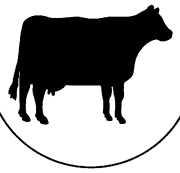

Bacteriological culture of blood from critically ill neonatal calves

Gilles Fecteau, David C. Van Metre, Julie Paré, Bradford P. Smith, Robert Higgins, Charles A. Holmberg, Spencer Jang, Walter Guterbock

Abstract — The objectives of this study were to estimate the prevalence of bacteremia in critically ill, neonatal calves with severe diarrhea or depression, and to describe the variety of bacteria involved. Two studies were conducted in the summers of 1991 and 1993 involving 190 neonatal calves, 1-day to 19-days-old. Bacteremia was detected by blood culture in 31% (28/90) of calves in study 1, and in 24% (19/79) of ill calves and 0% (0/21) of control calves in study 2. Bacteria cultured from blood included *Escherichia coli* (51% of all isolates), other gram-negative enterics (25.5%), gram-negative anaerobes (5.9%), gram-positive cocci (11.8%), and gram-positive rods (5.9%). Among clinically ill calves, the average age was significantly lower in the blood culture-negative group (5.5 d) than in the blood culture-positive group (7.5 d) ($P = 0.004$). Mean serum IgG concentration was significantly ($P = 0.0001$) lower in blood culture-positive calves (1.146 g/L) than in blood culture-negative calves (3.077 g/L). The mortality rate was significantly ($P < 0.0001$) higher in the blood culture-positive group (57.4%) than in the blood culture-negative group (15.1%). Bacteremia appeared to be a frequent entity in this particular rearing situation. Early recognition of the problem, as well as appropriate treatment, may be beneficial in increasing survival rates. Results also support the need to address the failure of passive transfer of maternal antibodies to prevent bacteremia in calves.

Résumé — Culture bactériologique du sang de veaux nouveau-nés dangereusement malade. Les objectifs de cette étude étaient d'évaluer la prévalence des bactériémies chez des veaux nouveau-nés dangereusement malades et présentant une diarrhée ou une dépression graves ainsi que de décrire les types de bactéries en cause. Deux études ont été menées au cours des étés 1991 et 1993 et comprenaient 190 veaux nouveau-nés âgés de 1 à 19 jours. La bactériémie a été détectée par culture sanguine chez 31 % (28/90) des veaux de la première étude et chez 24 % (19/79) des veaux malades et 0 % (0/21) des veaux témoins de la deuxième étude. Les bactéries cultivées à partir du sang comprenaient des *Escherichia coli* (51 % de tous les isolats), d'autres entérobactéries gram-négatif (25,5 %), des anaérobies gram-négatif (5,9 %), des coques gram-positif (11,8 %) et des bâtonnets gram-positif (5,9 %). Parmi les veaux cliniquement malades, l'âge moyen était significativement plus bas dans le groupe des cultures sanguines négatives (5,5 jours) que dans le groupe des cultures sanguines positives (7,5 jours) ($P = 0,004$). La concentration moyenne des IgG sériques était significativement plus basse ($P = 0,0001$) chez les veaux ayant des cultures sanguines positives (1,146 g/L) que chez ceux ayant des cultures sanguines négatives (3,077 g/L). Le taux de mortalité était significativement plus haut ($P < 0,0001$) dans le groupe des cultures sanguines positives (57,4 %) que dans le groupe des cultures sanguines négatives (15,1 %). La bactériémie semblait être une condition fréquente dans cette conjoncture d'élevage. Une reconnaissance hâtive du problème ainsi qu'un traitement approprié peuvent en augment les taux de survie. Les résultats appuient également le besoin de questionnement suite à l'insuccès rencontré dans la prévention de la bactériémie chez les veaux par le transfert passif des anticorps maternels.

(Traduit par docteur André Blouin)

Can Vet J 1997; 38: 95–100

Département de sciences cliniques (Fecteau), Département de pathologie et de microbiologie (Higgins), Faculté de Médecine Vétérinaire, Université de Montréal, C.P. 5000, Saint-Hyacinthe (Québec) J2S 7C6; School of Veterinary Medicine, University of California, Davis, California 95616 USA (Van Metre, Paré, Holmberg, Jang, Guterbock).

