PEER REVIEWED

Association of Seroconversion with Isolation of Agents in Transtracheal Wash Fluids Collected From Pneumonic Calves Less than Three Months of Age

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Abstract

The objective of this study was to investigate the potential association between seroconversion to organisms important in respiratory disease and isolation from transtracheal wash fluids (TTW) collected from pneumonic dairy calves less than 3 months of age. These calves had naturally acquired infections and represented 18 commercial dairy farms. Each calf had at least one respiratory pathogen cultured from the TTW, which was carried out at the time of clinical diagnosis. Blood samples were first collected at the time of diagnosis and a second sample was collected 3 weeks later. Most calves had evidence of maternal antibodies (at 2 days of age) to the same pathogens isolated from the TTW at the time of respiratory disease diagnosis.

Evidence of an active immune response (seroconversion) to the organism in the TTW was a specified rise in titer between paired sera. False-negative results, i.e. failure to seroconvert to the organism isolated in the TTW, were seen in 8/9 (89%) calves with bovine virus diarrhea virus in their TTW, 43/75 (57%) calves with *Mycoplasma* sp. in their TTW, 20/53 (38%) calves with *Pasteurella multocida*, 4/16 (25%) calves with *Pasteurella haemolytica*, and 5/13 (38%) calves with *Haemophilus somnus* isolates in their TTW. We do not recommend reliance on paired-serological testing to identify the respiratory pathogens present in the respiratory tract of clinically pneumonic dairy calves less than 3 months of age.

Introduction

The level of the immune response is controlled in part by a negative feedback whereby a specific antibody inhibits further production of more antibody of the same specificity.[20] Passive transfer of maternal antibody also inhibits the development of measurable antibody response.[4,6,19,20] If calves fail to suckle and are hypogammaglobulinemic, they begin to actively synthesize immunoglobulin (Ig) earlier in life.[10,19] If they obtain colostrum, this production begins at about 4 weeks of age.[19]

Diagnostic laboratories frequently receive paired serum samples collected from young calves with pneumonia or other diseases for confirmation of infection and etiologic diagnosis. The objective of this study was to describe the sensitivity of seroconversion between paired acute and convalescent serum titers and infection with specific respiratory pathogens in dairy calves less than 3 months of age.

Material and Methods

The study population, study design, selection criteria for calves with respiratory-tract disease, and labo-

ratory examinations have been described previously.[24] The study population was a convenience sample of 410 dairy heifer calves based on the willingness of 18 dairy producers to participate. All farms were located in the practice area of the study clinician (GDM), in the vicinity of the college of veterinary medicine. The first 35 newborn calves that were alive at the first visit to the farm in that month were selected by use of birth order from these herds each month, starting from alternating ends of the farm-visiting route each week. Calves were enrolled in the study between Jan 1, 1990 and Dec 31, 1990 and followed for 3 months. Clinical examinations of the calves were done on weekly visits. In a sub-study, a case series was used to compare specific antibody titers and microbial isolations from transtracheal wash (TTW) samples between 105 respiratory cases and 59 non-respiratory control calves. In this substudy, only pneumonic calves with a specific TTW isolation were used, but a calf could have had several pathogens isolated at the same time.[24] Respiratory tract disease was defined as detection of abnormal clinical signs related to the respiratory tract, such as inducible cough on tracheal massage, abnormal sounds on auscultation of the respiratory tract, fever (>103.1°F, 39.5°C), depression, and lack of involvement of other body systems that might explain the fever. A calf was not required to have all abnormalities for the diagnosis of respiratory tract disease.

TTW samples were obtained from calves at the time pneumonia was diagnosed. Blood samples collected from jugular venipuncture were obtained at the time of diagnosis (acute samples) and 3 weeks later (convalescent samples). Serum samples were analyzed at the New York State Diagnostic Laboratory at Cornell, according to standard procedures for virus-neutralizing antibodies to bovine viral diarrhea (BVD), infectious bovine rhinotracheitis (IBR), and parainfluenza 3 (PI-3) viruses.[7,15] Antibodies to *Pasteurella multocida*, *Haemophilus somnus*, and the leukotoxin of *P. hemolytica* were analyzed by ELISA.[8,23] Indirect hemagglutinating antibodies against *Mycoplasma bovis* and *M dispar* were determined.[5,13] The association between TTW isolation and homologous antibody change between convalescent and acute periods for pneumonic calves was studied. *Mycoplasma* sp. isolated from TTW samples were not speciated. Therefore *Mycoplasma* sp. could be related to either *Mycoplasma dispar* or *Mycoplasma bovis* antibody changes.

