A Recombinant Subunit Vaccine for Bovine RSV and *Histophilus somni* Protects
Calves against Dual Pathogen Challenge

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Abstract

Bovine respiratory syncytial virus (BRSV) and *Histophilus somni* synergize to cause respiratory disease in cattle. These pathogens cause enhanced disease during dual - infection and an IgE response to antigens of *H. somni* in dual-infected but not singly infected calves. Vaccines containing whole inactivated BRSV or *H. somni* have been associated with IgE responses. A vaccine strategy that avoids stimulation of IgE antibodies would provide superior protection from dual infection. We hypothesized that a subunit vaccine consisting of the nucleoprotein (NP) from BRSV and the recombinant antigen IbpA DR2 (a surface antigen of *H. somni* with two toxic fimbria motifs) in Quil A adjuvant would elicit protection without disease enhancement. Three groups of calves were vaccinated twice with either: Formalin inactivated BRSV (FI) plus Somnivac®, NP & IbpA DR2 plus Quil A or Quil A alone, followed by BRSV and *H. somni* challenge. Clinical scores and antibody levels (to whole pathogens and to the subunits) were evaluated. Lungs were examined at necropsy on day 23 after infection. Clinical scores were significantly greatest for the FI & Somnivac® group and both clinical scores and lung pathology were lowest for the subunit group. All calves shed BRSV in nasal secretions. FI & Somnivac® induced IgE antibodies to *H. somni* and BRSV, but not to NP or DR2. The subunit vaccine did not induce an IgE antibody response to IbpA DR2 antigen and induced little IgE to *H. somni*. It did not induce an IgG antibody response to BRSV and *H. somni*, but stimulated production of IgG antibodies against the subunits. In summary, the subunit vaccine, consisting of the BRSV NP and *H. somni* IbpA DR2 in Quil A,

protected against severe clinical signs and decreased lung pathology but did not prevent viral shedding. Importantly it prevented synergistic disease expression in response to dual infection.

Keywords: BRSV, *Histophilus somni*, nucleoprotein, subunit, vaccine, IgE

Introduction

The interaction of bovine respiratory syncytial virus and *Histophilus somni* in the respiratory tract of calves causes enhanced disease compared to infection with either pathogen alone [1]. Both pathogens are among those that cause bovine respiratory disease complex, a common cause of economic loss to the cattle industry despite availability of vaccines for BRSV and *H. somni* individually and in multiple pathogen combinations. [2]. We identified an IgE response to *H. somni* in calves that were unvaccinated and dually infected with BRSV and *H. somni* ; this response was associated with prolonged clinical signs and persistence of *H. somni* in the lung [3]. An association between vaccination and disease exacerbation has been observed for formalin inactivated BRSV and RSV vaccines [4,5]. For both BRSV and *H. somni* an IgE response to vaccination has been documented [6,7]. IgE antibodies are typically associated with allergic and anti-parasite reactivity not with protection from viral and bacterial pathogens; therefore it is not generally considered desirable for viral and bacterial vaccines to elicit IgE. We previously showed that the recombinant immunoglobulin binding protein from *H. somni* (IbpA) DR2 subunit induced little IgE response and protected calves against experimental *H. somni* pneumonia [8]. The nucleoprotein (NP) from BRSV has been associated with stimulating a cell mediated anti-viral response and protection of the lung [9]. Therefore, we hypothesized that a subunit vaccine consisting of IbpA DR2, and the NP of BRSV would induce protection against both pathogens and prevent enhanced disease from dual infection. The objective of the study was to develop the subunit vaccine

and to test it in dual experimentally infected calves for protection from clinical signs, pathological lesions, and control of viral shedding. We compared subunit vaccinated calves with calves that received a formalin inactivated BRSV vaccine plus a commercial *H. somni* bacterin, and with adjuvant controls. A second objective was to examine the immunoglobulin isotypes in serum and bronchoalveolar lavage fluid (BALF) against both whole organisms and subunits elicited by the vaccine and subsequent infection.. We were interested in both the correlation of IgG antibodies with protection and the IgE antibodies contributing to pathology.

