Summary

Pneumonia is a population-limiting disease of bighorn sheep (BHS; Ovis canadensis) and a recognized disease entity in domestic sheep (DS; Ovis aries) worldwide. Respiratory disease in BHS lambs can persist for years after all-age outbreaks, resulting in suppressed lamb recruitment. It has been suggested that inadequate passive transfer (PT) of maternal antibodies may play a role in BHS lamb pneumonia, although inadequate PT is often associated with illness prior to 4 weeks of age in DS, while BHS mortality predominantly occurs >4 weeks of age. The purpose of this study was to analyze PT of total colostral IgG in BHS lambs and DS lambs born and hand-raised under similar conditions. Total IgG concentrations were quantified for ewe sera, colostrum, and lamb sera collected at multiple time points following ingestion of a known quantity of colostrum. No significant interspecies differences were observed for total IgG concentrations in ewe sera or colostrum, or in the calculated apparent efficiency of IgG absorption. Waning kinetics of IgG in DS and BHS lamb sera was similar post-colostrum ingestion. BHS lambs produced IgG sooner than DS following waning of maternal IgG and had significantly higher serum IgG at 16 weeks, 20 weeks, and 24 weeks. Significant differences were observed in birth weight (DS > BHS) and amount of time to ingest colostrum (BHS > DS). Findings of this study, as well as consideration of the age of mortality in wild BHS lambs, do not support inadequate PT of total IgG as a primary contributor in BHS lamb pneumonia.

Key words: Passive Transfer; Colostrum; Bighorn Sheep; Domestic Sheep
Introduction

The bighorn sheep (Ovis canadensis, BHS) population in North America has declined from a broad estimate of five hundred thousand to two million at the beginning of 19th century to an estimated 15,000 to 18,000 in 1960, with an increase to an estimated 72,000 reported in 2007 (Buechner, 1960; Rominger, 2008). More recent population numbers are seemingly elusive in the literature, with unofficial numbers reported in the media as “nearly 200,000”, as per a recent New York Times article entitled “The Ultimate Hunt: Sheep” (Branch, 2017)(Boeskorov, 2011 #682). The most pronounced population decline of BHS occurred in the second half of the 19th century and early 20th century and is proposed to be due to a combination of factors, including unregulated hunting, loss of habitat, competition for forage and domestic livestock, and diseases, particularly pneumonia (Grinnell, 1928; Marsh, 1938; Besser et al., 2013b). All age epizootic pneumonia outbreaks continue to sporadically afflict wild BHS herds. Poor lamb recruitment, due to bacterial respiratory disease afflicting primarily lambs in years subsequent to all-age pneumonia epizootics, is reported to be the biggest impediment to BHS all-age pneumonia outbreaks historically (Marsh, 1938; Besser et al., 2013b). All age pneumonia is reported to be the biggest impediment to BHS herd productivity. Respiratory disease in BHS are scarce to absent in the literature.

A previous publication indicated that one factor in the reportedly heightened susceptibility to pneumonia in bighorn lambs is due to lower PT of maternal antibodies against Mannheimia haemolytica, a member of the Pasteurellaceae family, in BHS as compared to DS (Herndon et al., 2011). The design of that study had limitations in comparatively examining PT in DS and BHS lambs, including examination of colostrum that was collected up to 24 hours postpartum (presumably after sucking had occurred in at least some of the ewes) rather than immediately following parturition. That study also lacked data regarding amount of colostrum ingested and therefore apparent efficiency of absorption (AEA) could not be calculated. Additionally, the authors focused only on the transfer of antibodies to just 2 microbes (M. haemolytica and parainfluenza virus-3) rather than including total PT in the analyses. The present study provided testing to determine if there is a significant difference between PT of total IgG in BHS and DS and to examine serum IgG concentrations over time (kinetics) in lambs. To our knowledge, this is the first study designed to measure total PT and kinetics or waning of IgG in BHS over time and comparatively assess PT with that of DS cohorts born and raised under similar environmental conditions.

Materials and Methods

Experimental sheep and care

Animals described in this report were housed and maintained at Washington State University (Pullman, Wash., USA) and experiments were carried out according to the guidelines of the Institutional Animal Care and Use Committee and Association for Assessment and Accreditation of Laboratory Animal Care. All lambs were born at Washington State University; domestic lambs described in this study were born in April and bighorn lambs were born May through June.