To account for the effect of postcolostral antibodies, seroconversions were stratified by postcolostral serum antibody. Postcolostral serum antibody titers were determined at a mean of 5.7 days of age. All postcolostral serum antibody titers were extrapolated to day 2 values so that a single titer cut-point could be used to define the presence of maternal serum antibody (IgG at day 2=(IgG at day D) $\cdot e^{(D-2)\cdot(\ln 2/\text{half-life of IgG})}$).[24] Day 2 postcolostral serum antibody level ≥ 8 (standard serum dilutions with titers being expressed as the reciprocal of the dilution that is determined to be the end-point of the assay) against the pathogen studied was considered positive. Otherwise, the calf was considered not to have postcolostral antibodies against this pathogen. Postcolostral serum samples were taken earlier than TTW isolations because the peak period of pneumonia diagnosis was in the fifth week of life.

Descriptive statistics were calculated with the procedures UNIVARIABLE and MEANS in SAS.[16,17] The definition of seroconversion was a 4-fold rise in serum antibody titer from samples taken 3 weeks apart.[21,22]

Results

Homologous isolations and paired serum samples were obtained from 9 calves with bovine viral diarrhea virus (BVDV), 75 calves with *Mycoplasma* sp., 53 calves with *Pasteurella multocida*, 16 calves with *Pasteurella haemolytica*, and 13 calves with *Haemophilus somnus* isolation. Results for calves with detectable postcolostral serum antibody titers are presented separately from those without detectable postcolostral serum antibody titers. There were no isolations in the TTWs of bovine respiratory syncytial virus (BRSV), IBRV, and PI-3 virus. Similarly, paired serological testing failed to demonstrate any measurable antigen-specific serum antibody response for these agents.

For calves with detectable serum postcolostral antigen-specific serum antibody titers against the particular pathogen (Table 1), 0% (BVDV) to 73% (*P. haemolytica*) of calves seroconverted to the organisms present in their TTW. For the most common organisms causing pneumonia in these study calves, 33% of the calves seroconverted to *M. dispar* and 61% to *P. multocida*. The percentages of seroconversions were not significantly different between calves with and without detectable postcolostral antibody titers (Table 1).

Discussion

In response to natural challenge with BVDV, *Mycoplasma* sp., *P. multocida*, *P. haemolytica*, or *H. somnus*, at least 27% of the calves in this study population failed to seroconvert. From these 104 calves, 9 had BVDV isolations, 1 was from a non-pneumonic calf, 3 were solitary isolations (that is, nothing else was isolated), and the rest also had mycoplasmas isolated. There were 3 pneumonic calves persistently-infected (PI) with BVDV (BVDV isolated from mononuclear cell preparations of whole blood on 2 occasions at least 3 weeks apart, regardless of serologic status) and 5 PI-suspect calves. Houe and Heron studied immune responses in 5 clini-

Table 1. Seroconversion against natural challenge with common respiratory pathogens in young (< 3 mo of age) pneumonic Holstein dairy calves with detectable postcolostral serum antibody titers against the same pathogen. Results for calves without detectable postcolostral antibody titers are shown in parentheses.

Pathogen	Minimum rise in titer to equal seroconversion	Calves with positive TTW and acute and convalescent sera			
		Total	Seroconversions		
		N[a]	N	%	95% CI[b] (%)
Bovine virus diarrhea virus	4x	7 (2)	0 (1)	0 (50)	1 to 44 (3 to 97)
Mycoplasma dispar (*M.* sp. was isolated)	4x	63 (12)	21 (7)	33 (58)	22 to 46 (28 to 83)
M. bovis (*M.* sp. was isolated)	4x	3 (72)	0 (4)	0 (6)	0 to 69 (2 to 15)
Pasteurella multocida	4x	51 (2)	31 (2)	61 (100)	46 to 74 (20 to 100)
P. haemolytica	4x	15 (1)	11 (1)	73 (100)	45 to 91 (6 to 100)
Haemophilus somnus	4x	13 (0)	8 (0)	62 (0)	33 to 85 (not calculated)

[a]N = number of isolations obtained from transtracheal wash samples, [b]CI = confidence interval for % seroconversions.

cally healthy PI calves 1 to 1.5 yr old; 5 non-PI calves were used as controls.[9] In that study, the response of PI calves to the various types of antigenic stimuli (other than BVDV) was not significantly different from that of the control calves. For this reason, the PI and PI-suspect calves were included in our analyses.

