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Respiratory Tract Infections in Dairy Calves from Birth to Breeding Age: Detection by Laboratory Isolation and Antibody Responses

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Abstract

This observational study followed 30 dairy heifers from birth to breeding to identify when exposure to potential respiratory pathogens occurred. Following the standard heifer development protocol for the dairy farm studied, heifers were housed in larger group pens, termed "groupings", based upon individual growth and development. Samples were collected at each grouping to determine when potential respiratory pathogens were acquired. Nasal swabs for viral and bacterial culture, peripheral blood leukocytes for virus isolation, and sera for antibody titers were collected and assayed three times (days 0, 7 and 14) at each grouping, beginning at 24-36 hours of age. Nasal bacterial isolates included *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni* (formerly *Haemophilus somnus*) and *Mycoplasma* spp. *M. haemolytica* and *P. multocida* were occasionally isolated in the first 24-36 hours of life. Isolation of *M. haemolytica* and *P. multocida* increased substantially in the second and fourth groupings, respectively. Bovine herpesvirus-1 (BHV-1), parainfluenza-3 virus (PI_3V), *M. haemolytica* and *Mycoplasma* spp were isolated with increased frequency when heifers were housed with nose-to-nose contact with adult cattle, and when clinical respiratory disease that responded to treatment occurred. Heifers were vaccinated with modified live virus vaccine while in hutches and at 5-6 months of age (third and fourth groupings). Colostrum-derived antibodies to viruses had diminished by the second grouping (age 95.2 ± 25.4 days), and during that time PI_3V and BHV-1 were isolated. Viral neutralizing antibody titers did not significantly increase until after the second vaccination, which may have reflected interference from maternal antibody. Calves spontaneously developed antibodies to *P. multocida* between the 14th day in the hutch and the second grouping, and to *M. haemolytica* during the second (age 95.2 ± 25.4 days) and third groupings (age 134.5 ± 36.1 days). In conclusion, transmission of potential respiratory pathogens was documented in young dairy heifers despite commonly accepted health management programs, including vaccination.

Résumé

Cette étude longitudinale depuis la naissance jusqu'à l'âge reproducteur tentait de cerner le moment où l'exposition aux agents pathogènes respiratoires potentiels prenait place chez 30 taures laitières. En accord avec le protocole courant de développement des taures dans la ferme laitière concernée, les taures étaient logées dans de grands enclos communs, sous le nom de regroupements, selon le niveau individuel de croissance et de développement. Des échantillons ont été recueillis dans chacun des regroupements pour déterminer quand les agents pathogènes respiratoires potentiels étaient acquis. Des écouvillons nasaux ont été recueillis pour des cultures virales et bactériennes, des leucocytes sanguins d'échantillons périphériques ont été prélevés pour l'isolement de virus ainsi que du sérum pour les titres d'anticorps. L'échantillonnage a été fait trois fois (jours 0, 7 et 14) dans chacun des regroupements à partir de 24-36 heures d'âge. Les bactéries isolées à partir des écouvillons nasaux comprenaient *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni* (autrefois *Haemophilus somnus*) et *Mycoplasma* spp. *M. haemolytica* et *P.*

multocida étaient isolées occasionnellement lors des premières 24-36 heures suivant la naissance. L'isolement de *M. haemolytica* et de *P. multocida* augmentait substantiellement dans le deuxième et quatrième regroupement, respectivement. L'herpèsvirus bovin 1 (BHV-1), le virus parainfluenza 3 (PI_3V), *M. haemolytica* et *Mycoplasma* spp ont été isolés plus fréquemment lorsque les taures avaient un contact nez à nez avec des bovins adultes et lorsque des maladies cliniques respiratoires traitables prenaient place. Les taures ont été vaccinées avec des vaccins à virus vivants modifiés dans leur enclos et plus tard à l'âge de 5-6 mois (troisième et quatrième regroupements). Les anticorps contre les virus dérivés du colostrum diminuaient lors du second regroupement (95.2 ± 25.4 jours d'âge) durant lequel les agents PI_3V et BHV-1 ont aussi été isolés. Les titres d'anticorps viraux neutralisants n'ont pas augmenté substantiellement jusqu'à la seconde vaccination reflétant peut être l'interférence causée par les anticorps maternels. Les veaux ont développé spontanément des anticorps contre *P. multocida* entre le 14^{me} jour dans les enclos de naissance et le second regroupement et contre *M. haemolytica* dans le second regroupement (95.2 ± 25.4 jours d'âge) et le troisième regroupement (134.5 ± 36.1 jours d'âge). En conclusion, la transmission d'agents pathogènes respiratoires potentiels a été documentée chez de jeunes taures en dépit de l'utilisation de programmes de gestion courants incluant la vaccination.

