Epidemiological Study of Enzootic Pneumonia in Dairy Calves in Saskatchewan

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ABSTRACT

A field study involving 325 calves from 17 dairy herds in Saskatchewan was conducted to determine the risk of enzootic pneumonia and to assess its association with a number of factors. Two different case definitions of pneumonia were used in the analyses: the first was based on producers' treatment risk (CASE1) and the second was based on semimonthly clinical examinations of calves by the research veterinarian (CASE2). The risk of pneumonia based on CASE1 was 39% and on CASE2 was 29%. The measure of agreement between CASE1 and CASE2 at the calf level of analysis was poor (kappa = 0.24, SE = 0.02) and at the herd level of analysis was moderate (kappa = 0.40, SE = 0.12). The mortality risk from pneumonia was 1.8% and a variety of infectious organisms were isolated from pneumonic lungs.

Twenty-seven percent of the calves had inadequate (total IgG ≤ 800 mg/dL) levels of passively acquired antibodies as measured by radial immunodiffusion. The proportion of seropositive titers in calves within the first two weeks of age was 94% to parainfluenza 3 virus (PI3V) and bovine respiratory syncytial virus (BRSV), 73% to *Pasteurella haemolytica* (Ph), 68% to bovine viral diarrhea virus (BVDV), 67% to infectious bovine rhinotracheitis virus (IBRV), 46% to *Mycoplasma dispar* (Md), 44% to *Haemophilus somnus* (Hs), and 21% to *Mycoplasma bovis* (Mb).

At the calf level of analysis and after adjusting for clustering, there was a negative association (p = 0.10) between the diagnosis of pneumonia based on CASE2 and total IgG levels and Ph titers (rPh). There were no significant associations between serological titers in calves within the first two weeks of age and CASE1 at the calf level of analysis or CASE1 and CASE2 at the herd level of analysis.

At the calf level of analysis, girth growth during the first month of age was positively associated with serological titers to total IgG (SPIgG), Ph (SPPh), and Md (rMd) and negatively associated with CASE1 and CASE2. At the herd level of analysis, girth growth was negatively associated with titers to Mb (SPMb), Hs (median) and PI3 (median).

At the herd level of analysis, CASE1 and CASE2 were positively associated with the number of calves in the herd and the proportion of Holstein calves relative to dairy and beef crossbred calves in the herd.

In summary, enzootic pneumonia was a common disease in calves from 17 dairy herds in Saskatchewan based on producers' treatment records and semimonthly clinical examinations of calves by the research veterinarian. The measure of agreement in clinical diagnosis for pneumonia between the producers and the veterinarian, however, was poor, indicating that a more objective case definition of disease should be developed to help improve the validity of epidemiological studies. Furthermore, pneumonia clustered strongly within herds, which indicates that future studies may need many more farms than studied here to get a better understanding of the factors associated with disease.

RÉSUMÉ

Une étude faite sur le terrain et impliquant 325 veaux provenant de 17 troupeaux laitiers de Saskatchewan a été réalisée pour déterminer le risque de pneumonie enzootique et pour évaluer son association avec divers facteurs de risque. Deux définitions d'un cas de pneumonie furent utilisées, l'une basée sur le taux de traitement par le producteur (CASE1) et l'autre sur l'examen clinique effectué 2 fois par mois par le vétérinaire (CASE2). Le risque de pneumonie selon le producteur fut de 39 % et de 29 % selon l'examen vétérinaire. L'association entre les deux mesures était faible au niveau de l'animal (Kappa = 0,24, SE = 0,02) et modérée au niveau du troupeau (Kappa = 0,4, SE = 0,12). Le risque de mortalité spécifique due à la pneumonie fut de 1,8 % et plusieurs agents infectieux furent isolés des poumons.

L'immunodiffusion radiale des IgG a montré un niveau inadéquat (800 mg/dL) des anticorps acquis passivement chez 27 % des veaux. La proportion de veaux séropositifs pendant leurs 2 premières semaines de vie fut de 94 % contre les virus parainfluenza 3 (PIV3) et syncitial respiratoire bovin (BRSV), de 73 % contre le virus de la diarrhée bovine (BVDV), de 67 % contre le virus de la rhinotrachéite infectieuse bovine

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(IBRV), de 46 % contre *Mycoplasma dispar* (Md), de 44 % contre *Haemophilus somnus* (Hs) et de 21 % contre *Mycoplasma bovis* (Mb). Une association négative (p = 0,1) fut trouvée, au niveau du veau et après correction pour l'aggrégation, entre le diagnostic de pneumonie (CASE2) et le niveau de IgG totale et le titre contre Ph. Dans l'analyse de niveau troupeau, aucune association ne fut trouvée chez les veaux entre les titres trouvés jusqu'à deux semaines d'âge et les diagnostics de pneumonie.

