An Epidemiological Study of Selected Calf Pathogens on Holstein Dairy Farms in Southwestern Ontario

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ABSTRACT

Fecal samples from calves on 78 randomly selected Holstein dairy farms in southwestern Ontario were screened for *Salmonella, Campylobacter jejuni/coli*, enteropathogenic *Escherichia coli*, rotavirus and coronavirus.

Based on the observed prevalence, 22% of farms had calves infected with *Salmonella*, 13% with *Campylobacter jejuni/coli*, 41% with enteropathogenic *E. coli*, 19% with rotavirus and 5% with coronavirus. These estimates can be modified, using a method developed by Mullen and Prost (1983) for the World Health Organization, to account for the nature of the laboratory test used. If the test is assumed to have no false positives (that is, if an organism is detected it must be there), then the observed prevalence estimates seen on this study may greatly underestimate the true prevalence of infected premises.

The use of nipple feeders for calves was associated with an increased probability of farms having calves shedding detectable fecal levels of *Salmonella, E. coli*, or one of the two viruses. The use of group pens was associated with an increased odds of finding *C. jejuni*. Calves with diarrhea on these farms tended to have increased odds of shedding rotavirus, and *E. coli* with the K99 antigen. However, at the farm level, none of the organisms was associated with above median levels of morbidity. Farms positive for one or other of the viruses had increased odds of experiencing calf mortality relative to virus-negative farms, and farms positive for *C. jejuni/coli* had decreased odds of mortality.

In a separate study utilizing calves from some of the survey farms, scouring calves were observed to be more likely to shed rotavirus and *E. coli* positive for K99 than appropriately matched nonscouring calves from the same farms. A comparison of an indirect fluorescent antibody test for K99 with a commonly used serological method for screening for enterotoxigenic *E. coli* found no significant relationship between the results of the two tests.

Key words: Dairy calf, *Salmonella, Campylobacter, Escherichia coli*, rotavirus, coronavirus, prevalence, epidemiology.

RÉSUMÉ

Cette expérience consistait à rechercher dans les fèces de certains veaux issus de 78 troupeaux Holstein du sud-ouest de l'Ontario, choisis au hasard, les microorganismes suivants : *Salmonella* spp., *Campylobacter jejuni/coli*, des souches pathogènes d'*Escherichia coli*, ainsi que les virus rota et corona.

La recherche de la prévalence des microorganismes précités dans les troupeaux expérimentaux, donna les résultats respectifs suivants : 22%; 13%; 41%; 19% et 5%. Ces approximations pourraient subir des modifications, si on utilisait la méthode mise au point par Mullen et Prost (10) pour l'Organisation mondiale de la Santé, méthode qui rend compte de la nature de l'épreuve de laboratoire utilisée. Si on présume que cette épreuve ne donne pas de faux positifs, les approximations précitées pourraient sous-estimer grandement la véritable prévalence de troupeaux infectés.

L'alimentation des veaux avec des chaudières munies d'une tétine augmenta la probabilité qu'un plus grand nombre de troupeaux compte des veaux dont le fumier contiendrait une quantité décelable des microorganismes suivants : *Salmonella* spp., *E. coli*, ainsi que l'un ou l'ature des deux virus précités. Le fait de regrouper les veaux dans des parcs s'accompagna d'une plus grande probabilité de détecter *C. jejuni*. Le risque de l'élimination fécale de rotavirus et de colibacilles dotés de l'antigène K99 s'avéra plus grand, chez les veaux diarrhéiques de ces troupeaux. Dans les troupeaux pris individuellement, aucun des microorganismes précités ne se révéla toutefois relié aux moyennes de morbidité précitées. Les troupeaux où on isola l'un ou l'autre des deux virus couraient un plus grand risque de subir des mortalités chez les veaux, comparativement à ceux où on ne retrouva pas ces virus. Par ailleurs, les troupeaux où on isola *C. jejuni/coli* couraient un moins grand risque de subir des mortalités.

Dans une deuxième étude qui portait sur des veaux de certains des troupeaux de la première, les diarrhéiques se révélèrent plus susceptibles d'éliminer du rotavirus et des colibacilles dotés de l'antigène K99 que leurs congénères du même âge qui ne souffraient pas de diarrhée. La comparaison d'une méthode d'immunofluorescence indirecte, destinée à démontrer l'antigène K99, avec une méthode couramment utilisée pour déceler les colibacilles entérotoxinogènes, ne révéla pas de relation significative entre les deux.