Introduction

Many factors have been identified that increase the risk of bovine respiratory disease (BRD) for young dairy calves. Major risks for respiratory disease in dairy calves from birth to six months of age include poor air circulation and introduction of new cattle into a herd.[28] Low serum protein, as a measure of colostral immunoglobulin absorption, is also associated with increased risk for respiratory disease.[8,21,30] Total IgG colostral-acquired antibodies to specific respiratory pathogens were a predictor of protection against respiratory disease.[30]

BRD affects production and health in cattle, including dairy operations. In the 1996 Dairy Management Practices report by the USDA National Animal Health Monitoring System (NAHMS), dairy producers reported respiratory disease as the second major cause of death in unweaned heifer calves (24.5%).[18] Researchers found a similar prevalence in New York dairy calves.[30] In the NAHMS report, producers ranked respiratory disease as the number one cause of mortality in weaned heifers (44.8%).[18] Investigators described that dairy calves treated for pneumonia within the first three months of life were 2-5 times more likely to die by 90 days of age than were healthy, untreated calves.[31] *Pasteurella multocida*, *Mannheimia haemolytica*, *Histophilus somni* (formerly *Haemophilus somnus*), *Mycoplasma* spp and various viruses including bovine viral diarrhea virus (BVDV), bovine herpesvirus-1 (BHV-1), parainfluenza type 3 virus (PI_3V) and bovine respiratory syncytial virus (BRSV), are associated with pneumonia in this age of dairy calves.[1,2,15,25,27,28] These pathogens can exist as single or mixed infections.[30]

Health and management decisions in modern dairy production are frequently based on individual animal growth rates instead of age. Grouping by growth allows more efficient use of labor, facilities and other resources. In most modern dairy enterprises, neonatal calves are initially housed in individual calf hutches, resulting in improved calf health by reduced exposure to pathogens and by allowing more individual attention to calves during the critical neonatal period. However, resources to maintain this type of individual management beyond 8-9 weeks of age are impractical. Therefore, dairy heifers are housed or grouped into multiple animal pens at various times based on individual calf growth rate and development. Facilities are normally designed to utilize space and resources and to capitalize on management efficiencies, thus dictating that pens and pastures will be adjacent to each other. Cattle in adjacent pens and pastures are frequently in different phases of production.

It is assumed that calves acquire respiratory pathogens via the nasal route at a young age due to nose-to-nose contact with mature cattle. In the case of dairy calves that are not allowed to nurse and removed from the dam almost immediately after birth, initial sources of respiratory pathogens are not always obvious. This observational study was conducted to investigate the natural ecology of respiratory pathogens in 30 dairy heifers from one day to 15 months of age to determine whether a relationship exists between respiratory disease agent exposure, routine production management and clinical disease.

Materials and Methods

Animals

The dairy herd located on the Oklahoma State University - Stillwater campus was selected as a representative model for this study. This herd is managed by university staff and is comprised of approximately 180 adult Holstein-Friesian cows. Pregnant cows were selected based on expected calving date, targeting those that would start calving in early March of the given year. Heifer calves born from selected cows were enrolled in the study and sampled according to protocol until grouped into a replacement heifer pen and bred artificially. This experiment was conducted following the common health management practices of the dairy; there

was no attempt to interfere with or alter the established health program.

All post-parturient cows were transitioned into the lactating herd. Heifer calves were removed from their dams as soon as observed by personnel, not exceeding six hours after birth. All calves were administered colostrum via an esophageal feeding tube, with each calf receiving approximately 10 percent (10%) of her individual body weight of colostrum. Half the calculated amount was administered shortly after birth and the second half was administered by the next feeding time, generally 12 hours or less from the first feeding. Colostrum quality of each aliquot had been screened for immunoglobulin concentration using a colostrometer.[a,11] All colostrum administered to the calves was determined to be of superior quality based on the standard of the Colostrometer™. Determination of the quality of colostrum administered to each calf was performed according to the manufacturer's specifications.

At the time of the first feeding calves were given a permanent identification ear tag, and individual records with comments were initiated. Calves were moved into individual commercial fiberglass hutches that measured approximately 6 x 3 x 3.3 ft (2.1 x 1.1 x 1.2 m). All calves were fed a commercial milk-replacer formula until their next grouping at approximately eight to nine weeks of age.

Experimental design

A total of 30 heifer calves were enrolled in the study. Heifers were initially sampled at 24-36 hours of age, and then one week and two weeks later. At each sampling, nasal swabs were collected for viral, bacterial and *Mycoplasma* spp isolation. Blood samples were collected via jugular venipuncture for peripheral blood leukocytes (PBL) for BVDV isolation, and sera for measurement of total immunoglobulin concentration as well as viral and bacterial serology. Total serum immunoglobulin concentrations were determined by commercial radial immunodiffusion kits.[b]

Heifers were vaccinated according to the standard health care protocol for the dairy. While housed in their individual hutches, heifers received a modified live virus (MLV) intranasal BHV-1, PI_3V vaccine[c] at 10 days of age, and a *Clostridium perfringens* types C and D bacterin/toxoid with tetanus[d] at weaning. At 5-6 months of age (corresponding to the third or fourth grouping), heifers were administered a MLV vaccine containing BHV-1, PI_3V, BVDV and BRSV with 5-way leptospira bacterin,[e] 7-way clostridial bacterin/toxoid[f] and *Brucella abortus* live vaccine.[g] Thirty days prior to breeding, all heifers were administered a *Mannheimia (Pasteurella) haemolytica/Pasteurella multocida* bacterin/toxoid,[h] and a booster vaccination consisting of MLV BHV-1, PI_3V, BVDV, BRSV vaccine and 5-way leptospira bacterin.[e]

Heifers were housed and, therefore, grouped according to each individual's growth rate and development as subjectively evaluated by the herd manager. As heifers became more mature and uniform in their development, they were housed in larger groups. Most heifers were grouped six times. The fastest growing heifer was only grouped five times before being bred, while another heifer was grouped eight times before being bred. Table 1 shows mean ages that correspond to each grouping. At each grouping, individual animals were sampled the day of movement (grouping) and one week and two weeks later. The same samples were collected as described above, except sera was not obtained for total immunoglobulin concentration.