Dans l'analyse au niveau de l'animal, la croissance pendant le premier mois de vie était positivement associée au niveau des IgG (SPIgG), aux titres de Ph (SPPh) et de Md (rMd), et était négativement associée aux deux diagnostics de pneumonie. Dans l'analyse du niveau troupeau, la croissance était négativement associée aux titres contre Mb (SPMb), Hs (médiane) et PI3 (médiane).

Au niveau du troupeau, les proportions des diagnostics positifs de pneumonie (CASE1 et CASE2) étaient positivement associées au nombre de veaux Holstein par rapport aux veaux croisés de type laitier ou boucher.

En résumé, la pneumonie enzootique était une maladie fréquente chez les veaux de 17 élevages de bovins de type laitier en Saskatchewan selon les dossiers de traitement des producteurs et les diagnostics vétérinaires faits aux deux semaines. Il y avait faible concordance entre la mesure diagnostique du producteur et celle du vétérinaire, ce qui indique la nécessité d'une définition de cas valide dans la pratique épidémiologique. Les cas de pneumonie présentaient une forte aggrégation correspondant aux troupeaux. Ceci indique la nécessité de conduire des études épidémiologiques sur le sujet comportant le suivi d'un plus grand nombre de troupeaux. (*Traduit par Dr Michel Bigras-Poulin*)

INTRODUCTION

Enzootic pneumonia is one of the most common infectious diseases of dairy calves (1,2). The disease occurs frequently in calves from one to five months of age (3), with the highest incidence during the fall and winter seasons in housed calves (1,2). In severe outbreaks of pneumonia, 80% to 90% of the calves in a herd may be affected, yet death rates are usually less than 5% (1,2).

The occurrence and severity of enzootic pneumonia may depend on a series of complex interactions between several different infectious agents, environmental factors, and the immunological status of the calf (1–4). To date, there have been no reported attempts to describe the incidence or identify risk factors of enzootic pneumonia which occur under the type of management systems used in Saskatchewan.

The purpose of this field study was to describe the risk of pneumonia in calves in some Saskatchewan dairy herds, and to investigate both individual calf and herd risk factors for pneumonia. The serological factors we investigated for their relationship to risk were passively acquired total IgG levels and specific antibody titers to: infectious bovine rhinotracheitis virus (IBRV), parainfluenza 3 virus (PI3V), bovine respiratory syncytial virus (BRSV), bovine viral diarrhea virus (BVDV), *Pasteurella haemolytica* (Ph), *Haemophilus somnus* (Hs), *Mycoplasma bovis* (Mb), and *Mycoplasma dispar* (Md). Additional factors investigated for their association with risk of pneumonia included the number of calves in the herd, calf breed (BREED), calf sex (SEX), treatment for scours (SCOUR), cow vaccination (IBRVAC, BRSVVAC, BVDVAC), calf housing (HOUSING), and girth growth.

MATERIALS AND METHODS

STUDY DESIGN

A questionnaire was given to 250 dairy producers in the vicinity of Saskatoon via their milk truck drivers to gather preliminary baseline information on the occurrence of enzootic calf pneumonia and to identify willing participants for a more detailed study. From the 57 respondents, 20 were chosen to participate in a detailed field study based on their willingness to cooperate and their close location to Saskatoon.

Beginning in November 1989, the 20 dairy herds were visited every two weeks until April 30, 1990. All calves born from November 1, 1989 to March 12, 1990 were included in the study and followed during that time frame until six months of age or April 30, 1990. At each visit, calves were examined by the first author for the presence of pneumonia by using a clinical scoring system. The following clinical signs were recorded: rectal temperature, depression, respiration rate, ocular and nasal discharges, abnormal lung sounds on auscultation of the thorax, and cough illicited by tracheal palpation. A heart-girth measurement was taken to assess calf growth. Blood samples were collected from all calves when they were between 1 and 14 days of age to measure total serum IgG levels and specific antibodies to IBRV, PI3V, BRSV, BVDV, Ph, Hs, Mb and Md. Factors affecting colostrum intake were not assessed because of the difficulty in measuring them accurately on farms.