Materials and Methods

Animals:

Eighteen 6-7 week old Holstein bull calves were obtained from a dairy and transported to the University of California, Davis, where they were housed in an enclosed barn. An approved IACUC protocol was followed. Calves were fed milk replacer, calf starter, and hay twice daily and ad libitum water. Prior to shipment calves were tested for BRSV infection by qRT-PCR on nasal swabs and BRSV antibody titers by indirect immunofluorescence. Only calves with low maternal BRSV titers and negative for BRSV were used. Upon arrival each calf received a 3 consecutive daily injections of the antibiotic Naxcel® to prevent respiratory disease associated with shipping stress.

Calves were divided randomly into 3 vaccine groups of 6 animals each.

One group received BRSV NP purified from yeast and *H. somni* lbpA DR2 protein purified from *E. coli*, in Quil A adjuvant. A second group received formalin inactivated (FI) BRSV in alum adjuvant and Somnivac® bacterin. The third group received Quil A adjuvant as control. Infection was spread over three days with two animals from each vaccine group infected on each day

Vaccine Preparation:

BRSV NP was commercially produced (My Bioscience Inc.) by expression of the nucleoprotein gene in yeast, followed by purification. The lbpA DR2 was expressed in *E. coli* and purified as previously described [10]. For the subunit vaccine, 200 ug of NP was mixed with 200ug lbpA DR2 and 100ug Quil A (vaccine 1) or 1mg (vaccine 2) of Quil A (BRENNTAG, Denmark) was added to constitute a 2ml dose. The FI BRSV vaccine was prepared as previously described using BRSV strain (CA-1) grown on BT cells [3]. The *H. somni* bacterin was purchased commercially (Sominovac®). The adjuvant control consisted of 100ug of Quil A for dose 1 and 1mg for dose 2 in a volume of 2 ml.

Experimental Protocol:

Calves were vaccinated twice subcutaneously on the lateral neck with a three week interval between doses. Challenge infection was performed two weeks after the final vaccine. BRSV was grown on bovine turbinate cells as previously described [1]. On day 0 the calves received 3.82×10^6 , 1.75×10^6 or 9.99×10^5 TCID₅₀ (for first,

second, and third infection day) of BRSV (CA-1) in 5 ml of tissue culture medium; the virus was aerosolized via Devilbis air compressor/nebulizer and face mask.

On day 6 each calf received viable *H. somni* (previously calf passaged (vial #5166 of strain 2336). The culture of *H. somni* was grown as previously described [10]. Bacterial numbers were confirmed by plate counts of the inoculum and a final dilution in RPMI1640 was made so as to have approximately 10^8 bacteria in the 10 ml inoculum. The inoculum was instilled into the mid-trachea using a foal sized stomach tube with an internal polyethylene tube attached to a 3-way stopcock as previously described. [1]. The *H. somni* inoculum was 0.8×10^8 , 1.8×10^8 , and 1.78×10^8 CFU in 10 ml (for first, second, and third infection day).

Sampling:

Before each vaccine and on days 0, 6, 10, 14, 21 and 23, blood was collected for serum. Bronchoalveolar lavage was performed at pre-infection (day -3), and on days 14 and 23 as previously described [1]. BALF was stored at -20°C until assayed. Deep nasal swabs were collected daily from day 0 through day 14 and on days 21 and 23 as previously described [11]. Nasal epithelial cells were harvested from these nasal swabs and pelleted by centrifugation at $500 \times g$ for 10 min and frozen -80°C until conversion to cDNA and performance of qRT-PCR for detection and quantitation of shed virus.

Monitoring of Clinical Signs:

The clinical scoring system for respiratory disease described by Collie and modified by us was used [3] to create a quantitative assessment of health status. A veterinarian who was blinded to the identity of the vaccine group performed daily examinations. This scoring system assigns points for temperature, depression, cough, induced cough, respiratory rate, respiratory character, nasal and ocular discharge, and other features of health status. Each calf was assigned a daily score and mean daily scores were tabulated for each group.