Serology

Viral neutralizing antibody titers for BHV-1, PI_3V, BVDV and BRSV were determined by standard microtiter virus neutralization assay as previously described.[13] Antibodies to *M. haemolytica* whole bacteria and leukotoxin, and to *P. multocida* outer membrane proteins (OMPs), were determined by enzyme-linked immunosorbent assays (ELISAs) as previously described.[6]

Virus isolation using PBL and nasal swabs

Peripheral blood leukocytes (PBL) were obtained from whole blood by centrifugation, harvesting the buffy coat and treating with 0.17M NH_4Cl to lyse all red blood cells. The resulting PBL were stored frozen until inoculation for virus isolation. Nasal swabs were expunged into 2 ml of minimum essential medium (MEM), filtered through a 0.45 µ filter and stored frozen at -94°F (-70°C) until inoculation. For virus isolation, samples were inoculated (150 µl/well) into 24-well cell culture plates seeded the previous day with bovine turbinate (BT) cells

Table 1. Mean age for heifers at each grouping and associated contact with other cattle.

Groupings	Mean age[a] (days ± SD)	Age ranges (days)	Nose-to-nose contact
Hutch			
1	62.3 ± 5.9	47-73	no
2	95.2 ± 25.4	54-144	yes
3	134.5 ± 36.1	89-211	yes
4	192.3 ± 60.2	119-287	yes
5	245.3 ± 67.7	156-419	yes
6	347.2 ± 105.1	198-490	yes
7	441.9 ± 84.8	260-629	yes
8[b]	454.0	n/a	yes

[a] At time of grouping

[b] Contains one heifer

n/a - not applicable, only 1 animal involved in grouping 8

at 120,000 cells/well. Inoculated cultures were subcultured after 5-7 days incubation by trypsinization. After incubating the second passage for another 5-7 days, the presence of BVDV was detected by trypsinizing the cells, then spotting cells onto teflon-coated slides,[i] followed by fluorescent antibody staining using a BVDV-specific monoclonal antibody, as previously described.[22]

Bacterial isolation and identification

Nasal swabs were inoculated onto 5% sheep blood agar[j] following standard procedures.[20] Blood agar plates were incubated in a 5% CO_2 incubator at 98.6°F (37°C) for 18 hours. Bacterial isolates were identified by standard protocols.[20] For *Mycoplasma* isolation, nasal swabs were inoculated to FRIIS mycoplasma broth and agar[k] directly and at 1:100 and 1:1000 dilutions.

Statistical analysis

All serology data were analyzed using PC SAS Version 8.2 (SAS Institute, Cary, NC). Geometric means were used for the analysis. Analysis of variance procedures using PROC MIXED were performed assuming a completely randomized design with repeated measures. A REPEATED statement was used and an autoregressive period 1 covariance structure assumed. The factors in question were GROUP, which served as the main plot factor, and DAY, which served as the repeated measures factor. Individual cow's calves served as main plot experimental units. The interaction of GROUP and DAY (day of grouping) were assessed by the use of an LSMEANS statement with SLICE and DIFF options.

Colostral, vaccine-induced and infection-induced antibody titers (viruses) or antibody concentrations (bacteria) to the various pathogens were compared between days 0 through the sixth grouping by paired *t* tests.[19]

Results

Heifer morbidity

In 28 heifers (93.3%), passive colostrum-derived IgG_1 concentrations were >12 mg/ml, indicating adequate passive transfer.[3] In two heifers (#216 and #218) immunoglobulin concentrations were 0 or 6 mg/ml, indicating failure of passive transfer. All heifers remained clinically normal during the hutch and first-grouping periods. Space availability dictated the movement of eight heifers (#200, #203-209) for grouping two into a pen that had not been previously used for heifer development, and coincidently was located adjacent to several mature dry cows that were within two weeks of calving. Nose-to-nose contact and aerosolized transmission of potential pathogens was possible between the heifer calves and mature cows. Approximately one week after movement to this pen, all of these heifers developed clinical signs of BRD consisting of depression, weakness, decreased appetite, cough and nasal discharge. The heifers were treated with florfenicol[l] (18.2 mg/lb [6 mL/100 lbs] BW SQ; 40 mg/kg) and were clinically normal within seven days of treatment.

During the third grouping, two heifers (#216 and #222) developed clinical signs of BRD and were treated with ceftiofur hydrochloride[m] (1.0 mg/lb BW SQ; 2.2 mg/kg; dosage repeated in 48 hr). These heifers were clinically normal within five days of treatment.

During groupings four and five, one heifer (#195) was diagnosed with BRD and treated twice with antimicrobials, first with florfenicol and then with ceftiofur hydrochloride during a second episode. After the second course of therapy, the heifer was clinically normal and the manager reported the heifer developed normally.

Viral isolates in relation to groupings

No viruses were isolated while the heifers were housed or grouped in individual hutches (Figure 1), and BRSV was not isolated from any of the heifers during the study.

PI_3V was isolated 11 times during the study, beginning in the second grouping. Eight (72.7%) of those isolates occurred the day the heifers were moved into a new grouping (Figure 1). Two of the 11 PI_3V isolates were identified one week and two weeks after movement to a new grouping.

BVDV was isolated six times during the study, beginning in the first grouping (Figure 1). No BVDV was isolated more than one time from an individual heifer. Three isolates were identified in individuals in their first grouping, and the other three isolates were identified in individuals in their fifth grouping. The three heifers in the fifth grouping in which BVDV was isolated had been vaccinated one week earlier with a MLV vaccine containing BVDV.