Two male and two female Suffolk DS lambs and four male and one female Rocky Mountain BHS lambs were gently removed from the birth canal of four DS ewes and five BHS ewes during late parturition; two additional female BHS lambs were delivered by Caesarian section from a sixth BHS ewe. Each lamb was cleaned and dried with a towel and had no further contact with the ewe. Lambs were separated by species and housed in an indoor vivarium. Immediately following parturition, colostrum was collected from each ewe using a handheld vacuum pump (Udderly EZ; Lexington, Ky., USA). Immediately following, and for up to 18 hours post parturition, each lamb was fed colostrum from their dam, therefore the volume of colostrum ingested by each lamb was dependent on the amount that could be collected from each dam. Following consumption of the collected colostrum, DS lambs were switched to Land O’Lakes Ultra Fresh® milk replacer and BHS were switched to Purina ProNurse®, a more readily soluble, multispecies milk replacer. Each lamb was weighed and received a subcutaneous injection of vitamin E/Selenium (BO-SE®, Schering-Plough, Merck Animal Health; Madison, N.J., USA) and an intramuscular injection of vitamin A/D (Agri Laboratories LTD; St. Joseph, Mo., USA) between 20 and 30 hours after birth. At 3 days of age, each lamb was subcutaneously vaccinated with CD-T (Clostridium perfringens Types C & D – tetanus toxoid (Boehringer Ingelheim Vetmedica, Inc., Bar Vac®, St. Joseph, Mo., USA), tail docking was performed on each DS lamb, and the 2 male DS lambs were castrated. Volume of colostrum and milk replacer consumed was recorded for each lamb through weaning. DS lambs were fed 3 to 5 times, and BHS lambs were fed 4 to 6 times, per 24-hour period (feedings/day decreased with age). Each lamb was weighed weekly until weaned at 6 weeks of age for the DS lambs and 12 weeks of age for the BHS lambs. Water and alfalfa hay were provided ad libitum throughout this study.

Sample collection and sheep IgG ELISA

Blood was collected from each ewe within 4 weeks prior to parturition and colostrum was collected immediately following parturition. Blood from each lamb was collected at 1 day (20 to 32 hours post-parturition) and at 3 weeks, 6 weeks, 9 weeks, 12 weeks, 16 weeks, 20
weeks and 24 weeks of age. All blood was collected by jugular venipuncture. Serum was separated by centrifugation (800 x g for 20 minutes) and stored along with colostrum samples at -80°C. Duplicate samples of ewe serum, colostrum, and lamb sera, from each time point, were diluted and total IgG (mg/mL = g/L) measured using a commercially available ELISA, following manufacturer's recommendations and protocol (Sheep IgG ELISA kit, Alpha Diagnostic International, San Antonio, Texas, USA). Colostrum and sera samples were diluted 1:1000 and 1:500, respectively, with diluent provided in the kit prior to performing the ELISA. Each sample was tested in duplicate in three independent assays, and the mean value was used for each sample for further statistical analyses. The IgG ELISA kit was confirmed for use in DS and tested for cross reactivity in BHS using purified serum IgG. Purified IgG was obtained from pooled sera collected from lambs at 24 weeks of age using the NAb™ Protein G Spin Kit following the manufacturer's recommended protocol (Thermo Scientific; Rockford, Ill., USA). The solution containing IgG was dialyzed against phosphate-buffered saline containing 0.02 percent sodium azide using a 3.5 kD Slide-A-Lyzer cassette (Thermo Scientific; Rockford, Ill., USA). Isolation of purified IgG was confirmed by gel electrophoresis (Invitrogen NuPAGE 3 to 8 percent Tris-Acetate pre-cast gel; Grand Island, N.Y., USA) and stained with coomassie blue for visualization (Bio-Rad Bio-Safe Coomassie Blue G250; Hercules, Calif., USA) (Fig. 1, panel A). A NanoDrop 200C (Thermo Scientific; Rockford, Ill., USA) was used to spectrophotometrically quantify isolated IgG in order to test the accuracy (validity) of the ELISA kit for use in both species.

