Herd-based Testing for Young Stock

Sheila M. McGuirk, *DVM, PhD*
Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, WI 53706

Abstract

Investigation of calf morbidity or mortality problems usually requires an accurate assessment of the colostrum-feeding program as well as documentation of the potential enteric and respiratory pathogens to which the pre-weaned calves have been exposed. The approach to herd-based problem solving and assessment is quite different than testing individual calves. Conclusions and recommendations are based, not just on recent history, impressions, written protocols, examination of affected animals and post-mortem results, but on farm data collection. Accurate conclusions require appropriate sample sizes, discriminating tests and an appropriate population of calves to test. The purpose of this paper is to describe the farm data collection and analysis that determines whether there is a herd problem of failure of passive transfer of immunity (FPT), the level of calf exposure to potential fecal and respiratory pathogens and the determination of antimicrobial susceptibility of potential respiratory pathogens.

Herd-based Testing for Failure of Passive Transfer of Immunity in Calves

The goal of feeding colostrum to dairy calves is to transfer specific and non-specific immune factors, nutritional elements and growth factors that provide disease resistance until the calf has a functionally mature immune system. In individual, healthy calves, the transfer of immunity is generally viewed as being adequate if serum immunoglobulin G (IgG) concentration is ≥ 1,000 mg/dl or the serum total protein concentration is ≥ 5.2 g/dl.[10,11] When there are calf health problems in a dairy herd, one of the first concerns is recurring FPT. A herd with a large number of non-immune shedding calves creates susceptibility and environmental reservoirs for enteric and respiratory pathogens. To test a herd for FPT, we use serum total protein concentration as the discriminating test. The measurement of total protein concentration in calves is practical, economical and has been validated as a herd test for passive immunity.[10,11] The test can be accurately applied to calves less than one week of age and more than six hours after colostrum administration.[2,4] A concentration of 5.5 g/dl is adopted as the cut point and, in herd testing, we are interested in the proportion of calves that fall below the cutpoint. To interpret the data, we set an alarm level of 20%. That is, greater than 20% of calves falling below the cutpoint is indicative of a herd problem of failure of passive transfer. Using a proportional outcome based test, a minimum of 12 calves should be sampled to yield a 75% confidence interval. For smaller herds, it is important to accumulate test results until 12 have been run. If the results in any herd are close to the cut-point, more tests should be done. The interpretation of tests for total protein concentration obtained from 12 calves is shown in Table 1. The success of a dairy's colostrum feeding program is judged by regular testing, with delivery and discussion of results. Used appropriately, test results provide an opportunity for constructive feedback or can serve as the basis for an incentive program for calf feeders.

Assessing Colostrum Bacterial Concentration

Bacterial contamination of colostrum has a negative impact on acquisition of passive immunity and is a common problem on many dairies.[6,7,8] On dairies with calf health concerns, bacterial numbers and fecal coliform counts in colostrum have exceeded 1,000,000 and 10,000 cfu/ml, respectively, in many of the samples tested (McGuirk SM, unpublished observations). Bacterial contamination can be avoided by appropriate udder preparation of colostrum donors prior to collection of colostrum, satisfactory function of the milking equipment used to milk fresh cows, and good sanitation of collection, storage and feeding equipment used for colostrum. To monitor the bacterial quality of colostrum, samples can be submitted to the laboratory for bulk tank culture. Because bacterial counts in colostrum are typically high compared to bulk tank milk, a series of four dilutions (1:50, 1:500, 1:5,000 and 1:50,000) is necessary to obtain accurate colony counts. Dilutions can be made by dispensing 1.8 ml of sterile diluent into each of four tubes. Vortex the colostrum well and deliver 200 µl to the first tube (1:50 dilution). Repeat the procedure and deliver 200 µl from tube 1 to tube 2 (1:500 dilution), from tube 2 to tube 3 (1:5,000 dilution) and from tube 3 to tube 4 (1:50,000 dilution). Colostrum culture goals and herd examples are shown in Table 2.

Table 1. Herd-based testing for failure of passive transfer (FPT).