BHV-1 was the most commonly isolated virus as it was detected 28 times from 17 calves beginning in the first grouping (Figure 1). Twenty-two of the 28 (78.6%) isolates were identified in the second or third grouping. When the BHV-1 virus was isolated, 14 of 17 (82.4%) calves were either in the pen adjacent to adult cows for one week or had just been moved from this pen to a new group. The other isolates were from three calves in grouping one, two or five; however, these heifers had no contact with the pen adjacent to the adult cows.

Bacterial isolates in relation to groupings

One-hundred sixty-one (161) nasal swabs were culture-positive for potentially pathogenic bacteria (Figure 1). Of those isolates, 125 (77.6%) were *M. haemolytica*, 34 (21.1%) were *P. multocida* and two (1.2%) were *H. somni*. Twelve of these isolates (nine *M.*

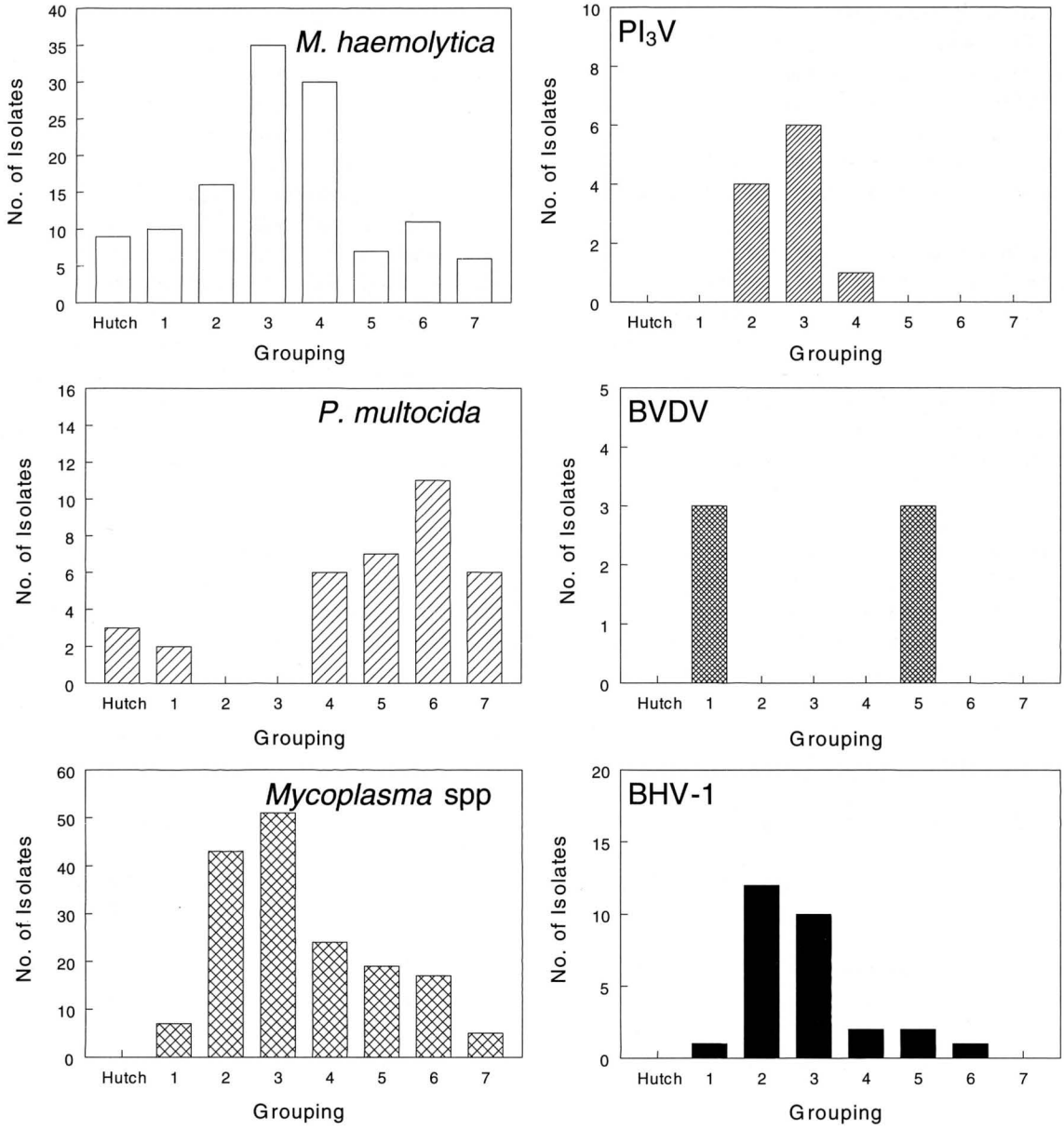

Figure 1. Isolations of infectious agents from calves at each grouping.[a]
[a]At each grouping, there were three isolation attempts (day 0, 7, 14) for each calf (30 heifers). Therefore, the number of isolations reflects a total of 90 attempts per grouping.

haemolytica and three *P. multocida*) were from 12 heifers while housed in individual hutches. *H. somni* was not isolated during this time. Two heifers were culture-positive for *M. haemolytica* at the 24-36 hour sampling period, when the calves had just been moved to their individual hutch from the maternity pasture.

M. haemolytica was isolated from at least one heifer in every grouping during the study, and every heifer enrolled in the study had at least one positive nasal culture for this bacterium (Figure 1). There was a distinct increase in number of isolates (16) starting in grouping two, with the peak number of isolates (35) occurring in grouping three. Twenty-one (70%) heifers in the study had at least one positive nasal culture for *M. haemolytica* during the second or third grouping. Of those heifers, 20 (95.2 %) were housed in the pen adjacent to adult cows and pregnant first-calf heifers, or had just been moved from that pen and placed into another grouping. There were 30 *M. haemolytica* isolates in grouping four, 21 (70%) of which were from individuals that had been culture-positive during grouping two or three. The other seven isolates were from six heifers previously negative for *M. haemolytica* during the second or third grouping. Of these six culture-positive heif-

ers, four had not been culture-positive for *M. haemolytica* in any of the previous groupings.