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Mots clés : veau laitier, *Salmonella, Campylobacter jejuni/coli, Escherichia coli*, rotavirus, coronavirus, prévalence, épizootiologie.

INTRODUCTION

Rotavirus, coronavirus, *Salmonella, Campylobacter jejuni* and enteropathogenic *Escherichia coli* possessing the K99 antigen are all either demonstrated or hypothesized pathogenic agents in dairy calves (1,2,3,4,5,6). The geographic distribution and biological importance of these organisms have been inferred from diagnostic laboratory submissions and sporadic culturing from selected premises, usually only of diseased animals (1). Only rarely have any attempts been made to conduct scientifically designed surveys and/or formal analytic studies of the prevalence, distribution and effects of these organisms. Certainly, no such formal studies have been carried out on Canadian dairy farms.

This study was designed to determine the prevalence of infection with rotavirus, coronavirus, *Salmonella, Campylobacter jejuni/coli* and enteropathogenic *E. coli* among Holstein dairy farms in southwestern Ontario. The intent was to identify the prevalence of premises with infected calves; no attempt was made to identify the level of infection within those premises.

A second objective was to determine if the presence or absence of these organisms in the feces of calves was associated with any specific calf management policies on these premises. A third objective was to examine the impact of these organisms on calf morbidity and mortality rates at both the farm and the individual calf level.

MATERIALS AND METHODS

PREVALENCE SURVEY

The prevalence of infected premises was estimated by means of a survey of calf feces collected from a random sample of Holstein dairy farms in southwestern Ontario. The formal process for selecting the farms on the study has been described previously (7). In all, 104 randomly selected dairy farms were enrolled on the survey. In the late fall of 1982, fecal samples were collected from the youngest one or two (maximum) calves under two weeks of age at the time of the farm visit. A maximum of two calves were cultured in order not to bias the prevalence estimates in favour of farms with large calf populations. As well, it was thought that a small number of samples per farm would be sufficient to detect premises with a level of infection sufficiently serious, either in terms of within farm prevalence or in terms of intensity (dose effect) to be considered a potential health hazard. All fecal samples were taken directly from the rectum of the calf. Two thin smears were made from each fecal sample, fixed in alcohol, and then stored in a refrigerator. The remainder of each sample was submitted for laboratory analysis in a plastic photographic film container. Where possible, a pooled sample was also submitted, to gain an impression of how such pooling might affect subsequent screening programs.

The fecal samples from this survey were submitted to the Clinical Microbiology Laboratory at the Ontario Veterinary College (OVC) and screened for enteropathogenic *E. coli, Salmonella* and *C. jejuni/coli.*

For *Salmonella* isolation, one gram of feces was inoculated into tetrathionate broth to which five drops of iodine were added. This was incubated for 24 hours at 42°C, and then subcultured to Brilliant Green agar plates. After 24 hours incubation at 37°C, suspect colonies were identified as *Salmonella* or not, using General Diagnostics Micro-ID^R identification system (General Dynamics, Division of Warner-Lambert Co., Morris Plains, New Jersey 07950, USA). Positive cultures were subcultured to triple sugar-iron (TSI) medium for storage and to tryptose-soy-agar (TSA) slopes for transport to the Toronto Public Health Laboratory for serotyping. Antimicrobial sensitivities were done on all positive *Salmonella* cultures.

For enteropathogenic *E. coli*, feces were streaked onto MacConkey plates and incubated for 24 hours at 37°C. Slide agglutination tests were performed using a bovine antibody pool containing Myers' strains 483, 490, 505, 524, 559 and Wi-1, all of which contain the K99 antigen. Six colonies were tested before calling a sample negative.

For *C. jejuni/coli* detection, feces were streaked onto a selective medium containing the antimicrobials vancomycin, polymyxin B, amphotericin, cephalothin and trimethoprim. These plates were incubated microaerophilically at 42°C for 48 hours. Suspect colonies were gram-stained to check for the typical *Campylobacter* morphology. No further typing was carried out on these organisms; that is, *C. jejuni* was not differentiated from *C. coli.*

The virology analyses were carried out at the Veterinary Infectious Disease Organization (VIDO) in Saskatoon, where they were screened using monoclonal antibody capture enzyme-linked immunosorbent assay (ELISA)(8,9).