**Statistical analysis**

Intra- and inter-assay coefficients of variability were calculated for all test sample results to determine assay precision for the Sheep IgG ELISA kit (108 samples total: 10 ewe sera, 10 colostrum samples, and sera from 11 lambs at 8 time points). Mean values and standard deviations were calculated for each species for ewe sera, colostrum, and lamb sera IgG concentrations, and for lamb birth weight (kg), volume of colostrum ingested (mL), and time to ingest colostrum (hours). Apparent efficiency of absorption (AEA) for each lamb was calculated as follows: AEA = [(g IgG / L serum) (0.06 x kg body weight) (100 percent)] / [(g IgG / L colostrum) (L colostrum ingested)] (Husband et al., 1973). This calculation makes the assumption that both DS and BHS lambs have a plasma or sera volume that is 6 percent of body weight, as previously described for DS lambs (Gratama et al., 1992). A two-tailed t-test was performed, with Welch’s correction for data sets having unequal variance, for each interspecies analysis, except for repeated sampling over time of the lamb sera, for which a two-way repeated measures ANOVA was performed and statistical significance was determined using the Holm-Sidak method with alpha = 0.05. Statistical analyses were performed using GraphPad Prism 6 (GraphPad Software, Inc., La Jolla, Calif, United States) and results were considered significant for all analyses at P ≤ 0.05.

**Results**

Validation of commercial sheep IgG ELISA for use in BHS

Analysis of purified IgG from sera, pooled by species from lambs at 24 weeks of age, showed similar interspecies results (Fig. 1, panel B) that were consistent with the standards provided with the kit (Fig. 1, panel C). The intra- and inter-assay coefficients of variability for the ELISA for the 108 test samples (10 ewe sera and colostrum...
Table 1. Interspecies comparative analyses of maternally derived, passively transferred total IgG in domestic sheep (*Ovis aries*) and bighorn sheep (*Ovis canadensis*)

<table>
<thead>
<tr>
<th></th>
<th>DS Median ± SD</th>
<th>BHS Median ± SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ewe</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum IgG (mg/mL)</td>
<td>14.7 ±1.2</td>
<td>19.0 ±6.6</td>
<td>0.3583</td>
</tr>
<tr>
<td>Colostrum IgG (mg/mL)</td>
<td>57.2 ±14.6</td>
<td>69.9 ±16.3</td>
<td>0.1209</td>
</tr>
<tr>
<td>Colostrum/Sera IgG ratio (mg/mL)</td>
<td>4.5 ±2.0</td>
<td>4.6 ±2.1</td>
<td>0.9443</td>
</tr>
<tr>
<td><strong>Lamb</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth weight (BW, kg)</td>
<td>6.1 ±0.5</td>
<td>4.1 ±0.2</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Volume colostrum ingested (mL)</td>
<td>384.4 ±59.1</td>
<td>232.3 ±98.7</td>
<td>0.0217*</td>
</tr>
<tr>
<td>Time to ingest colostrum (hours)</td>
<td>4.2 ±0.38</td>
<td>10.1 ±3.89</td>
<td>0.0065*</td>
</tr>
<tr>
<td>Colostrum ingested/BW (mL/kg)</td>
<td>63.0 ±4.5</td>
<td>56.9 ±25.5</td>
<td>0.5559</td>
</tr>
<tr>
<td>IgG ingested (g)</td>
<td>21.5 ±4.0</td>
<td>16.8 ±8.7</td>
<td>0.3390</td>
</tr>
<tr>
<td>IgG ingested/BW (g/kg)</td>
<td>3.6 ±0.8</td>
<td>4.12 ±2.2</td>
<td>0.6491</td>
</tr>
<tr>
<td>24 hour sera IgG (mg/mL)</td>
<td>11.5 ±4.6</td>
<td>8.23 ±4.6</td>
<td>0.2781</td>
</tr>
<tr>
<td>AEA (%)</td>
<td>20.8 ±11.4</td>
<td>11.7 ±3.7</td>
<td>0.2082</td>
</tr>
</tbody>
</table>

AEA = apparent efficiency of absorption; DS = domestic sheep (ewes: n=4, lambs: n=4); BHS = bighorn sheep (ewes: n=6, lambs: n=7); parenthetical BHS lamb data excludes data from twin ewe lambs.