The dairy producers identified each calf at birth with an eartag and maintained calving and individual treatment records for the occurrence of pneumonia and scours. We recorded the type of calf housing at each farm. Since calf housing was not the same for each calf on a farm and it changed depending on the environmental temperatures, we could only create a binary variable for this risk factor. Calf housing was considered a 1 if calves shared a common airspace with the cows and a 0 if they had a separate airspace from the cows. All calves that died were sent to the Western College of Veterinary Medicine for necropsy. All necropsies were performed by the same pathologist.

SEROLOGICAL METHODS

Total passive IgG levels in serum were measured by radial immunodiffusion (VMRD Inc., Veterinary Medical Research and Development, Pullman, Washington). Total IgG levels $\leq$ 800 mg/dL were indicative of failure of passive transfer (VMRD Inc.).

Sera were also analyzed for specific antibodies to IBRV, PI3V, BRSV, BVDV, Ph, Hs, Mb and Md using enzyme-linked immunosorbent assay

(ELISA) techniques described previously (5–7). The proportion of seropositive titers was defined as the proportion of positive titers in serum collected within the first two weeks of life. Titers to IBRV, PI3V, BRSV and BVDV > 10 units, and titers to Ph and Hs > 1000 units were considered to be positive titers (laboratory standards). Mycoplasma titers were considered to be positive if the optical density values were two standard deviations above the mean value of the negative controls.

Serological titers were analyzed as a continuous variable and as a binary variable (seropositive). As a continuous variable, titers were not normally distributed; therefore, they were transformed using rank procedures (8,9).

STATISTICAL METHODS

All data were entered into databases in the Statistical Analysis System (SAS/STAT 1989, Version 6, SAS Institute Inc., Cary, North Carolina) and the Statistix Analytical Software program (STATISTIX Version 3.5, 1991, Analytical Software, St. Paul, Minnesota). Three farms were deleted from the analysis because ≤ 5 calves were examined and we thought this number was insufficient to establish a reliable risk of disease on these farms. In total, 325 calves from 17 dairy herds were included in the final analyses.

The outcome variables were pneumonia according to the producers' treatment records (CASE1), pneumonia according to semimonthly clinical examinations by the research veterinarian (CASE2), and girth growth (cm/day) during the first, second and third month of life. The case definition of pneumonia, as diagnosed by the first author (CASE2), included the presence of depression, in addition to any two or more of the following clinical signs: cough, fever (rectal temperature > 39.5°C), tachypnea (respiration rate > 40 breaths/min), increased anteroventral lung sounds or wheezes. Pneumonia risks were measured over the entire study period.

For group level analysis, treatment risks for pneumonia and for scours were calculated as the proportion of calves treated by the producer during the study period. Recurrence of disease was defined as retreatment of a calf by the producer for the same disease at a later date. Disease risks, proportion of female calves, proportion of Holstein calves relative to dairy and beef crossbred calves, average girth growth, proportion of failure of passive transfer, proportion of seropositive titers, and median titers were calculated by herd for group level analysis.

SIMPLE ANALYSES

Separate analyses were performed for the two outcomes of pneumonia, CASE1 and CASE2, and girth growth. Simple associations between these outcomes and risk factors at both the individual and the herd level were investigated using chi-square tests, univariate logistic regression, *t*-test statistics, ANOVA and median or rank sum tests (8–11).

The measure of agreement between CASE1 and CASE2 was assessed using the kappa statistic (12). To assess agreement at the herd level, a case was defined as a farm with a morbidity risk of ≥ 10% with control farms having a morbidity risk of < 10%.

REGRESSIONS ANALYSES

Individual calf level — The association between pneumonia (CASE1 or CASE2) and risk factors was assessed using logistic regression (13) and the association between girth growth and risk factors was investigated using least squares linear regression (10). Farm, breed and sex were forced into the model of girth growth and pneumonia to control for confounding. For the same reason, farm was forced into the model of girth growth and serological titers.