In the context of this report, the terms viruses and viral shall refer specifically to rotavirus and coronavirus as detected by those tests. The terms *E. coli, Salmonella* and *C. jejuni/coli* shall refer only to strains of those organisms which were identified by the above methods.

Prevalence was estimated in two ways. Firstly, a direct estimate was calculated as the proportion of sampled farms with at least one positive fecal sample. Secondly, Mullen and Prost (10) have developed a method of estimating the true prevalence of an agent when two samples are taken from each unit, and the test used does not yield any false positives. The latter assumption is to some extent met by this survey (since it should not be possible, presumably, to detect an organism which isn't present). However, two fecal samples were obtained from only about 40% of the farms. Nevertheless, if it were assumed that each farm did yield two samples, and that the second samples on single-sampled farms followed the same patterns as second samples on farms where two samples actually were taken, then a reasonable upper limit to the true prevalence may be estimated using the Mullen and Prost method.

ASSOCIATIONS OF PATHOGEN STATUS WITH MANAGEMENT, MORBIDITY AND MORTALITY

Management data from the summer immediately preceding the microbiological survey were used to analyze the relationship between calf management policies and detectable levels of the selected calf pathogens in the calves. Calf morbidity (treatment days per liveborn calf) and mortality data from the fall and winter during which the survey took place were used to assess the impact of the organism at the farm level. The questionnaire used to gather management information, and the daily log sheets used by the farmers to record calf treatment and mortality information, are described in more detail elsewhere (7). Thus, while pathogen status of the farms was established on the basis of calves less than two weeks old, morbidity and mortality rates included calves up to the age of weaning.

For the association between pathogen status and management, the data were analyzed, firstly, by arranging them into two-way tables one for each management variable versus the presence or absence of each organism, and applying the chi-square test. Secondly, logistic regression (11,12) was applied to the data in order to be able to control for potentially confounding inter-relationships that might occur among the various management policies.

For the association of pathogen status with farm level morbidity and mortality, farms were classified as experiencing above or below median heifer calf mortality, the median being zero (i.e. no mortality), and above or below the median in treatment days per calf, the median being 0.57 treatment days per live-born heifer calf. Analysis was by logistic regression, so that management variables could be considered simultaneously with pathogen status.

Effects of rotavirus, coronavirus and *E. coli* were assessed at the individual calf level by means of a case-control study comparing the fecal excretion of K99, rotavirus and coronavirus in calves which were reported to scour at $\leq$ 14 days of age versus those which did not scour during that time period. The calves selected for inclusion in this part of the study were from farms participating in a field trial of an anti-scour vaccine (13). Any calf which was treated for scours during the first two weeks of life was initially included as a case. For each fecal sample from a scouring case, a fecal sample from a control calf, not treated for scours during the first two weeks of life and matched by farm and closest birthday, was selected. Appropriate controls were not found for nine of the 52 cases which had fecal samples; hence the final analyses included 43 scouring cases and 43 nonscouring control calves. Wherever possible, fecal samples were obtained from diarrheic calves before they were treated.

Fecal samples from cases and controls were coded so that laboratory personnel would have no *a priori* basis for differentiating them. Samples were stored at -20° C and submitted to the Veterinary Infectious Disease Organization (VIDO) for viral analyses. Fecal smears from all these calves were screened for K99 antigen using a monospecific anti-K99 antibody as part of an indirect fluorescent antibody test (IFAT) (14). As well, the sensitivity and specificity of the fluorescent antibody test relative to the standard culture and serology (CS) technique was determined on a random sample of eight fecal samples from each of the 15 farms participating in the vaccine field trial (a total of 120 samples).