Figure 2. Comparative analyses over time of domestic (DS) and bighorn (BHS) lamb sera IgG concentrations (mg/mL). Analysis performed by ELISA. Graph (A) includes all lambs (DS n=4; BHS n=7). Graph (B) excludes the twin female BHS lambs as they had 24 hour (hr) serum IgG concentrations considered inadequate passive transfer of IgG (1.9 and 2.6 mg/mL). The only notable difference between the two graphs is at the 24 hour age in which error bars are wider for BHS. Two-way repeated measures ANOVA, statistical significance was determined using the Holm-Sidak method with alpha = 0.05 and denoted by an asterisk (*) at 16, 20, and 24 weeks (wk) of age (P = 0.0050, 0.0004, and 0.0115, respectively).

Interspecies analysis

Table 1 summarizes the results of interspecies comparative analyses for PT, including IgG concentrations in samples (ewe sera, colostrum, 24 hour lamb sera), volume of colostrum ingested by the lambs, lamb birth weights (BW), and amount of time to ingest colostrum, as well as calculations based on these data (volume ingested/BW, IgG ingested/BW, and AEA). Data analysis was performed both including and excluding the twin BHS lambs, as these 2 lambs were considered to have insufficient IgG at 24 hours of age (2.6 mg/mL and 1.9 mg/mL). All other lambs had >6 mg IgG per mL serum, a value considered to be sufficient PT, with serum concentration ranging from 7.1 mg/mL to 17.9 mg/mL in DS lambs and 6.4 mg/mL to 11.6 mg/mL in BHS lambs (McGuire et al., 1983). No significant difference was identified for total IgG (mg/mL) in the ewes' sera, colostrum, or lamb sera at 24 hours of age. No significant difference in lamb sera was identified until 16 weeks of age, at which time the BHS lambs had significantly higher concentration of
total serum IgG ($P = 0.0050$; Table 1 and Fig. 2). This higher concentration of total serum IgG in BHS persisted at the final two time points, weeks 20 and 24 ($P = 0.0004$ and $0.0115$, respectively; Fig. 2). Comparative evaluation of serum IgG concentrations over time from repeated serum sampling indicates that DS and BHS lambs have a similar waning kinetics of IgG following consumption of colostrum, with the nadir identified in serum samples collected at 6 weeks and 9 weeks of age (Fig. 2). DS lambs weighed significantly more at birth and at each of the following weekly weight measurements, collected for both species through 6 weeks of age ($P \leq 0.0122$). The average weekly weight gain and volume of milk replacer ingested was significantly greater for DS lambs as compared to BHS lambs ($P \leq 0.0202$ and $P \leq 0.00003$, respectively; Fig. 3). No significant intraspecies gender differences in weight or weekly weight gain were observed ($P \geq 0.2031$). All lambs were observed drinking water and eating hay at will within the first week of age.

**Discussion**

This study was performed to assess total maternal IgG (serum and colostrum) and to compare kinetics of total IgG in lamb sera following consumption of colostrum in DS and BHS lambs hand-raised under the same controlled environmental conditions (indoor isolation facility). While the sample size for both BHS (6 ewes, 7 lambs) and DS (4 ewes and 4 lambs) hold statistical limitations, a larger study under such controlled parameters would be difficult to achieve, particularly for a wildlife species with limited availability. Previous studies investigating PT in DS have reported a great deal of variability between individual DS and between different breeds; our findings are within reasonable limits in comparison (McGuire et al., 1983; Vatankhah, 2013). The twin female BHS lambs in this study had serum IgG concentrations indicative of inadequate PT (<6 mg/mL), at 2.6 mg/mL and 1.9 mg/mL at 24 hours, and also had the two lowest calculated %AEA values (8.3 percent and 8.2 percent) (McGuire et al., 1983). Dystocia, delivery by caesarian section with delay in colostrum ingestion, hypoxia, and hypothermia in the 1.9 mg/mL lamb, and having to divide the ewe’s colostrum, which had a lower IgG concentration compared to the other BHS ewes (48.1 mg/mL versus ≥68.1 mg/mL for the other BHS ewes), may have all contributed to the inadequate 24-hour serum IgG in these lambs. Considering the inadequate transfer in these two lambs, interspecies comparisons and statistical analyses were performed with and without their values, as described.