Only 35 isolates of *P. multocida* were identified from 20 calves during the study (Figure 1). Three isolates (8.6%) were cultured from three different heifers during the hutch period. Two of these isolates were identified one week after movement to the hutches, and the other isolate was identified two weeks after movement. *P. multocida* was not isolated from these three individuals during the remainder of the study. *P. multocida* was not isolated from any nasal specimen in the second, third or eighth grouping. The number of isolates dramatically increased from grouping four through six. *P. multocida* was isolated more than once in nine (30%) heifers. In these nine heifers, 24 isolates were identified during the study. *P. multocida* was isolated only once from 11 (36.7%) and not at all from 10 (33.3%) heifers during the entire study.

A total of 167 nasal samples were culture-positive for *Mycoplasma* spp isolates (Figure 1), with the first isolates occurring in grouping one. *Mycoplasma* spp were isolated the least number of times (twice) from heifer #193, and the most number of times (10) from heifer #221 and heifer #222. A dramatic increase in isolates occurred in groupings two and three. Twenty-eight (93%) heifers were identified as having at least one positive *Mycoplasma* spp culture during the second and/or third grouping. Overall, *Mycoplasma* spp were isolated at least once from all 30 calves.

Antibody responses within groupings

PI_3V neutralizing antibodies decreased significantly ($P < 0.05$) by day 14 while calves were in their hutches, with further significant reduction ($p < 0.05$) by day 0 of the first grouping (Figure 2). PI_3V neutralizing antibodies declined significantly in heifers in grouping two from day 0 to days 7 ($p < 0.01$) and 14 ($p < 0.05$). An interesting and significant increase in neutralizing titers was observed during the fifth grouping from day 0 to days 7 ($p < 0.05$) and 14 ($p < 0.01$). This increase occurred in the grouping after the heifers were vaccinated with a MLV vaccine containing PI_3V (during grouping four). The nadir for geometric mean PI_3V antibody titer occurred during the third grouping, and was followed by a small yet significant increase ($p < 0.01$) in titer following vaccination during the fourth grouping. A significant increase in neutralizing antibody titers was observed during the fifth grouping from day 0 to days 7 ($p < 0.05$) and 14 ($p < 0.001$). This increase occurred after the heifers were vaccinated during grouping four

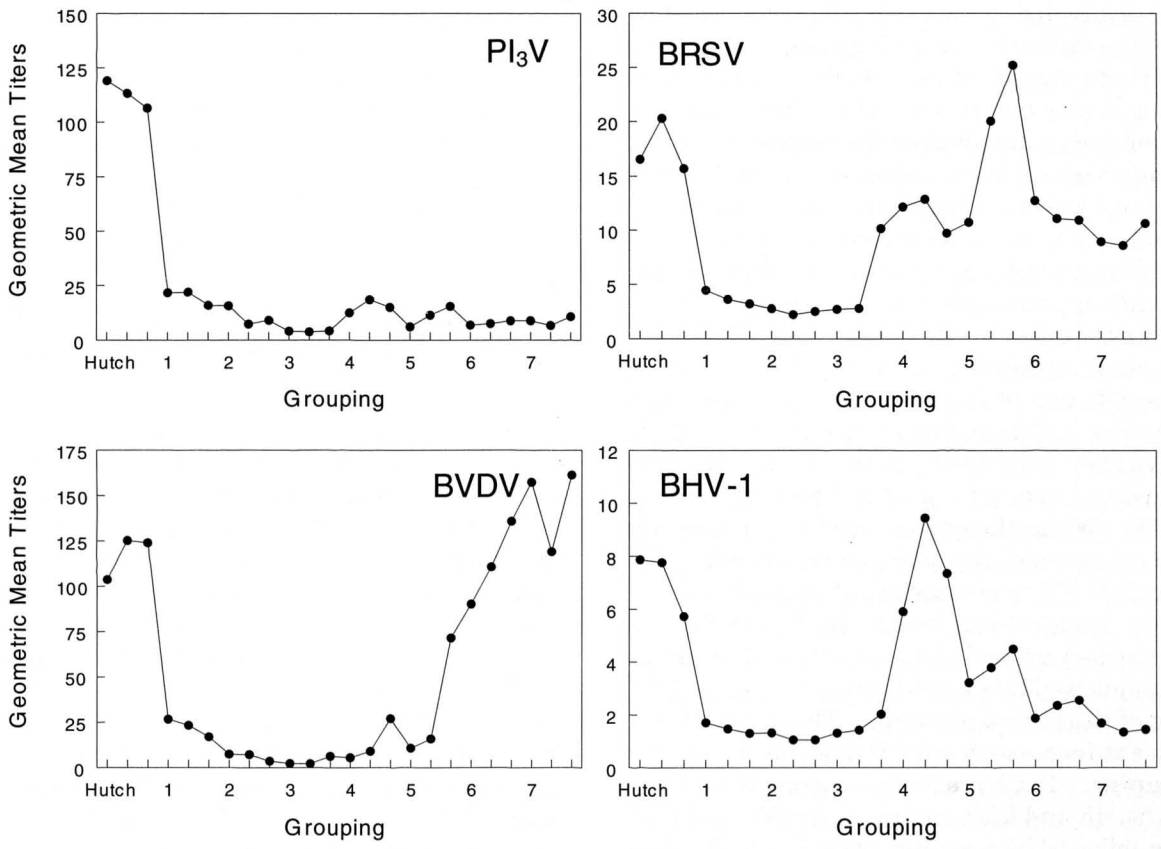

Figure 2. Geometric mean neutralizing antibody titers from calves at each grouping.

using a MLV vaccine containing PI_3V. Subsequently, antibody titers declined and remained low throughout the study.