RESULTS

PREVALENCE ESTIMATES

Fecal samples were obtained from calves on 78 of the 104 participating farms. Some of the fecal samples were too scant for splitting, hence only 59 were screened for rotavirus and 56 for coronavirus. All samples were screened for *Salmonella, C. jejuni/coli* and *E. coli.*

Direct estimates of the proportions of farms positive for the various pathogens are displayed in Table I, column 2. Given the manner in which this microbiological survey was carried out, these figures probably underestimate the true prevalence of "contaminated" farms. The estimate using the method of Mullen and Prost, on the other hand (Table I, col 3), may overestimate the true prevalence, especially of premises infected with *E. coli*, since the assumptions made to carry out the calculations in this case are only partially met. The Mullen and Prost estimate could not be calculated for coronavirus because there were no farms on which both fecal samples yielded the virus. The "true" field prevalence of farms positive for these organisms probably lies somewhere in

TABLE I. Prevalence of Holstein Dairy Farms with Selected Calf Pathogens in Southwestern Ontario, 1982

Organism	Number Farms Sampled	Oberved Proportion Positive (p)[a]	Estimated Proportion Positive (p*)
Salmonella[b]	78	0.218	0.419
C. jejuni[c]	78	0.128	0.463
E. coli[d]	78	0.410	1.000
Rotavirus[e]	59	0.186	0.382
Coronavirus	56	0.054	-

[a] $p = \frac{N-N_0}{N}$; $p^* = \frac{(N \quad N_2)^2}{4NN_2}$

where N = total number of farms sampled
N_0= number of farms with no positive fecal cultures
N_1= number of farms with one positive fecal culture
N_2= number of farms with two positive fecal cultures

p* is the estimate of true prevalence using the method of Mullen and Prost (see Text)

[b] All but one of the 17 farms positive for *Salmonella* yielded *S. muenster.* One farm yielded *S. typhimurium*

[c] *C. jejuni* were not identified in more detail

[d] Strains of *E. coli* were agglutinated by antibodies to Myer's strains 483, 490, 505, 524, 559 or W_i-J. See text for explanation

[e] Rotavirus and coronavirus were identified by ELISA tests

the range between the direct and the Mullen and Prost estimates.

If only those farms on which two calves were actually sampled are considered, prevalence estimates similar to the overall direct estimates are arrived at. The proportions of farms classified positive on the basis of a pooled fecal culture were compared to the proportion positive on the basis of two individual fecals. Pooled samples tended to underestimate the proportion of positive farms, especially for *E. coli*, but, on the basis of chi-square tests, none of these differences were significant (Table II).

The antimicrobial sensitivities of the *Salmonella* isolates are reported in Table III.

TABLE II. Prevalence of Holstein Dairy Farms with Selected Calf Pathogens in Southwestern Ontario, 1982: Effect of Pooling Fecal Samples

Organism	Number Farms Sampled	Proportion Positive (individual)[a]	Proportion Positive (pooled)
Salmonella[b]	30	0.200	0.157
C. jejuni	30	0.133	0.100
E. coli	30	0.567	0.367
Rotavirus	18	0.278	0.278
Coronavirus	17	0.000	0.000

[a]Either one or both fecals cultured were positive
[b]See text for how organisms were indentified

TABLE III. Antimicrobial Sensitivities for *Salmonella* found in Heifer Calves on 17 Holstein Dairy Farms in Southwestern Ontario, 1982[a]

	% Sensitive	% Resistant	% Intermediate
Ampicillin	100	-	-
Penicillin	-	100	-
Tetracycline	35	-	65
Neomycin	59	-	41
Chloramphenicol	100	-	-
Nitrofurazone	82	6	12
Cephaloridine	94	6	-
Triple Sulfa	41	18	41
Gentamicin	94	-	6
Trimethoprim-sulfa	100	-	-
Kanamycin	76	-	34

[a]Percent of farms in each category are presented. If two isolates from one farm differed in sensitivity patterns, the farm was classified according to the isolate which was resistant to more antimicrobials

TABLE IV. Proportion of Holstein Dairy Farms in Southwestern Ontario Positive for *Salmonella*, 1982: by County

County	Number of Farms Sampled	Number Positive	Proportion Positive
Bruce	14	3	0.214
Grey	6	0	0.000
Huron	9	4	0.444
Oxford	18	1	0.056
Perth	17	3	0.176
Waterloo	3	0	0.000
Wellington	10	6	0.600
Middlesex	1	0	0.000
Total	78	17	0.218

$\chi^2 = 17.017$, 7 df
$p = 0.0173$

ASSOCIATION OF PATHOGEN STATUS WITH FARM MANAGEMENT

Because so few farms yielded coronavirus, the rotavirus-positive and coronavirus-positive farms were pooled into one category, virus-positive. In these analyses, a farm was considered positive for a particular organism if at least one of the two fecals on that farm yielded the organism.