While the AEA between the DS and BHS lambs in this study were not significantly different, the lower mean %AEA for BHS lambs (calculated with and without the twins’ values) as compared to DS lambs may have been at least in part attributable to the significantly longer time it took for the BHS to ingest the colostral IgG under the condition of being handfed. This is supported by research that has shown a linear decrease in immunoglobulin absorption by neonatal ruminants from birth to 24 hours of age, with optimal absorption reported to occur within 4 hours of parturition (Besser et al., 1985; Weaver et al., 2000; Nowak and Poindron, 2006). As the neonate’s ability to absorb maternal antibodies decreases over time, so does the concentration of IgG in colostrum as it is diluted with new mammary secretion, gradually becoming milk. The concentration of IgG that is concentrated in colostrum prior to parturition is reported to decrease rapidly,

![Figure 3](image-url)
with a decrease to 50 percent at 6 hours and to less than 10 percent by 24 hours following parturition when DS lambs are allowed to suckle (Shubber and Doxey, 1979). It is therefore important to evaluate the mammary content before or at the lamb’s first feeding in order to accurately investigate the colostral component of PT. This rapid decrease in colostral IgG concentration explains results reported by Herndon, et al. (2011), in which ewe serum samples from all DS and BHS were reported to have similar or greater mean titer values to specific pathogens/antigens, (M. haemolytica, leukotoxin (a primary virulence factor of M. haemolytica), and parainfluenza 3 virus) than did the colostrum collected up to 24 hours post-partum, likely after the lambs suckled, as the authors acknowledge may have occurred. Herndon et al. also reported 24 hour DS and BHS lamb sera to have higher titers than did the colostrum, which would indicate an impossible %AEA of greater than 100 percent, as neonatal ruminants have negligible serum IgG prior to colostrum ingestion due to the epitheliochorial cotyledonary placentation, which limits transfer of immunoglobulin from the dam to the fetus (Hunter et al., 1977).

Findings in this study indicate that following ingestion of colostrum, total serum IgG in BHS and DS at 1 day of age are similar, as are the waning kinetics of IgG with the nadir or “immunity gap” observed at 6 weeks to 9 weeks of age followed by an increase in IgG after 9 weeks of age, interpreted to be endogenous production (Fig. 2). Mortalities due to pneumonia typically occur in wild BHS lambs greater than 4 weeks of age, peaking between 6 weeks and 11 weeks, while inadequate PT is typically associated with mortality in the first weeks of life, when maternally derived antibodies should be at the highest concentration with adequate PT (Sawyer et al., 1977; Cassirer et al., 2001; Cassirer et al., 2013). Supporting our findings and conclusions is a 2001 publication by Cassirer, et al. that reported “free-ranging lambs appeared especially vulnerable to pasteurellosis from 6 weeks to 11 weeks of age, near the time that passively-acquired agglutinating and leukotoxin neutralizing antibody levels wane” (Cassirer et al., 2001). Additionally, the 2001 publication by Cassirer, et al. reported that 36 BHS ewes, sampled from three Hells Canyon herds, had high-serum agglutinating and neutralizing titers to M. haemolytica and leukotoxin, respectively, and high titers did not correlate with increased lamb survival. Herndon, et al. contradict this 2001 publication by concluding that BHS ewes, both wild (from Hells Canyon) and captive, are "deficient in Ab production" against M. haemolytica and leukotoxin. Herndon et al. go on to make the conclusion that the lower titers observed from 12 captive and 12 wild BHS ewes in their study is "representative of BHS in general", which seems an unreasonable conclusion based not only on the 2001 Cassirer et al. publication, but also when considering the overall population of BHS, located in western Canada, western United States, and northern Mexico, is estimated to be greater than 72,000. While the present study has limitations due to group size, our findings indicate a similar transfer of maternal total IgG in DS and BHS and similar serum IgG concentration kinetics in both species following colostrum ingestion. Our finding of similar PT in DS and BHS is supported by the findings of Herndon, et al. describing similar interspecies PT of antibodies directed at a specific-pathogen to which both BHS and DS ewes had serum titers.

Conclusions

This study provides a controlled interspecies comparative analysis of maternal total IgG in DS and BHS ewe serum and colostrum and kinetics of total serum IgG concentrations in DS and BHS lambs following colostrum ingestion. Not evaluated in the present study and vastly absent from the literature overall are factors that may impede BHS lambs from mounting an effective endogenous protective immune response against ovine respiratory pathogens. This seems a valid consideration in light of documentation that peak BHS lamb mortality coincides with the timing of the immunity gap identified in this study.

Literature Cited