Quantitative RT-PCR for BRSV:

Quantitative RT-PCR was performed on RNA from cell pellets that were obtained from daily nasal swabs as previously described [11].

Pathology:

Calves were euthanized on day 23 with an intravenous overdose of pentobarbital after mild sedation with Rompun®. The respiratory tract was removed, gross pathology was examined, and percent of consolidated lung surface area was estimated. The left lung was separated from the upper respiratory tract at the bronchial bifurcation and was immediately lavaged with three 60 ml aliquots of Hank's balanced salt solution. The BALF was placed immediately on ice and the lung was cannulated for infusion with 10% neutral buffered formalin solution at 15cm pressure for approximately 18 hours. After infusion samples were taken from each lung lobe according to a pre-determined site map and lesions were also sampled. Similar sections were taken from the un-infused right lung, as well as sections of

trachea and associated lymph nodes. Bacterial cultures were taken from the respiratory tract during necropsy, for isolation of *H. somni* and any other bacteria present. Histopathology was evaluated and described after sectioning and staining with Hematoxylin & Eosin. To develop a quantitative assessment of lung histopathology twelve predetermined sites in the lung of each animal were evaluated and rated 0 -3 for the presence and magnitude of specific pathological features. A detailed list of the histopathological lesions that were used to calculate histopathological scores is contained in Table One. The compartments scored were: septae, pleura, alveolus, interstitium, trachea, and bronchial lymph nodes. For each of these cellular accumulations were classified by type of cell. These lesion scores were summed for each animal and each lung compartment. Mean lesion scores were determined and statistical analysis was performed to determine significant differences between groups.

ELISA for Antibody Responses:

ELISA was performed on serum and BALF for whole virus BRSV antigen, for NP antigen, for whole bacteria *H. somni* antigen, and for the subunit IbpA DR2 antigen. For BRSV ELISA both whole purified BRSV antigen and recombinant NP were used in separate assays to evaluate IgG1, IgG2, IgE, and in BALF also IgA. These assays were performed as previously reported by us with some modification [12]. For the NP antigen the concentration was 100 ng/well for IgG1 and IgG2 and 250 ng/well for IgE. For BALF assays the concentration of BRSV used was 1 ug/well (all isotypes) and for NP it was 0.1 ug/well (all isotypes).

H. somni specific IgG1, IgG2, IgE, and IgA were measured by ELISA. For antibody responses to *H. somni* and to IbpA DR2 our previous reported protocols were followed [3,8,12]. For all IgE assays the anti-bovine epsilon chain monoclonal antibody E5 initially produced by us [13] was used. Results of BRSV and NP antibody ELISA on serum were reported as absorbance values. Antibody in lung lavage fluid was reported as units per mg of protein. Because there may sometime be unequal recovery of fluid from lung lavage and therefore differences in dilution of respiratory secretions, protein concentration was measured in each lavage sample and the absorbance from ELISA was multiplied by 100 and then divided by the mg of protein in the sample to correct for differences in dilution.

Statistical Analyses:

The InStat program was used to perform nonparametric Kruskal-Wallis testing on the clinical scores. Prism® was used to calculate ANOVA with Bonferroni testing to determine effects of day and treatment group for all of the serum and BALF ELISA data. Histological lesion scores were compared using the non-parametric Mann-Whitney test (InStat).

BRSV Serum Neutralization Assays:

Sera were sent to the University of Colorado, Veterinary Diagnostic Laboratory (Ft. Collins, CO) where BRSV virus neutralization titers were performed using standard procedures

Results

Clinical Signs:

Mean clinical signs for the FI & Somnivac group were very significantly greater than the NP & IbpA DR2 group ($p < 0.0001$), and greater than the control group ($p = 0.0024$) during the first eight days of the infection (Figure 1). The clinical signs during the first six days after viral infection reflect the effects of the BRSV; after day six both *H. somni* and BRSV contributed to the clinical signs. This data confirms the vaccine enhancement effect of the FI vaccine. One calf in the FI & Somnivac® group was extremely sick with open mouth breathing and inability to stand and eat; he was euthanized on day 10 of the experiment. Clinical signs during days 9 through 23 remained elevated and not significantly different from each other, for the control and the FI & Somnivac® groups (figure 1). Importantly, mean clinical signs for the NP and IbpA DR2 vaccine group remained significantly lower than the other groups, demonstrating a protective response.