At the farm level, using a chi-square test, the presence or absence of any particular organism was not significantly associated ($p \leq 0.05$) with the presence or absence of any other organism. A farm positive for *Salmonella*, for instance, was no more likely to be positive for enteropathogenic *E. coli* than a farm negative for *Salmonella*. Some "dual organism" farms occurred, of course, just by chance.

In the unconditional (two-way) analysis, there was significant ($p < 0.05$) county to county variation in the proportion of farms positive for *Salmonella* (Table IV). In the logistic regression, however, because of management-county associations, only the age at which pail feeding of calves was introduced exhibited a significant association with the presence of *Salmonella*. The introduction of pail feeding after two weeks, but before weaning, was associated with the presence of *Salmonella* in the young calves.

In both two-way and logistic regression analyses, farms were more likely to have *E. coli* positive calves if the farmer had a policy of force-feeding first colostrum to calves from either a nipple pail or a nipple bottle. As well, waiting to introduce pail feeding to calves (that is, keeping them on nipple feeders) until they were older than two weeks was significantly associated in the multiple logistic regression with the presence of *E. coli*..

Farms were more likely to be virus-positive if the farmers delayed pail feeding, and if they delayed calf-starter feeding, to beyond two weeks.

The *C. jejuni/coli* positive farms presented some peculiar analytical problems. In two-way analysis, only calf housing was associated with being *C. jejuni/coli* positive: there were more positives among farms where calves were housed in group pens than in hutches or individual pens. In the logistic regression, however, as one variable would enter the model (and hence be "controlled" for), several other significant factors would be uncovered, until almost as many variables had entered the model (nine)

as there were positive farms (10). It was apparent that there were too many positive farms for the large number of management variables being considered (31), and that no reliable multivariable model could be built on the basis of these data.

ASSOCIATION OF PATHOGEN STATUS WITH FARM-LEVEL MORBIDITY AND MORTALITY RATES

Farms with detectable levels of *Salmonella, E. coli, C. jejuni/coli* and "virus" in their calves were no more, nor less, likely to experience high calf treatment rates in general or scours rates in particular, than farms on which these organisms were not found. However, farms with *C. jejuni/coli* were more likely to have below median calf mortality rates, and farms with rota-and/or coronavirus were more likely to have above median calf mortality rates.

COMPARISON OF TESTS FOR DETECTING *E. COLI*

The IFAT and CS tests were compared as methods of identifying calves carrying K99-positive *E. coli.* Of the 120 calves screened, 25.8% were positive by IFAT and only 7.5% by CS. These percentages are significantly different (McNemar's chi-square statistic 13.8; $p < 0.0005$). Using CS as the standard, the relative sensitivity and specificity of IFAT are 44% and 94.4% respectively. The degree to which the two tests agreed with one another, based on the kappa and standard chi-square statistics, is given in Table V. The kappa statistic indicates the degree (proportion) to which the two tests agree beyond what one would expect on the basis of chance alone (15). It is apparent that, in this study, the observed agreement (73%) was not significantly greater than the agreement one would expect on the basis of chance alone (71%).

For classifying farms, as opposed to individual calves, the tests were in somewhat better agreement. As part of the anti-scour vaccine trial from which the calf fecals in this study were derived, 15 farms were classified as either *E. coli* positive or negative using the CS test. Eight randomly selected fecals from each farm during the trial, as well as those obtained during the survey described in this chapter, were used for this purpose. On this basis, 13 of the 15 farms were classified as *E. coli* positive. These same 13, plus one more, were classified as K99 positive using IFAT, using 168 fecal samples obtained from calves during the trial. However, if the farm-classifying performance of the two tests is compared using only the eight randomly-selected field trial samples, the amount of agreement is considerably less (though still better than at the individual calf level); 12 were positive on IFAT and seven on CS (Kappa = 0.102, Fisher's Exact p = 0.877).

ASSOCIATION OF PATHOGEN STATUS WITH SCOURS IN INDIVIDUAL CALVES

Data from scouring calves and nonscouring control calves were analyzed using both matched and unmatched formats. Since there were no substantive differences in results using the two methods, and since the latter is more generally understood, both the data and the analyses are presented in unmatched formats. The breakdown of scouring and nonscouring animals according to pathogen status is given in Table VI. There were no marked differences, in general, between scouring and nonscouring calves with regard to the presence or absence of any of the three organisms.