There were so few calves without detectable postcolostral antigen-specific serum antibody titers (only 0 to 2 calves for each pathogen) that there was insufficient power to detect whether calves with and without postcolostral titers differed in percent seroconversion. In general, maternally-derived antibody suppresses antigen-specific serum antibody response.[2,4,10,14,19] The precise mechanisms of suppression are not clear, but it is probably due both to central suppression and to antigen masking and sequestration.[19] Elevated corticosteroid concentrations, increased numbers of T suppressor cells, and transfer of maternal anti-idiotypic antibody contribute to the suppression of the immune response of the neonate.[1] After infection, however, the decline of maternally derived antibodies appears to be slower, which could be proxy for an active antibody response.[3]

There are only a few reports in which vaccines stimulated active immunity in young calves exhibiting high maternally derived antibody levels. Brar *et al* interpreted their data to indicate that calves developed an antigen-specific serum antibody response to BVDV vaccine when maternally derived BVDV antibody titers remained between 1:20 and 1:96.[2] There were no evident seroconversions (that is, titer increases) to IBRV vaccine until maternal antibodies were undetectable. However, they suggested that there was priming of some immunologically competent memory cells to IBRV since the calves responded anamnestically to a second vaccination after maternal antibodies were undetectable. Similarly, serum and local antibody responses to BRSV infection were strongly suppressed by maternal antibodies and after reinfection there was clear evidence of mucosal memory.[11,12]

Failure to seroconvert to the organism isolated in the TTW as seen in some of the calves in this study may

have been due to the suppressive effect of maternally derived antibodies. However, it is possible (as shown with some vaccines) that there was some priming of mucosal memory that could have been investigated only after reinfection. Another possibility is that the assays used weren't sensitive enough to detect immunoglobulin produced by an active immune response. Howard *et al* reported that calves with high maternally derived antibodies had no detectable IgG_1 seroconversion (neutralization assay or ELISA assay); however, an ELISA assay that detected IgG_2 had a 7-fold increase 6 weeks after challenge.[10] In a primary response, IgM rises first.[18] In theory, neutralization tests detect IgA, IgG, and IgM antibody activity.[21] However, assays detecting pure IgM activity might better reflect primary antibody responses.

In conclusion, clinicians should be judicious in requesting serologic determinations from young pneumonic dairy calves for diagnosis of respiratory infections, because calves often fail to seroconvert to agents commonly associated with respiratory disease. Also, the tests that are usually used in diagnostic laboratories cannot differentiate between active or passively-derived Ig isotypes. Cost of serological sampling, selection of pathogens for serological testing, the limited ability of the diagnostic lab to test for a wide array of pathogens, and the time lost between acute and convalescent periods also serve to question the merit of serological testing. Collecting TTW from a sample of calves will provide the clinician with quick and accurate identification of agents associated with respiratory disease in a group of calves.