BRSV neutralizing antibody titers were relatively low after ingestion of colostrum compared to PI_3V and BVDV titers. By day 14 in grouping three, the neutralizing antibody titers were significantly higher than during the preceding sampling times for this grouping ($p < 0.0001$). In the fifth grouping, the mean titer on day 0 was significantly less than on the two subsequent samplings ($p < 0.05$ day 7, $p < 0.01$ day 14). There were decreased neutralizing antibody titers on day 14 of grouping two, and increased antibody titers on day 14 of groupings three, four and five compared to day 0 ($p < 0.05$, <0.01, <0.01, <0.01, respectively) and day 7 ($p < 0.05$, <0.01, <0.01, <0.01, respectively).

BVDV neutralizing antibodies decreased significantly ($P < 0.001$) by the first grouping (Figure 2). The nadir for geometric mean BVDV antibody titer occurred during the third grouping, and was followed by a small yet significant increase ($p < 0.05$) in titer following vaccination during the third grouping. Subsequently, antibody titers significantly increased through the sixth grouping. There was a significant increase ($p < 0.05$) in antibody titers in grouping four, followed by a further increase in titers ($p < 0.05$) during grouping five, reaching a maximum by grouping seven.

BHV-1 neutralizing antibody titers were the lowest of any of the viral titers after ingestion of colostrum (Figure 2). Those titers decreased significantly ($p < 0.05$) by the first grouping and reached the nadir for geometric mean antibody titer during the second grouping. Neutralizing antibody titers increased significantly ($p < 0.01$) by day 14 of the third grouping and further increased ($p < 0.05$) by day 7 of the fourth grouping. This was followed by a significant decline ($p < 0.05$) in antibody titers during the fourth and fifth groupings.

Serum antibodies to *M. haemolytica* whole cells (WC) were significantly higher on day 14 in grouping two from day 0 to day 14 ($p < 0.05$), grouping three from day 0 to day 7 ($p < 0.01$) and day 0 to day 14 ($p < 0.01$), grouping five from day 0 to day 14 ($p < 0.05$) and grouping six from day 0 to day 14 ($p < 0.05$) and day 7 to day 14 ($p < 0.05$). While the calves were in hutches, the serum antibodies to *M. haemolytica* whole cells (WC) and leukotoxin (LKT) decreased significantly ($p < 0.001$) below day 0 concentrations by day 14 (Figure 3). The nadir was reached around the time of the first grouping, and was followed by a significant increase ($p < 0.05$) by the time of the second grouping. This was followed by a significant increase ($p < 0.05$) in antibodies by the fourth grouping. Peak antibody responses occurred during the fourth and fifth groupings for WC and LKT, respectively, followed by a significant decline by the sixth grouping.

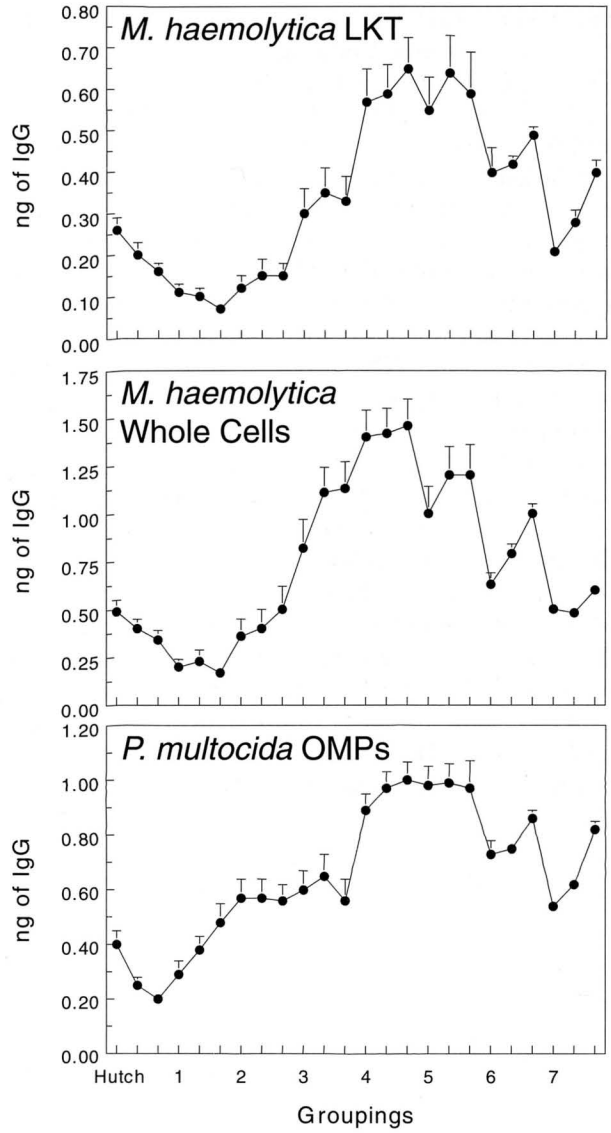

Figure 3. Mean antibody responses (± SEM) to *M. haemolytica* leukotoxin (LKT) and whole cells and to *P. multocida* outer membrane proteins (OMPs).