Number of calves < 5.5 g/dl total serum protein	Percentage of calves tested	Interpretation
0/12	0	FPT is **not** a herd problem
1/12	8.3	FPT is **not** a herd problem
2/12	16.7	**Borderline** concern for FPT
3/12	25	**Borderline** concern for FPT
4/12	33.3	FPT **is** a problem
5/12	41.7	FPT **is** a problem
6/12	50	FPT **is** a problem

Table 2. Colostrum bacterial contamination – goals and examples.

Count (cfu/ml)	Goals (cfu/ml)	Herd 1	Herd 2	Herd 3
Total bacteria	<100,000	285,000	1,150	4,100,000
Fecal coliforms	<10,000	0	0	2,400,000
Other gram negs	<50,000	270,000	0	0
Strep. non-*ag.*	<50,000	10,000	50	1,700,000
Coag neg *Staph*	<50,000	5,000	1,100	0
Other	<5,000	0	0	*Salmonella uganda*

Herd Based Testing for Calf Diarrhea and Respiratory Disease Problems

Armed with disease or treatment records and having examined or scored calves to clarify the herd problem and the population at-risk, it is possible to determine the enteric pathogens to which calves have been exposed and/or to solidify a treatment plan for respiratory disease problems in calves. Amongst the at-risk age group of calves, diagnostic samples are obtained from a minimum of six untreated calves or 10% of eligible calves in the group.

For a calf diarrhea investigation, a fecal sample is collected into a 4 oz specimen cup. Calves are stimulated to defecate by gentle rectal massage with a gloved finger. Four cotton swabs are used to obtain a rectal smear from calves that do not produce manure. Sampled calves are identified by ear tag, birth date and pen location and fecal consistency is graded as follows:

0) normal
1) semi-formed, pasty
2) loose, enough consistency too stay on bedding
3) watery

Prior to leaving the farm, a 1-2 gm fecal sample (or one cotton swab each) is inoculated into tetrathionate and selenite enrichment media. Just prior to fecal inoculation, a pre-aliquoted 200-µl vial of iodine is added to the tetrathionate medium. Fecal smears are made upon return to the veterinary hospital and are submitted for *Cryptosporidium parvum* detection by acid-fast stain. The remaining fecal sample is submitted for electron microscopic detection of rota- and corona virus particles. Proper sample preparation and submission is essential for accurate assessment of results, such as those shown in Table 3. With an anticipated rate of exposure to potential fecal pathogens in the calf environment, up to 20% of the tested calves may be shedding *Cryptosporidium parvum*, rota- or corona virus. No calves should be exposed to or shedding *Salmonella* spp. From Table 3 data, we know that there is an environmental reservoir of *Cryptosporidium parvum*, the most likely cause of the endemic calf diarrhea problem. With this knowledge, appropriate measures can be taken to by-pass, dilute or distance calves from the source of infection.

One aspect of working up a calf pneumonia problem is to establish an effective treatment protocol. Collection of nasal swabs from a group of at-risk, untreated calves can yield antimicrobial susceptibility patterns that help determine the best treatment protocol for the herd. While nasal swabs are not necessarily useful in predicting the etiologic agents in a herd pneumonia problem, they are useful in predicting antibiotic susceptibility of the pathogens. Two deep nasal swabs (BBL™ Culture Swab Plus™, Becton Dickinson, Sparks, MD) are obtained from each calf. One swab is submitted for bacterial and the other for *Mycoplasma* culture. From each calf's nasal swab bacterial culture, the antibiotic susceptibility patterns for *Pasteurella multocida, Mannheimia*

haemolytica and *Histophilus somni* are determined, as shown in Table 4. From these data, ceftiofur, florfenicol, trimethoprim-sulfonamide combination (if affected calves are less than 2-weeks of age) or tilmicosin are potentially useful antibiotics. Ceftiofur or other beta-lactam antibiotics are not appropriate if there is a high risk of *Mycoplasma bovis* infection in the calves. In a low risk herd, the proportion of calves that culture *M. bovis* from the nasal flora is less than 10%.[1,5] In an endemic *M. bovis* herd, 49-91% of calves have positive nasal cultures.[9] From a herd sample size of six calves, if there is more than one positive nasal isolate for *Mycoplasma bovis,* we look for a source of exposure and revise the antibiotic protocol to reflect its potential etiologic role. *Mycoplasma* susceptibilities are not performed routinely in most laboratories and field strain resistance is problematic. The three flouroquinolones (danofloxacin, enrofloxacin and marbofloxacin), all of which are illegal for use in dairy calves in the US, are the only antibiotics to have consistent efficacy against *M. bovis.* Tiamulin, approved only for the treatment of swine dysentery in the US, has demonstrated efficacy against most *M. bovis* field isolates.[3] Newer generation macrolide antibiotics may have more promise for clinical efficacy in the future.