Tests for homogeneity of binomial samples were used to determine whether the proportion of calves with pneumonia varied significantly from herd to herd. The tests were significant ($p < 0.05$), indicating there was a "herd effect" with respect to pneumonia. Such a herd effect, if left uncorrected in the individual level analysis, would lead to an underestimate of the size of the standard error in a statistical model, which could result in spuriously significant variables being included in the final model (14). To correct for this herd effect, the intracluster correlation coefficient was calculated for CASE1 and for CASE2 and used to adjust the standard error of variables within the respective logistic models (15).

Group (herd) level — Least squares regression analysis (10) was used at the herd level for each pneumonia outcome, CASE1 and CASE2. The logit of pneumonia[1] was regressed on serological data (proportion of seropositive titers or median titers) or on other risk factors, namely herd size (number of calves), proportion of Holstein calves relative to dairy and beef crossbred calves, proportion of female calves, treatment risk for scours, and proportion of failure of passive transfer. These models were weighted by the inverse of the adjusted variance[2].

Two models were developed in a forward stepwise manner for average girth growth. Girth growth was regressed on serological data (proportion of seropositive titers or median titers), and it was regressed on the proportion of Holstein calves, proportion of female calves, proportion of failure of passive transfer, and risk of pneumonia (CASE1 or CASE2).

The fit of regression models was assessed using the Pearson Chi-Square and deviance, and graphical examination of the residuals (10,13).

RESULTS

The risk of CASE1 was 39% (SE = 0.03) and the reoccurrence risk was 56% (SE = 0.04). The median age of CASE1 was 27 days and the age at reoccurrence was 34 days. The risk of CASE2 was 26% (SE = 0.02) and the reoccurrence risk was 29% (SE = 0.05). The median age of CASE2 was 36 days and the age at reoccurrence was 58 days. The risk of CASE1 and CASE2 varied significantly ($p < 0.05$) among herds (Table I). The measure of agreement (kappa) between CASE1

[1]logit of pneumonia = log [(pneumonia + 0.5)/(number of calves − penumonia + 0.5)]

[2]adjusted variance = variance * [1 + (number calves − 1) * intracluster correlation coefficient]. Variance = [(number calves + 1) * (number of calves + 2)]/[number calves * (pneumonia + 1) * (number calves − pneumonia + 1)].

TABLE I. Disease risks, girth growth, passive seropositive proportions and median antibody levels to several respiratory pathogens in dairy calves at the herd level

Outcome	Median	Range
Number of calves	15	6–57
CASE1 %[a]	17	0–91
CASE2 %[b]	17	0–55
Pneumonia mortality %	0	0–7
Scour treatment risk %	27	0–62
Girth growth (cm/day)		
— 1st month of age	0.27	0.17–0.37
— 2nd month of age	0.31	0.18–0.39
— 3rd month of age	0.30	0.21–0.38
Seropositive proportion %[c]		
— IgG (> 800 mg/dL)	84	33–100
— IBRV	67	5–100
— PI3V	100	79–100
— BRSV	100	63–100
— BVDV	64	32–100
— Ph	80	0–92
— Hs	27	1–100
— Mb	16	0–64
— Md	45	0–79
ELISA titer		
— IgG (mg/dL)[d]	1400	600–2600
— IBRV	21	1–77
— PI3V	71	38–98
— BRSV	74	23–91
— BVDV	25	−5–93
— Ph	1500	250–16.000
— Hs	500	0–3000
— Mb[e]	0.07	0.01–0.17
— Md[e]	0.11	0.04–0.24

n = 17 dairy herds
[a]CASE1 = producer diagnosed pneumonia
[b]CASE2 = veterinarian diagnosed pneumonia
[c]See text for definition of proportion of seropositive titers within the first 2 weeks of life
[d]Radial immunodiffusion
[e]Optical density

TABLE II. Level of agreement between producer (CASE1) and veterinarian (CASE2) diagnosis for enzootic pneumonia in dairy calves

		Producer diagnosed pneumonia			
		Yes	No	Total	Kappa (SE)
Calf level of analysis					
Vet diagnosed	Yes	50	34	84	0.24 (0.02)
pneumonia	No	78	163	241	
Total		128	197	325	
Herd level of analysis[a]					
Vet diagnosed	Yes	8	3	11	0.40 (0.12)
pneumonia	No	2	4	6	
Total		10	7	17	

[a]Herd was considered positive if the risk of pneumonia > 10%

and CASE2 at the calf level was poor (Table II) and at the herd level was moderate (Table II).

