni* 2336 and 129Pt
fimA suspected to be part of Pilus components or secretion mechanism
Homologous to pilA displayed by *H. ducreyi* and *H. influenza* and many others

Sample: **Hs_LGD1_061812-2**
Comments: ~38kDa immunoreactive OMP observed to correlate to efficacy in proof-of-concept OMP Vaccine studies.

Sequence: A P Q A N T F Y A G A ? L (SEQ ID NO: 4)
BLAST Results
E-value = $1e^{-6}$: **ompA**
Highly conserved in *Pasteurellaceae*, Porin acting as non-specific channel for small hydrophilic molecules

Sample: **Hs_LGD1_061812-3**
Comments: ~23kDa immunoreactive OMP observed in all vaccinate animals regardless of efficacy
Sequence: S I N I A P Q I T E I L A I N G L ? Q ? (SEQ ID NO: 5)
BLAST Results
E-value = 0.19: **Hypothetical Protein Hs_0779 of H. somni 129Pt**

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<120> OUTER MEMBRANE PROTEINS OF HISTOPHILUS SOMNI AND METHODS THEREOF

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