Viral shedding:

BRSV shedding was evaluated daily from nasal swabs. On day 0 prior to infection no virus was detected. Viral shedding was greatest between days 3 and 10. All vaccine groups shed virus. There was no significant difference between groups in duration or magnitude of virus shedding.

Pathology:

Adjuvant controls had a significantly greater percentage of lung consolidation than the vaccine groups. Overall consolidation in the vaccinated calf lungs was minimal, (figure 2A). The subunit vaccine group showed the least amount of consolidation. Examination and quantitation of lung histopathology revealed a very significant difference in overall lung histopathology between the controls and the subunit vaccine group (figure 2B). Three of the specific anatomic areas examined and rated showed a significant difference between the groups. Lung areas most differentially affected include the bronchiolar, alveolar, and septal compartments of the lung (figure 2C,D,E). In contrast to the subunit vaccine group the FI & Somnivac® group did not show a significant difference from the control group, consistent with the lack of protection by this vaccine. These compartments showed increases in cellular infiltrates in both the control and FI/Somnivac calves, indicating the presence of inflammation in these dually infected calves on day 23 after viral infection. It should be noted that exacerbated lung pathology induced by FI BRSV vaccine has previously been reported at day 7 and day 10 after BRSV infection. By day 23 in this dual pathogen infection the pathological lesions present represent the residual effect of the virus together with the pathology induced by the concurrent *H. somni* infection.

Bacterial Isolation:

Aerobic and microaerophilic culture of all airways and related lung structures was performed only on necropsy samples with the following results: *H. somni* was isolated from the lung and/or bronchial swab of four out of five control calves, two

out of six NP & IbpA DR2 calves, and none of six FI & Somnivac® calves. The calf that was euthanized on day 10 had *Pasteurella multocida* isolated from the lung. These cultures were taken after the clinical disease had subsided, so are not as relevant as cultures taken throughout the infections would have been (as for BRSV data).

BRSV Serology:

The first vaccine administration elicited a slight BRSV specific IgG1 response from the FI vaccine immunized group and no detectable response from the other groups. The second vaccine elicited IgG1 and IgG2 responses which persisted through day 23 in the FI vaccine group and were significantly greater than the other groups (figure 3A, B). A slight IgE response to BRSV was observed after the second vaccine and sustained through day 23. Neither of the other groups showed an IgE response to whole virus antigen (figure 3C).

BRSV Serum Neutralization Test:

The results of the SVN testing on sera taken before the first vaccine, before day 0 when virus infection occurred, and on day 14 after BRSV infection are shown in Table Two. All titers were very low and there was no significant difference between the three groups.

BRSV Antibody Responses in BALF:

BRSV specific IgG1 responses in BALF were not detected in the control group (Figure 3D). Antibody responses were detected only on day 23 when the FI vaccine group showed a slight but insignificant response. Overall the difference between groups and the influence of day on the antibody response was significant ($p < 0.0001$) (figure 3D). IgG2 was not detected in any group at any time point. Although specific IgA antibodies were detected through the experiment, only on day 14 the subunit vaccine group showed a significant amount of BRSV specific IgA in BALF (figure 3E). Importantly, the FI vaccine group showed some BRSV specific IgE in the pre-infection BALF and the levels were significantly greater than in the other groups on days 14 and 23 (figure 3F).

NP Subunit Serology:

IgG1 antibodies specific for NP were very significantly higher ($p < 0.001$) in the serum of the subunit vaccine group than in the other two groups (figure 4A). No NP specific IgG2 was detected. Subunit vaccinated calves made IgE against NP in response to vaccination and infection (figure 4B).

NP Antibody Responses in BALF:

IgG1 antibodies were made in response to the subunit and FI vaccines (figure 4C). No NP specific IgG1 was detected in the control group. IgA responses to NP were detected in all groups with a significantly higher response in the day 14 BALF from the FI vaccine group (figure 4D). NP specific IgE in BALF of the subunit vaccinated group only on day 23 was significantly higher than the other groups on (figure 4E).