The mortality risk from pneumonia was 1.8% (SE = 0.007). A variety of different infectious agents were isolated from the pneumonic lungs of each calf, namely, BRSV, PI3V, Ph, *Pasteurella multocida*, and *Actinomyces pyogenes*.

The treatment risk for scours was 29% (SE = 0.02) and the median age at first treatment was eight days. The mortality risk from scours was 1.2% (SE = 0.006).

Twenty-seven percent of the calves had failure of passive transfer and this proportion ranged from 0% to 67% among herds (Table I). The median total IgG level in 325 calves was 1400 mg/dL. The overall proportion of seropositive titers was 67% to IBRV (SPIBR), 94% to PI3V (SPPI3) and BRSV (SPBRS), 68% to BVDV (SPBVD), 73% to Ph (SPPh), 44% to Hs (SPHs), 21% to Mb (SPMb), and 46% to Md (SPMd). The overall median ELISA serological titers were 26 to IBRV, 75 to PI3V, 74 to BRSV, 42 to BVDV, 1000 to Ph, 500 to Hs, 0.06 (optical density) to Mb and 0.11 (optical density) to Md. There were significant ($p < 0.05$) correlations ($r > 0.80$) between specific serological titers. The proportion of seropositive titers and median serological titers varied significantly ($p < 0.05$) among herds (Table I).

The effect of cow vaccination on serum antibody levels in calves within the first two weeks of age is shown in Table III. Calves from cows vaccinated with an IBRV vaccine (IBRVAC) were 2.44 times more likely to be seropositive to IBRV than those from unvaccinated cows ($p < 0.05$). There were no significant associations between PI3V (PI3VAC), BRSV (BRSVAC) and BVDV (BVDVAC) vaccination of cows and respective passively acquired antibody levels in calves.

The simple association between pneumonia (CASE1 or CASE2) and passive antibody levels in calves is shown in Table IV. CASE1 was positively ($p < 0.05$) associated with the proportion of seropositive titers to IBRV (SPIBR) and CASE2 was negatively ($p < 0.05$) associated with the proportion of seropositive titers to IgG (SPIgG) and to Ph (SPPh).

The significant ($p < 0.05$) associations at the calf level between CASE1 and risk factors are shown in Table V and between CASE2 and risk factors are shown in Table VI. After adjusting for clustering, there were no significant associations between CASE1 and any risk factors, whereas CASE2 was positively associated with BRSVVAC ($p = 0.07$) and negatively associated with SPIgG ($p = 0.10$) and ranked titers to Ph (rPh) ($p = 0.10$).

The significant ($p < 0.05$) unconditional associations between girth growth and risk factors at the calf level are shown in Table VII. Results of the conditional analysis for girth growth are shown in Table VIII. Girth growth was positively associated with SPIgG, SPPh, ranked titers to Md (rMd) and negatively associated with CASE1 and CASE2 (Table VIII).

At the group (herd) level of analysis, CASE1 and CASE2 were positively

($p < 0.05$) associated with the number of calves in the herd and the proportion of Holstein calves relative to dairy and beef crossbred calves in the herd (Table IX). Average girth growth was negatively ($p < 0.05$) associated with the proportion of seropositive titers to Mb and median titers to Hs and PI3V.

DISCUSSION

Enzootic pneumonia was a common disease in calves studied from 17 dairy herds in Saskatchewan based on producers' treatment records and semimonthly clinical examinations of calves by the research veterinarian. An important finding of this study was the poor agreement in clinical diagnosis of pneumonia between the producers and the veterinarian. The most likely explanation for this discrepancy is the frequency of observations of calves and misclassification bias (16). It is possible that calves were sick and recovered within the two week time frame that we examined them and that we misdiagnosed calves. Similarly, producers may have misdiagnosed cases of pneumonia. We observed that some producers would mass treat all calves when a few were sick whereas others failed to treat any calves which appeared ill. Since there is no gold standard for the diagnosis of enzootic pneumonia, we do not know which case definition is a more accurate reflection of the true disease status. Therefore, we have presented and discussed the findings for both outcomes of pneumonia with the hope that each case definition may teach us something about enzootic pneumonia, yet realizing that these two different subjective definitions of disease may in turn compromise the ability of this study to investigate risk factors associated with pneumonia.