Serum antibodies to *P. multocida* OMPs decreased significantly ($p < 0.001$) below day 0 concentrations by day 7 while in hutches, reaching the nadir by the first grouping (Figure 3). This was followed by a significant increase in antibodies by the second grouping, followed by a further significant increase ($p < 0.05$) by the fourth grouping. Outer membrane protein antibodies (OMPs) were also lowest within groupings four and five at day 0 than other sampling times during the respective groupings ($p < 0.05$ day 0 to day 14 grouping four, < 0.01 day 0 to day 14 grouping five, and < 0.05 day 7 to day 14 grouping five). This was followed by a significant decline by the sixth grouping. There was a significant increase in OMPs antibodies

in grouping seven day 0 to day 14 ($p < 0.01$) and day 7 to day 14 ($p < 0.01$).

Discussion

To keep growing heifers healthy, they are commonly isolated from older cattle during their first year of life. Heifers are often moved from hutches to an isolated area on the dairy for the first grouping. To better understand transmission of respiratory disease among dairy calves, we conducted this study to determine critical times of exposure to respiratory pathogens, and which pathogens are commonly transmitted to dairy calves. Similar to the findings of other researchers,[29] we found that infection with respiratory pathogens was not always associated with clinical disease in a closed dairy herd.

Isolation of PI_3V or antibody response to the virus in healthy cattle is not uncommon. In several studies, investigators failed to associate PI_3V infection with clinical respiratory disease.[4,27,28] The source of PI_3V in the four heifers that were positive during the first grouping (age 62.3 ± 5.9 days) was unknown. A positive PI_3V culture potentially could have been from vaccine virus; however, heifers were not vaccinated until the fourth grouping (age 192.3 ± 60.2 days). It is possible that handlers or fomites transmitted the virus. A shortage of space dictated movement of some heifers for grouping two into a pen not previously used for heifer development. This pen allowed nose-to-nose or aerosol contact with mature cows, and PI_3V was isolated from several heifers. Ten of the 11 PI_3V isolates were from heifers that were housed in this pen prior to the positive samples obtained the day of movement to a new grouping. It is possible that acute subclinical PI_3V infection was ongoing within the mature cow herd, and the adult cows shed the virus to the younger calves.

BRSV can cause severe respiratory disease in dairy cattle.[2,5,9,27] Calves developed antibodies to BRSV during their third and fourth groupings (age 134.5 ± 36.1 and 192.3 ± 60.2 days, respectively), indicating either response to vaccine previously administered or natural exposure to BRSV-infected adult cattle. Because vaccination times varied somewhat among cattle, it is not certain if calves developed antibodies to vaccine or from natural exposure. It is known that BRSV infections can persist indefinitely in dairy herds.[7] There was no corresponding clinical evidence of BRSV infection in these heifers.

BVDV is an important pathogenic virus because it can produce acute, chronic or persistent infection in cattle.[14] BVDV's association with respiratory disease is well documented.[13] Because BVDV was not isolated more than once from any individual animal, we concluded there were no persistently infected heifers in this group.[14] In the first grouping (age 62.3 ± 5.9 days), three calves tested positive for BVDV by isolation in PBL. BVDV was only recovered once from each of the heifers. No heifer received any vaccine nor were they housed adjacent to adult cattle or any other animals that could have been a source of infection at that time. Persistently infected animals have not been identified in this herd.

In the rare event any animal from the farm was transported to an exhibition or to a veterinary hospital for treatment, the animal was isolated from the rest of the herd to minimize potential disease transmission. The source of exposure to BVDV that occurred in the group-one heifers is, therefore, unknown. The other three isolates of BVDV occurred in three heifers in grouping five (age 245.3 ± 67.7 days), one week after movement and vaccination with a MLV vaccine containing BVDV. It was probable that these three BVDV isolates were not field viruses, but instead vaccine virus isolated one week after vaccination.[13]

BHV-1, like other herpesviruses, can develop latent infections.[10] Cattle that have recovered from BHV-1 infection are generally considered immune but latently infected; under stressful conditions, latent carriers can shed the virus. In the present study, the majority (78.6%) of BHV-1 isolates were recovered from heifers in a pen adjacent to the periparturient adult cows, or shortly after being moved from this pen. Impending parturition is a stressful time in a cow's life, and may have stimulated shedding of BHV-1 to the heifer calves in the adjacent pen.

Results of this study, both via culture and serological evidence, indicate viruses were acquired after heifers were weaned from hutches and moved to different groupings. Housing young calves in individual hutches minimizes or eliminates exposure to viral respiratory pathogens, and therefore improves health.

In contrast, *M. haemolytica* and *P. multocida* were both isolated from calves while housed in hutches. To the authors' knowledge, there is no evidence that *M. haemolytica* is transmitted vertically during gestation from the dam to the offspring. Susceptible calves most likely acquired the pathogen during contact with cattle shedding the organism in nasal secretions or from contact with nasal droplet-contaminated fomites.[12] *M. haemolytica* then becomes part of the normal nasopharyngeal flora. The two positive nasal cultures of *M. haemolytica* isolated at the first sampling were most likely acquired shortly after birth and before movement of the calves to their individual hutches. Most dairies, including the herd in this study, house cows and/or pregnant heifers in the same area, pen or pasture. The *M. haemolytica* isolates may have originated from the calf's dam or from another cow in the same pen that was shedding the organism. Alternatively, an animal handler

may have mechanically transmitted the organism from another animal to the newborn calf during movement from the maternity pen to the calf hutch.