304

305 *H. somni* Serology:

306 The Somnivac® group produced significant IgG1 and IgG2 to whole *H. somni*
307 antigen in serum through day 23 (figure 5A,B). An increase in serum specific IgG1
308 and IgG2 was apparent in the control group only on days 21 and 23. Specific serum
309 IgE was detected on days 14, 21 and 23 for all groups (Fig. 5C). The Somnivac®
310 group had a significantly higher IgE response than controls on days 14 and 21 and
311 significantly greater than the IbpA DR2 group on day 23 (figure 5C). Timing of these
312 responses indicates that Somnivac primed the IgE response, while the later
313 responses of the control and subunit group were stimulated by infection.

314

315

316 *H. somni* Antibody Response in BALF:

317 On day 23 Somnivac® immunized calves showed a significant *H. somni* specific
318 IgG1 and IgG2 response in BALF (figure 5D,E), unlike subunit and control groups.
319 The Somnivac® group made significantly more IgE specific for *H. somni* on days 14
320 and 23 than the control group (figure 5F).

321

322 IbA DR2 Subunit Serology:

323 The subunit vaccine group responded to vaccination by making IgG1 antibody to
324 Ibpa DR2, which was significantly elevated over both other groups from day -3
325 through day 10 (figure 6A). Little Ibpa DR2 IgG2 antibody was detected until day 21
326 when the subunit group had significantly more IgG2 antibodies than the control

group, and on day 23 when subunit vaccinated calves showed significantly more antibody than both other groups (figure 6B). No IbpA DR2 specific IgE was detected (data not shown).

IbpA DR2 Specific Antibody Responses in BALF:

IgG1 specific for IbpA DR2 was detected in BALF from the subunit vaccinated group on day -3 pre-infection and on days 14 and 23, but the difference with other groups was only significant on day 23 (figure 6C). BALF IgG2 antibodies were significantly higher than controls in both the subunit and Somnivac® vaccinated groups on day 23. (figure 6D).

Discussion

This study shows that viral and bacterial recombinant subunit antigens can protect against a synergistic pneumonic infection in calves. We evaluated a subunit vaccine for both BRSV and *H. somni* because these pathogens synergize to create severe respiratory disease and because there is no consistently protective commercial vaccine available for both pathogens. Evidence that IgE plays a role in pathogenesis of this dual infection [3] and recognition of the propensity that both killed BRSV and *H.somni* vaccines have for eliciting an IgE response prompted the choice of controls. We chose to compare the subunit vaccine with a FI alum adjuvant vaccine that has been shown to induce IgE, coupled with a commercial *H. somni* bacterin. The choice of IbpA DR2 as the subunit for *H. somni* immunization was based on a previous

349 study in which we showed that the DR2 subunit induced IgG1, IgG2, but very little
350 IgE antibodies and was protective against single pathogen challenge [10]
351 Although the FI vaccinated group made high levels of IgG1 and IgG2 antibodies to
352 BRSV and *H. somni*, we saw enhanced disease rather than protection. This is
353 probably related to the IgE antibodies to both BRSV and *H. somni* demonstrated in
354 the FI vaccinated group. This is consistent with previous reports of IgE responses
355 after vaccination with both killed BRSV and *H. somni* vaccines [7].
356 IgA is recognized as an effective virus neutralizing antibody in respiratory
357 secretions. We found that the subunit vaccine group showed a higher level of BALF
358 IgA antibodies to BRSV than control calves on days 14 and 23. This may have
359 contributed to the lower clinical scores observed in this group. Also in the BALF on
360 day -3 as well as on days 14 and 23 after infection the FI group showed a very
361 significant IgE response to the whole BRSV antigen, possibly contributing to the
362 higher clinical signs in that group.
363 The NP subunit vaccine group was the only group to respond significantly to the NP
364 protein after vaccination. The NP specific IgE response in serum of this subunit
365 vaccine group was unexpected. However, the BRSV nucleoprotein was the target of
366 an IgE response (shown on Western Blots of sera from FI BRSV/alum vaccinated
367 and BRSV challenged calves) in a previous experiment [6]. Importantly, this IgE
368 response to an internal viral protein does not appear to have caused a vaccine
369 enhanced disease, possibly because the internal protein is not available for IgE
370 cross-linking of mast cell receptors