Among the scouring calves, *E. coli* positive for K99 and coronavirus appeared to be shed from prescouring or untreated scouring calves, and rotavirus from scouring calves, both untreated and treated. When diarrheic, untreated calves were compared with appropriate nonscouring calves (same farm, closest birthdate), the diarrheic calves were shown to have a greater tendency to shed K99-positive *E. coli* than nonscouring calves (Fisher's exact = 0.060; odds ratio = 4.9). When diarrheic calves, treated or untreated, were compared with appropriate nonscouring calves, the diarrheic calves are shown to have a tendency toward greater rotavirus shedding than the nonscouring calves ($p = 0.10$; odds ratio = 4.3). The rates of coronarivus shedding between prescouring or scouring cases and

TABLE V. Culture and Serology (CS) versus Indirect Fluorescent Antibody (IFAT) as Means of Identifying Enteropathogenic *E. coli* with the K99 Antigen in Dairy Calf Fecal Samples

		IFAT		
		+	-	Totals
CS	+	4	5	9
	-	27	84	111
		31	89	120

$\chi^2 = 0.866$, $p = 0.36$
Kappa = 0.0947

TABLE VI. The Association between Rotavirus, Coronavirus and K99 *E. coli* in the Feces of Heifer Calves, and Calf Health Status, on Holstein Dairy Farms in Southwestern Ontario, 1982-83

Status	Number Calves Sampled	Number of Calves with Selected Organisms							Mean Age[a] (days)
		K99[b]	R	C	KR	KC	RC	KRC	
No scours	43	8	3	3	3	0	0	0	8.3
Prescours[c]	12	5	0	2	0	1	0	0	4.8
Scours	14	6	1	0	2	0	1	0	9.1
Scours-treated	9	0	5	0	0	0	0	0	11.4
Postscours	8	0	0	0	0	0	0	0	11.8
All scouring calves[d]	43	11	6	2	2	1	1	0	8.9

[a] Mean age of calves at time of fecal sampling
[b] K99 = fecals positive to the K99 antigen by IFAT
R = fecals positive for rotavirus by ELISA
C = fecals positive for coronavirus by ELISA
KR = fecals positive for both K99 and rotavirus
KC = fecals positive for both K99 and coronavirus
RC = fecals positive for both rotavirus and coronavirus
KRC = fecals positive for K99, rotavirus and coronavirus
[c] Prescour cases were sampled shortly after birth, before any indication of disease was present. They were only later classified as controls or prescour calves, according to their subsequent experiences
[d] Includes prescours, scours, scours-treated and postscours

appropriate nonscouring calves could not be formally compared because of the small numbers involved.

DISCUSSION

The farm level survey of potential calf pathogens indicates that the calves on many dairy farms in southwestern Ontario are infected with *Salmonella, C. jejuni/coli,* enteropathogenic *E. coli* or rotavirus. While pooling of fecal samples did not significantly alter the direct prevalence estimates, a more precise estimate of prevalence was obtainable using two samples per farm, and using the estimation methods derived by Mullen and Prost.

The antimicrobial sensitivities of the *Salmonella* isolates in this survey indicate that the *S. muenster* cultured from these calves was sensitive to a wide variety of antimicrobials, including ampicillin, chloramphenicol and trimethoprim-sulfa.

In both the prevalence survey and the observational study it is clear that farms classified as "positive" and "negative" were not so in an absolute sense. That is, some of the "negative" farms may well have had calves harbouring the organisms being looked for, but at very low levels. During the previously mentioned field trial, for instance, it was apparent that enteropathogenic *E. coli* were probably present on all farms, and that their detection depended at least in part on the number of calves sampled and the test used. This is in keeping with the work of Ueda *et al* (16) who detected *E. coli* in both healthy and diarrheic cattle. Thus while it cannot be proven on the basis of this data, it may be safe to assume that the comparisons being made are between farms with higher and lower levels of infection.