Enzootic pneumonia, according to both case definitions, occurred most frequently within the first two months of life and the recurrence risk was high, similar to previous studies (17,18). Calves with pneumonia, according to either case definition, had reduced girth growth in the first month of life. However, we do not know whether reduced girth growth increased the risk of pneumonia or

TABLE III. Association between cow vaccination and serological titers in dairy calves within the first two weeks of life

Agent	Cow vaccination	n herds	Seropositive proportion %[a]	Odds ratio[b]	Chi-square[c]
IBRV	yes	8	77	2.44	12.79
	no	9	58		
	total	17	67		
PI3V	yes	8	96	2.06	2.04
	no	9	92		
	total	17	94		
BRSV	yes	1	97	2.91	1.15
	no	16	93		
	total	17	93		
BVDV	yes	2	58	0.62	2.46
	no	16	70		
	total	18	68		

[a]Any calf having a positive serological titer to the respective viral agent. See text for cutpoints
[b]If odds ratio > 1, proportion of seropositive titers is higher in the calves from vaccinated cows than from unvaccinated cows; if odds ratio < 1, proportion of seropositive titers is lower in calves from vaccinated cows than from unvaccinated cows
[c]$p \leq 0.05$ if $X^2 \geq 3.84$

TABLE IV. The association between passive seropositive proportions to several respiratory pathogens and enzootic calf pneumonia as diagnosed by the producer (CASE1) or by the veterinarian (CASE2). Odds ratio comparing sick calves to healthy calves

Agent	Seropositive[a] CASE1 % sick/healthy	Odds[b] ratio	Chi-[c] square	Seropositive CASE2 % sick/healthy	Odds ratio	Chi-square
IgG[d]	39/43	0.83	0.43	24/43	0 .41	9.44
IBRV	76/61	2.04	7.66	67/67	0 .98	0.003
PI3V	94/94	0.87	0.08	93/95	0 .72	0.42
BRSV	95/92	1.69	1.10	94/93	1 .11	0.04
BVDV	71/66	1.26	0.87	71/67	1 .22	0.49
Ph	71/75	0.81	0.63	62/77	0 .49	6.55
Hs	49/40	1.43	2.26	40/45	0 .80	0.68
Mb	24/20	1.33	1.01	17/23	0 .70	1.18
Md	46/46	0.97	0.02	37/49	0 .60	3.60

[a]Any calf having a positive titer. See text for definition of proportion of seropositive titers
[b]If odds ratio > 1, proportion of seropositive titers is higher in sick calves than healthy calves; if odds ratio < 1 then proportion of seropositive titers is lower in sick calves than healthy calves
[c]$p \leq 0.05$ if $X^2 \geq 3.84$
[d]Seropositive if total IgG level > 800 mg/dL

TABLE V. Univariate logistic regression of variables unconditionally associated ($p < 0.05$) with producers' treatment (CASE1) for enzootic pneumonia at the calf level of analysis

Variable	Coefficient	SE	p value	CSE[a]	p value[b]
BREED	−0.78	0.29	0.007	0.89	0.38
SEX	−1.08	0.24	0.0001	0.73	0.14
SCOUR	0.99	0.25	0.0001	0.77	0.20
HOUSING	0.49	0.24	0.04	0.75	0.51
IBRVAC	0.55	0.23	0.02	0.70	0.43
BRSVVAC	2.20	0.44	0.0001	1.33	0.18
BVDVAC	1.53	0.33	0.0001	1.00	0.13
SPIBR	0.71	0.26	0.006	0.79	0.37
rIBR[c]	0.004	0.001	0.002	0.004	0.30

[a]Standard error adjusted for clustering. Intracluster correlation coefficient is 0.463
[b]p value of T test adjusted for clustering
[c]Ranked titer to IBRV

TABLE VI. Univariate logistic regression of variables unconditionally associated ($p < 0.05$) with veterinarian diagnosed enzootic pneumonia (CASE2) at the calf level of analysis

Variable	Coefficient	SE	p value	CSE[a]	p value[b]
BRSVVAC	1.17	0.35	0.0009	0.64	0.07
BVDVAC	0.68	0.32	0.03	0.58	0.24
SPIgG	−0.90	0.30	0.003	0.54	0.10
SPPh	−0.72	0.28	0.01	0.52	0.17
rPh	−0.005	0.002	0.003	0.003	0.10
rMb	−0.003	0.001	0.03	0.003	0.25