The serum antibody response to *H. somni* was greatest for the Somnivac® group, for both IgG1 and IgG2. These responses were induced by the vaccine and reinforced by infection. By day 14 both the subunit vaccine group and the Somnivac® group had developed an IgE response to the *H. somni* and by days 21 and 23 all three groups showed an IgE response to *H. somni*. The IgE response to *H. somni* that was documented in our previous dual infection studies is preserved in this study with the control group and now we show that calves concurrently infected with BRSV and *H. somni* develop even more *H. somni* specific IgE if they have been immunized with these two killed vaccines prior to infection . The slight increase in IgE anti-*H. somni* in the subunit vaccine group is not statistically different from the control group. This result is in agreement with the results of IbpADR2 immunization and challenge with *H. somni* single infection [10]. Importantly although the vaccine does not prevent the slight infection driven IgE response, it also does not exacerbate such a response.

Unlike the BRSV NP, which is an internal viral protein, the IbpA is a surface protein of *H. somni* and the DR2 domain has two conserved [14] toxic motifs with recognized pathogenic effect [10,15,16]. Stimulation of DR2 specific IgG1 and IgG2 would be expected to depress pathogenic effects through neutralization of these toxic motifs. Indeed, high levels of IgG1 were made in response to the subunit vaccine and it is likely that these antibodies in the serum and in the lavage fluid contributed greatly to the decreased clinical signs in the IbpA DR2 vaccinates. The IgG2 response peaked later in both compartments, but these were also a likely contribution to the overall improved clinical disease and the decreased lung

pathology. The toxic effects of IbpADR2 fic on lung epithelium and phagocytes [17] can enhance cell destruction and inflammatory cell accumulation. Neutralization of these motifs with antibody likely explains, at least partly, the reduced lung pathology in the subunit vaccinates.

The shedding of virus was unaffected by vaccination; this reflects a lack of induction of sterilizing immunity. The NP subunit would not be expected to induce virus neutralizing antibody and did not induce virus neutralizing antibody in this study; thus it is likely that replication in the upper airways was not impeded by this vaccine. We evaluated concentrations of Interferon γ by ELISA in sera and lung lavage that had been stored at -20°C and did not find any positive samples. Cell mediated responses to the NP remain to be examined by in vitro lymphocyte stimulation with viral antigen in future studies to determine whether reduction of clinical scores and lung pathology could be attributed to induction of T cell responses.

Conclusions: This study demonstrates that a subunit vaccine containing the nucleoprotein NP subunit from BRSV and the IbpA DR2 subunit from *H. somni* provides substantial protection from synergistic dual infection with both agents.

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Figure Legends

Figure 1 – Daily clinical scores are presented with mean and standard error for each group. On infection days 1 – 8 clinical scores for the FI & Somnivac® group were significantly greater than for the NP & IbpA DR2 group ($p < 0.0001$) and greater than the control group ($p < 0.0024$).

Figure 2 – The mean percentage of lung consolidation is shown for each group (A). Histological scores in several lung compartments; Mean scores were computed for each lung compartment in each group. Overall scores are presented (B) and in the three compartments with significant differences: bronchiolar (C), alveolar (D), and septal (E). In each compartment shown the NP & IbpA DR2 subunit vaccine group had significantly less lesions than the adjuvant control group.

Figure 3 – Mean serum levels of IgG1 (A), IgG2 (B), and IgE (C) antibodies specific for whole purified BRSV antigen as determined by ELISA. The FI & Somnivac group's values were significantly greater than for the other two groups ($p < 0.001$).