All positive farms — except for the *C. jejuni/coli* positive ones — could be distinguished by the fact that they were more likely to delay pail feeding to their calves, that is, they were farms which kept calves on nursing bottles or nipple pails for two weeks or more. This suggests that the nipples being used to feed the calves were not being properly sanitized between calf-feedings, and that they might have served as a source of horizontally transmitted infection. The association of first-colostrum feeding by nipple with *E. coli* isolation is compatible with this explanation, as is the association of virus-positive farms with delayed introduction of calf starter. This latter practice indicates that milk or milk substitute was the *only* source of feed intake for calves on those farms during that time. If feeding utensils were not properly cleaned greater opportunity for amplification of the virus population and for cross-contamination would occur.

The observation that group penning of calves is associated with *C. jejuni/coli* positive farms is biologically plausible. Calves kept in groups would be more likely to pass the organism back and forth among each other, allowing for a build-up of organisms. The finding that *C. jejuni/coli* positive farms tended to have below median mortality rates may be an artifact of the small numbers of positive farms involved, or may indicate that, although the organism was present, it was not causing any serious health problems. While not impossible, it seems biologically unlikely that the presence of *C. jejuni/coli* on a farm has a sparing effect on mortality.

The association of positive viral status with above median calf mortality rates suggest that, of the various organisms considered in this study, rotavirus (which comprised the bulk of the virus-positive samples) may have been associated with the most serious clinical problems in this population of calves. This interpretation assumes that mortality is a reflection of serious morbidity, while treatment days per calf reflect morbidity in a more general fashion. The particular strain of *Salmonella* found on these farms, *S. muenster,* was only rarely referred to in the literature prior to about 1982, and more research as to its effects on the health status of calves on Ontario dairy farms would be in order.

Aitken *et al* (17) isolated *Salmonella saint-paul* from neonatal calf feces 30 days before the onset of morbidity and mortality on two farms. In the study described in this paper, morbidity and mortality were recorded on calves well past this potential incubation period, and extended for several months beyond the end of the fecal-sampling survey. However, no increases in morbidity or mortality were detected that could be associated with the *Salmonella.*

With regard to the detection of enteropathogenic *E. coli,* the IFAT and CS tests do not appear to be measuring the same thing. The IFAT, using monoclonal anti-K99, would be expected to result in a more conservative estimate of *E. coli* positive fecals than CS, since the IFAT was structured to only look for one antigen, while CS screens for K99 as one of several antigens related to pathogenicity. The fact that the estimated prevalence based on IFAT was significantly higher than that based on CS is of some concern. It may be that the culture media used by the laboratory were not appropriate for the full expression of antigens, such as K99, associated with pathogenicity in *E. coli.* Lintermans and Pohl (18), in comparing a direct fluorescent antibody technique with conventional cultural methods, reported that the two tests agreed 75% of the time, with an expected agreement, by chance alone, of 67% (kappa = 0.245, $p < 0.05$). This was based on examination of 208 calf fecal specimens. The authors of that paper did not report whether or not their test readings and comparisons were done in a blind manner. They hypothesized that the discrepancy between the two tests was due, in part, to the ability of the immunofluorescence test to detect small numbers of organisms, including those that were nonviable. If that were the case in this study, one would have expected that fecals with a large number of organisms per field on IFAT would have been more likely to be classified as positive using CS. In fact, no such correlation was found. A further study, involving a large number of coded, randomly selected calf fecals subjected to both tests and perhaps judged by other criteria as well, should help clarify the utility of these two tests under normal diagnostic laboratory conditions.

The results of this study indicate that shedding of both K99 and rotavirus tended to be associated with scouring in individual calves on these southwestern Ontario farms. In both cases, the age at which calves were scouring was about ten days.

These results, in the context of the farm level data, where neither K99 nor rotavirus were associated with higher rates of morbidity, may mean one of two things. It may be that only *E. coli* possessing K99 were associated with diarrhea on these farms, and that the farm-level screening, using CS, was not sufficiently sensitive, microbiologically, to pick this up. On the other hand, farms with enteropathogenic *E. coli* may not have experienced more calf diarrhea problems than farms without the organism, but, within the *E. coli* positive farms those calves which had diarrhea were shedding *E. coli,* while diarrheic calves on *E. coli* negative farms were not; the diarrhea on those farms presumably being caused by other agents. This latter explanation would seem to be plausible with regard to rotavirus as well. However, since no association of morbidity with these organisms occurred at the farm level, this explanation assumes that mortality reflects the most serious cases of calf illness. There were not enough calves which died in this study (only four) to be able to explore this hypothesis at an individual calf level.

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