In summary, herd based testing protocols play an important role in solving recurring health problems of young stock.

References

1. Bennett RH, Jasper DE: Nasal prevalence of Mycoplasma bovis and IHA titers in young dairy animals. *Cornell Vet* 67:361-373, 1977.
2. Burton JL, Kennedy BW, Burnside EB, *et al*: Variation in serum concentrations of immunoglobulins G, A, and M in Canadian Holstein-Friesian Calves. *J Dairy Sci* 72:135-149, 1989.
3. Francoz D, Fortin M, Fecteau G, *et al*: Determination of *Mycoplasma bovis* susceptibilities against six antimicrobial agents using the E test method. *Vet Micro* 105:57-64, 2005.
4. Hancock DD: Assessing efficiency of passive immune transfer in dairy herds. *J Dairy Sci* 68:163-183, 1985.
5. Hirose K, Kobayashi H, Ito N, *et al*: Isolation of Mycoplasmas from nasal swabs of calves affected with respiratory diseases and antimicrobial susceptibility of their isolates. *J Vet Med B Infect Dis Vet Public Health* 50:347-351, 2003.
6. James RE, Polan CE, Cummins KA: Influence of administered indigenous microorganisms on uptake of I ^{125}-g-globulin in vivo by intestinal segments of neonatal calves. *J Dairy Sci* 64:52-61, 1981.
7 Poulsen KP, Hartmann FA, McGuirk SM: Bacteria in colostrum: impact on calf health. Abstract #52. Proc 20th Ann ACVIM; 773, 2002.
8. Staley TE, Bush LJ: Receptor mechanisms of the neonatal intestine and their relationship to immunoglobulin absorption and disease. *J Dairy Sci* 68:184-205, 1985.
9. Stipkovits L, Ripley P, Varga J, *et al*: Clinical study of the disease of calves associated with *Mycoplasma bovis* infection. *Acta Vet Hung* 48:387-395, 2000.
10. Tyler JW, Hancock DD, Parish SM, Rea DE, Bessser TE, Sanders SG, *et al*: Evaluation of 3 assays for failure of passive transfer in calves. *J Vet Int Med* 10:304-307, 1996.
11. Weaver DM, Tyler JW, Van Metre DC, Hostetler DE, Barrington GM: Passive transfer of colostral immunoglobulins in calves. *J Vet Int Med* 14:569-577, 2000.

Table 3. Fecal results from a dairy with calf scours at 7-10 days of age.

Animal ID	Age	Fecal score	EM for virus	*C. parvum* smear	*Salmonella* culture
740	10 days	2	None	+	Negative
742	9 days	0	None	++	Negative
743	9 days	2	Corona virus	+++	Negative
744	9 days	2	None	+++	Negative
747	8 days	1	None	++	Negative
749	7 days	3	Rota virus	+++	Negative
750	7 days	1	None	++	Negative

Table 4. Antibiotic susceptibility of nasal swab bacterial isolates.

Antibiotic	*Pasteurella multocida*	*Mannheimia haemolytica*	*Histophilus somni*
Amp/Amoxicillin	Sensitive	Resistant	Resistant
Ceftiofur	Sensitive	Sensitive	Sensitive
Florfenicol	Sensitive	Sensitive	Sensitive
Spectinomycin	Sensitive	Sensitive	Incomplete
Tetracycline	Resistant	Resistant	Incomplete
Trimethoprim sulfa	Sensitive	Sensitive	Sensitive
Tilmicosin	Sensitive	Sensitive	Sensitive