[a]Standard error adjusted for clustering. Intracluster correlation coefficient was 0.129
[b]p value of T test adjusted for clustering

TABLE VII. Variables unconditionally associated with calf girth growth during the first month of life (n = 325)

Variable	Status	Average girth growth (cm/day)	T test p value
Producer diagnosed pneumonia (CASE1)	yes	2.20	0.001
	no	2.62	
Producer retreatment for pneumonia	yes	2.00	0.02
	no	2.47	
Veterinarian diagnosed pneumonia (CASE2)	yes	2.06	0.0001
	no	2.60	
BVDVAC	yes	1.96	0.0002
	no	2.55	
BRSVVAC	yes	2.02	0.006
	no	2.51	
SPIgG[a]	yes	2.61	0.0001
	no	2.05	
SPPh	yes	2.54	0.004
	no	2.13	
SPMd[a]	yes	2.61	0.02
	no	2.33	
SPPI3	yes	2.48	0.05
	no	1.70	
BREED	Holstein	2.51	0.03
	crossbred	2.16	

[a]Girth growth also associated ($p < 0.05$) with ranked variables

TABLE VIII. Least-squares linear regression results of girth growth during the first month of age regressed on disease and serological variables in dairy calves at the calf level of analysis

Model	Independent variables	R^2
1	0.02 + HERD[a] + 0.03BREED + 0.004SEX + 0.05SPIgG − 0.04CASE1[b]	0.32
2	0.22 + HERD + 0.03BREED + 0.005SEX + 0.05SPIgG − 0.05CASE2[c]	0.34
3	0.25 + HERD + 0.05SPPh	0.25
4	0.26 + HERD + 0.0002rMd	0.25

n = 325 calves
[a]Sixteen dummy variables created for herd. Coefficients not shown
[b]Producer diagnosed pneumonia
[c]Veterinarian diagnosed pneumonia

resulted from pneumonia. The overall mortality risk from pneumonia was 1.8% but it was extremely variable among herds. A variety of infectious organisms were isolated from pneumonic lungs of fatal cases, suggesting that disease may be caused by multiple infectious agents (1,2,19).

The overall treatment risk for scours was 29% and those calves treated for scours were at increased risk of subsequent treatment for pneumonia ($p = 0.0001$), in agreement with previous work (20,21). After adjusting for clustering, however, scours was no longer significantly associated ($p = 0.20$) with CASE1. This may be due to a strong "herd effect" which caused a reduction in effective sample size and power (15). There was no association between treatment for scours and girth growth.

The proportion of failure of passive transfer was 27% and it ranged from 0% to 67% among herds. Calves with failure of passive transfer were at increased risk of pneumonia according to CASE2 and they also had reduced girth growth during the first month of age, similar to that reported previously (4,21–23). It is possible that CASE1 was not associated with passive transfer because of misclassification bias of disease and of failure of passive transfer (16). Since blood samples were collected between 1 and 14 days of age, older calves may have been misclassified as failure of passive transfer because of the rapid decline in serum passive antibody levels over time (21).

Passively acquired antibody levels in calves to several infectious agents of enzootic pneumonia varied among herds. Sixty-seven percent of the calves had colostral antibody levels to IBRV and calves from cows vaccinated for IBRV were 2.44 times more likely to have colostral antibodies to IBRV than calves from unvaccinated cows, similar to the findings of Mechor *et al* (24). Colostrally acquired antibody levels to PI3V and BRSV were high and they were not associated with cow vaccination, probably because of the ubiquitous nature of these viruses (2). Vaccination of cows with BRSV was positively associated ($p = 0.07$) with CASE2. Only one cow herd was vaccinated for BRSV and immunization occurred prior to the initiation of this study because of a chronic problem with calf pneumonia.

Sixty-eight percent of the calves had antibodies to BVDV; however, this level varied among herds. Vaccination of cows with killed BVDV was not associated with higher colostral antibody levels in their calves. This finding was not unexpected based on other work showing a poor antibody response to killed BVDV vaccines (25).

The colostrally acquired antibody level in calves to Ph was 73%, suggesting that the dairy cattle in this study were commonly exposed to this bacteria. Titers to Hs, Mb and Md were moderately low, which may reflect a low exposure rate to these agents or failure of passive transfer in some calves.