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References

1. Banks KL, McGuire TC: Neonatal immunity, in Halliwell REW and Gorman NT (eds): *Veterinary Clinical Immunology*. Philadelphia, WB Saunders Co, 1989, pp 193-204.
2. Brar JS, Johnson DW, Muscoplat CC, Shope REJ, Meiske JC: Maternal immunity to infectious bovine rhinotracheitis and bovine viral diarrhea viruses: Duration and effect on vaccination in young calves. Am J Vet Res 39: 241-244, 1978.
3. Butler JE: Bovine immunoglobulins: an augmented review. Vet Immunol Immunopathol 4: 43-152, 1983.
4. Carmichael LE: Immunization strategies in puppies - Why failures? Comp Cont Ed 5: 1043-1051, 1983.
5. Cho HJ, Ruhnke HL, Langford EV: The indirect hemagglutination test for the detection of antibodies in cattle naturally infected with mycoplasmas. Can J Comp Med 40:20-29, 1976.
6. Dixon FJ, Jacot-Guillarmod H, McConahey PJ: The effect of passively administered antibody on antibody synthesis. J Exp Med 125: 1119-1135, 1967.
7. Donis RO, Dubovi EJ: Molecular specificity of the antibody responses of cattle naturally and experimentally infected with cytopathic and noncytopathic bovine viral diarrhea virus biotypes. Am J Vet Res 48:1549-1554, 1987.
8. Harland RJ, Potter AA, van Drunen-Litter-van den Hurk S, *et al*: The effect of subunit or modified live bovine herpesvirus-1 vaccines on the efficacy of a recombinant Pasteurella haemolytica vaccine for the prevention of respiratory disease in feedlot calves. Can Vet J 33:734-741, 1992.
9. Houe H, Heron I: Immune response to other agents of calves persistently infected with bovine virus diarrhoea virus (BVDV). Acta Vet Scand 34: 305-310, 1993.
10. Howard CJ, Clarke MC, Brownlie J: Protection against respiratory infection with bovine virus diarrhoea virus by passively acquired antibody. Prev Vet Med 19: 195-203, 1989.
11. Kimman TG, Westenbrink F, Schreuder BEC, Straver PJ: Local and systemic antibody response to bovine respiratory syncytial virus infection and reinfection in calves with and without maternal antibodies. J Clin Microbiol 25: 1097-1106, 1987.
12. Kimman TG, Westenbrink F, Straver PJ: Priming for local and systemic antibody memory responses to bovine respiratory syncytial virus: Effect of amount of virus, virus replication, route of administration and maternal antibodies. Vet Immunol Immunopathol 22: 145-160, 1989.
13. Lauerman LH: Mycoplasmas of the bovine respiratory tract, in Whitford HW, Rosenbusch RF, Lauerman LH, (eds): *Mycoplasmosis in Animals: Laboratory Diagnosis*. Ames, Iowa, Iowa State University Press, pp 50-56, 1994.
14. McNulty MS, Bryson DG, Allan GM: Experimental respiratory syncytial virus pneumonia in young calves: microbiologic and immunofluorescent findings. Am J Vet Res 44: 1656-1659, 1983.
15. Rossi CR, Kiessel GK: Microtiter tests for detecting antibody in bovine serum to parainfluenza 3 virus, infectious bovine rhinotracheitis virus, and bovine virus diarrhea virus. Appl Microbiol 22:32-36, 1971.
16. SAS Institute Inc. The MEANS procedure, in SAS user's guide: basics, version 5 edition. Cary, NC, SAS Institute Inc, 1985, pp 959-965.
17. SAS Institute Inc. The UNIVARIATE procedure. in: SAS user's guide: basics, version 5 edition. Cary, NC, SAS Institute Inc, 1985, pp 1181-1191.
18. Tizard IR: B cells and their response to antigen, in Tizard IR (ed) *Veterinary Immunology: An Introduction*. 5th edition. Philadelphia, WB Saunders Company, 1996, pp 121-140.
19. Tizard IR: Immunity in the fetus and newborn, in Tizard IR (ed) *Veterinary Immunology: An Introduction*. 5th edition. Philadelphia, WB Saunders Company, 1996, pp 237-250.
20. Tizard IR: Regulation of the immune response, in Tizard IR (ed) *Veterinary Immunology: An Introduction*. 5th edition. Philadelphia, WB Saunders Company, 1996, pp 203-215.
21. Tizard IR: Serology: the detection and measurement of antibodies, in Tizard IR (ed) *Veterinary Immunology: An Introduction*. 5th edition. Philadelphia, WB Saunders Company. 1996, pp 216-236.
22. Van der Poel WHM, Kramps JA, Middel WGJ, Van Oirschot JT, Brand A: Dynamics of bovine respiratory syncytial virus infections, a longitudinal epidemiological study in dairy herds. Arch Virol 133: 309-321, 1993.
23. Van Donkersgoed J, Ribble CS, Boyer LG, *et al*: Epidemiological study of enzootic pneumonia in dairy calves in Saskatchewan. Can J Vet Res 57:247-254, 1993.
24. Virtala A-MK, Mechor GD, Gröhn YT, Erb HN, Dubovi EJ: Epidemiologic and pathologic characteristics of respiratory tract disease in dairy heifers during the first 3 months of life. J Am Vet Med Assoc 208: 2035-2042, 1996.