There was a marked increase in the number of *M. haemolytica* isolates from calves during the second and third grouping (age 95.2 ± 25.4 and 134.5 ± 36.1 days, respectively). Location of facilities that allows exposure of susceptible heifers to potential pathogens plays a substantial role in the pathogenesis of infectious diseases. Only three (10%) of the heifers did not shed detectable levels of *M. haemolytica* from the nasal mucosa during the second and third grouping. However, nasal culture for *M. haemolytica* can be negative when the organism is present in low numbers or when imprecise laboratory methods are used. Whether the *M. haemolytica* isolates were originally transmitted from one of the two heifers infected first or from adjacently housed cows and calves is not known. Employment of molecular epidemiological tools, such as ribotyping, may have answered that question.[17]

There were fewer isolates of *P. multocida* compared to *M. haemolytica* during the study. This was somewhat surprising because *P. multocida* is a more common isolate from dairy calf pneumonia than is *M. haemolytica*.[30] Heifers in this study experienced minimal clinical disease, which may have been reflected in the fewer number of isolates. It has been postulated that *P. multocida* is an opportunistic pathogen that overgrows the lung previously damaged by other bacteria, such as *M. haemolytica*.[1] In fact, *P. multocida* is usually associated with a subacute to chronic bronchopneumonia, whereas *M. haemolytica* is associated with more acute to subacute fibrinous pleuropneumonia.[16] Like *M. haemolytica*, *P. multocida* is considered to be a normal inhabitant of the nasal passages of cattle, and most of these heifers were exposed by nose-to-nose contact with adult cattle during the second or third grouping. Therefore, they should have had ample exposure to this pathogen and increased positive isolations during this time period. We did not, however, isolate this pathogen until later in the groupings. It is probable that there are other factors required for *P. multocida* to colonize the nasal passage in growing heifers. It is also possible that *P. multocida* was present, but it was not successfully isolated in the laboratory.

In previous studies, 19 to 91.4% of dairy calves were positive for *Mycoplasma* spp when nasal samples were cultured.[23,24,25] In our study, *Mycoplasma* spp were isolated from all heifers, and there was a marked increase in the number of *Mycoplasma* spp isolates between the second and third grouping (age 95.2 ± 25.4 and 134.5 ± 36.1 days, respectively). Only two (7%) of the heifers did not have a positive *Mycoplasma* spp nasal culture during the second and third groupings. This increase mirrored the increased number of *M. haemolytica* isolates. Not all *Mycoplasma* spp are pathogenic to cattle, and *Mycoplasma bovis* is considered to be among the most pathogenic bovine isolates.[26] Unfortunately, the laboratory lacked the capability to speciate the isolates. However, the information is still relevant because it suggests the spread can be rapid and substantial when susceptible heifers are housed in facilities adjacent to older cattle that may be carriers of *Mycoplasma* spp.

Although several viral and bacterial pathogens were isolated from the nasal cavity of these heifers, there was little clinical BRD observed during the study. Interestingly, the two heifers with failure of passive transfer did not have more clinical disease than the heifers with adequate colostral transfer. However, based on viral and bacterial isolations and antibody responses, it was obvious that subclinical infections occurred, and subclinical infections could potentially have a negative impact on growth and performance of developing heifers. The cause of the respiratory outbreak in the second grouping was not determined with certainty. There was no antibody response to any of the agents in the calves treated for BRD. During this time, however, *M. haemolytica*, *Mycoplasma* spp, PI_3V and BHV-1 were isolated from numerous calves. It is probable that the cause of clinical disease was multifactorial.

Conclusions

Despite the fact that calves did not have nose-to-nose contact with their dams or other cattle, *M. haemolytica* and *P. multocida* were isolated from the nasal cavities of several calves while they were in hutches. Other potentially important bacterial and viral respiratory pathogens were isolated somewhat later, with most occurring at 3.5-4.5 months of age in heifers that appeared clinically normal. Therefore, health management and disease prevention practices, including expeditious removal of the calf from the dam after parturition, earlier prophylactic vaccination, and isolation from adult animals as long as possible, may need to be incorporated on dairies earlier than commonly practiced. It is obvious that young, growing replacement heifers are exposed to potential pathogens throughout their development and groupings from the individual hutches to breeding.

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Footnotes

[a] Colostrometer™, Biogenics, Mapleton, OR 97453
[b] VMRD, Inc., Pullman, WA 99163
[c] TSV-2®, Pfizer Animal Health, New York, NY 10017
[d] Vision® CD-T with SPUR®, Intervet Inc, Millsboro, DE 19966
[e] Bovi-Shield® 4+L5, Pfizer Animal Health, New York, NY 10017
[f] Vision® 7 with SPUR®, Intervet Inc, Millsboro, DE 19966
[g] Brucella Abortus Vaccine, Strain RB-51 Live Culture, Professional Biological Co, Denver, CO 80216
[h] Presponse® HM, Fort Dodge Laboratories Inc, Fort Dodge, IA 50501
[i] Cell-line Associates, Newfield, NJ 08344
[j] Becton Dickinson and Company, Sparks, MD 21152
[k] Oklahoma Animal Disease and Diagnostic Laboratory, Stillwater, OK 74078
[l] Nuflor® Injectable Solution, Schering-Plough Animal Health Corp, Union, NJ 07083
[m] Excenel® RTU, Pharmacia & Upjohn Co, Kalamazoo, MI 49001

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