Mean levels of IgG1 (D), IgA (E), and IgE (F) antibodies specific for whole purified BRSV antigen as determined by ELISA in bronchoalveolar lung fluid (BALF). ELISA absorbance values are multiplied by 100 and divided by the protein concentration measured in the BALF. There was no BRSV specific IgG2 detected in BALF. BRSV specific IgE was significantly greater in the FI & Somnivac® group ($p < 0.001$).

Figure 4 – Mean serum levels of NP specific IgG1 (A) and IgE (B) determined by ELISA. Only the NP vaccine group developed a response to NP. No NP specific IgG2 was detected. Mean levels of IgG1 (C), IgA (D), and IgE (E) antibodies specific for recombinant NP antigen in broncho-alveolar lung fluid (BALF) as determined by ELISA. Absorbance values are multiplied by 100 and divided by the protein concentration measured in the BALF. BRSV infection was on day 0. There was no NP specific IgG2 detected in BALF. The NP & IbpA DR2 vaccine group developed a significant IgE response in BALF only on day 23 post infection.

Figure 5 - Mean serum levels of IgG1 (A), IgG2 (B), and IgE (C) for whole *H. somni* antigen were determined by ELISA. Infection induced an IgE response to *H. somni*, which was significantly greater in the FI & Somnivac group. Mean levels of IgG1 (D), IgG2 (E), and IgA (F) antibodies specific for whole *H. somni* antigen as determined by ELISA in broncho-alveolar lung fluid (BALF). There was no NP specific IgE detected in BALF.

Figure 6- Mean serum levels of IgG1 (A) and IgG2 (B) antibodies specific for recombinant DR2 antigen were determined by ELISA. No IgE was detected in serum. Mean levels of IgG1 (C) and IgG2 (D) antibodies specific for recombinant DR2 antigen as determined by ELISA for recombinant DR2 antigen in bronchoalveolar lung fluid (BALF). No IgA or IgE was detected.

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Table One – Histopathological Lesions Used
For Scoring

Bronchi	Neutrophilic exudate Deciliation Epithelial transmigration Intraepithelial pustules Inclusion bodies Mononuclear cell infiltrates submucosa Lymphoid nodules
Bronchioli	Neutrophilic exudate Fibrinous exudate Obliterating bronchiolitis necrosis of epithelium Inclusion bodies Epithelial transmigration Peribronchiolar mononuclear cell infiltrates Peribronchiolar lymphoid nodules
Alveolus	Neutrophilic exudate Fibrinous exudate necrosis Edema Hemorrhages Syncytial cells Hyperplasia of type II pneumocytes Granulomas Atelectasis
Interstitium	Thickening (monocytes) Thickening (pmn neu/eos) vasculitis Thrombosis Lymphoid nodules edema
Septae	Expansion edema Fibrin Mononuclear cell infiltrates PMN infiltrates Pleocellular infiltrates Arteritis

Table One – Histopathological Lesions Used
For Scoring

Pleura	Thickening Fibrosis Lymphatic dilation Mononuclear cell infiltrates PMN infiltrates Pleocellular infiltrates
Trachea	Deciliation Epithelial transmigration Mononuclear cell infiltrates submucosa Lymphoid nodules
Bronchial lymph node	Hyperplasia/reactive Serocellular sinus exudate Mononuclear cell infiltrates PMN infiltrates Pleo infiltrates

Table Two – BRSV Serum Neutralization Titers

Vaccine Group	Pre-Vaccine 1	Day 0	Day 14
Control	*<2 – 8 (mean 2.33)	<2 – 2 (mean 1.67)	<2 – 8 (mean 2.67)
BRSV FI & Somnivac	2 – 8 (mean 4.67)	2 – 4 (mean 2.67)	<2 – 8 (mean 3.6)
Np & Ibp DR2	<2 – 4 (mean 2.33)	<2 – 2 (mean 1.33)	<2 – 2(mean 1.33)

*indicates range of titers among 6 animals in the group

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Gershwin et al_ figure 2

Serum BRSV Specific IgG1

D BALF BRSV Specific IgG1

Serum BRSV Specific IgG2

E BALF BRSV Specific IgA

Serum BRSV Specific IgE

F BALF BRSV Specific IgE

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Gershwin et al. figure 5