After adjusting for "herd effects", the only significant associations between specific colostrally acquired antibody levels in calves and pneumonia at the calf level, were between

CASE2 and rPh ($p = 0.10$). As passively acquired antibody levels to Ph increased, the risk of CASE2 decreased, suggesting a protective role for colostral antibodies to Ph in enzootic pneumonia. This observation was further supported by a positive association between girth growth and SPPh. In feedlot respiratory disease, however, there are conflicting data on the association between sickness and titers to Ph and the reasons for these variations have been previously described by Allen *et al* (26).

At the calf level of analysis, girth growth was also positively associated with increasing levels of colostrally acquired antibodies to Md. Others (19,27,28) have associated Md with subclinical and clinical pneumonia in calves. At the herd level of analysis, girth growth was negatively associated with the proportion of seropositive titers to Mb and median titers to Hs and PI3V. The only plausible biological explanation we have for this finding is that colostral antibody levels reflected the presence of these agents in the herd and these agents caused subclinical disease which reduced girth growth (27).

Alternatively, this finding may represent a type 1 error (16). At the herd level of analysis, 69 multiple associations were evaluated and at the calf level of analysis, 81 multiple associations were evaluated. There could be about three "significant" ($p < 0.05$) associations at the herd level of analysis and four "significant" ($p < 0.05$) associations at the calf level of analysis that occurred only by chance. Therefore, any new associations should be considered only suggestive (16). However, previously reported associations should not become a weaker confirmation because they were accompanied by multiple comparisons (16).

Both CASE1 and CASE2, at the herd level, were positively associated with the number of calves in the herd. Herd size has been reported previously to be positively associated with the level of disease (4,29). In our study, the number of calves in the herd may be an indirect measure of stocking density, overcrowding, level of infectious agents, and the propensity of producers with larger herds to treat calves more frequently.

TABLE IX. Least-square linear regression results of pneumonia and girth growth on variables at the herd level of analysis

Outcome variables in model	Coefficient	SE	p > T	R^2
Producer diagnosed pneumonia (CASE1)				
intercept	−7.15	1.60	0.0005	0.69
number of calves	0.12	0.02	0.0001	
breed proportion	4.11	1.37	0.009	
Veterinarian diagnosed pneumonia (CASE2)				
intercept	−4.48	1.31	0.004	0.35
number of calves	0.04	0.02	0.04	
breed proportion	2.92	1.15	0.02	
Average girth growth 1st month				
intercept	0.29	0.02	0.0000	0.27
SPMb	−0.16	0.07	0.03	
Average girth growth 1st month				
intercept	0.17	0.04	0.0006	0.49
median HS	−0.00005	0.00001	0.004	
median PI3	−0.002	0.0006	0.02	

n = 17 herds

At the herd level of analysis, CASE1 and CASE2 were also positively associated with the proportion of Holstein calves relative to dairy and beef crossbred calves. This association may reflect differences in genetic susceptibility to disease or differences in the producers propensity to ascribe different values to calves of different breeds; thus to treat them differently. We did not observe any differences in herd size, management or environmental conditions to explain the association between breed and pneumonia.

Our study did not find any significant associations between specific antibody levels and CASE2 at the calf level of analysis nor CASE1 and CASE2 at the herd level of analysis. Other researchers have reported a protective effect of colostral antibodies to IBRV (24), PI3V (30), BRSV (31) and BVDV (32) for pneumonia, although the data are sparing. Our failure to show significant associations may be due to: 1) the multifactorial nature of disease (1–4); 2) misclassification bias of disease due to the subjectivity of both case definitions; the presence of subclinical disease; and the variable follow-up period among calves (16); 3) misclassification bias of antibody levels due to the variability in age that blood samples were collected; 4) failure to test infectious agents which caused disease (16); 5) multicollinearity between serological titers (13); 6) a lack of variability in PI3V and BRSV titers in calves (13); 7) too small a sample size at the herd level of analysis (12,16); and 8) a reduction in power after adjusting for clustering (15). As shown in Tables V and VI, the p values rose significantly after the variance was adjusted for clustering, which caused a shrinking of effective sample size (15). This indicates a strong "herd effect" or clustering, the nature of which could not be fully explained in this size of study. This finding is significant in that it indicates future studies would have to involve many more farms to get a better understanding of the factors associated with this disease. Furthermore, a more objective case definition for enzootic pneumonia should be developed to help improve the validity of the findings.

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