Introduction

Bacterial infections are an important cause of morbidity and mortality in the large animal neonate (1–7). Colostrum-deprived calves are more susceptible to developing infections during the neonatal period (8). The early environment of the dairy calf is an important source of microorganisms (corral maternity pen, hutches) (4,6,9). Moreover, calves allowed to nurse the dam often make erratic nursing attempts in heavily contaminated areas (tail, hock). This, often massive, oral contamination may lead to colonization of the gastrointestinal tract by different infectious agents, some of which may cause bacteremia or diarrhea. Other routes of entry of bacteria could be the umbilicus or the respiratory tract. Calves developing diarrhea of bacterial, viral, or parasitic origin often show clinical signs compatible with sepsis, including fever, scleral injection, pneumonia, inappetence, collapse, shock, and death. The prevalence of bacteremia in critically ill calves with diarrhea has not been reported.

Weinstein and Reller (10) reported that *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus pneumonia* were the most common bacteria isolated from the blood of ill humans (n = 500). Wilson and Madigan (3) reported that the bacteria most commonly isolated from septicemic foals (n = 47) were *E. coli*, *Actinobacillus* spp, and *Klebsiella pneumonia*. Dow and Curtis (11) reported that out of 100 critically ill dogs and cats, 49 (39 dogs, 10 cats) were bacteremic. Gram-negative rods were the most common organisms isolated from the bloodstream of dogs with bacteremia (46%), whereas, in cats, both gram-negative rods (especially *Salmonella enteritidis*) and anaerobic bacteria were most frequently isolated (11). In another report, the most common bacteria found in the blood of ill dogs were *Enterobacteriaceae* and coagulase-positive staphylococci (12). In neonatal calves presented at the University of Colorado Teaching Hospital with a confirmed diagnosis of septicemia, *E. coli* was the predominant bacterium isolated (1).

The objectives of this study were to: (1) estimate the prevalence of bacteremia among a nonrandom sample of ill neonatal calves on an intensive calf-raising farm, (2) examine the associations between bacteremia and total serum IgG concentration, age, and subsequent mortality (60 d following sampling), (3) describe the variety of bacteria found in neonatal bacteremic calves, and (4) examine the pattern of susceptibility to antimicrobial agents of these bacterial isolates.

Materials and methods

Study population

Two studies were undertaken in August 1991 and July 1993. Both studies were conducted on a calf-rearing farm housing over 15 000 calves in the San Joaquin Valley, California. Approximately 5000 calves were bottle fed at a time. Our sample consisted of 90 calves between 2 and 16 d of age in study 1, and 100 calves between 1 and 19 d of age in study 2 (79 ill and 21 controls).

Selection criteria

In study 1, calves were enrolled over a 6-day period. Only calves observed to have severe diarrhea or to be severely depressed were included in the study (n = 90). Calves were located in 7 different areas on the farm.

In study 2, calves were enrolled over a 5-day period, according to their total clinical score, which was made up of the 5 individual scores (feces, hydration, attitude, umbilicus, and scleral vessels) (13). The sample consisted of 21 control calves, with a total clinical score of 5 or lower, and 79 ill calves, with a total clinical score of 5.5 and above. Control calves were selected from calves located at least one stall away from an ill calf. Calves were located in 16 different areas on the farm.

Blood sample collection and processing

Two whole blood samples (a.m. and p.m.) were collected from each calf and submitted to the laboratory for culture. Prior to venipuncture, hair was shaved, skin was disinfected by vigorous scrubbing with povidone-iodine (7.5%) detergent, followed by one swabbing with isopropyl alcohol (99%) and one swabbing of povidone-iodine (10%) solution. Five milliliters of blood were then drawn from the jugular vein with a disposable syringe and an 18 gauge needle. After changing to a new needle and wiping the bottle stopper with isopropyl alcohol, the blood was injected into a commercially available 50-mL blood culture bottle of tryptic soy broth containing 0.03% sodium polyanethosulfonate and CO_2 (PML Microbiologicals, Tualatin, Oregon, USA). The bottles were transported to the laboratory, incubated at 37°C, and observed daily for 5 d. In study 1, bottles were vented over a flame on arrival at the laboratory, and anaerobic cultures were not performed. In study 2, bottles were kept under anaerobic conditions. Obligate anaerobic bacteria were identified, as previously described (14,15). Bottles were plated on regular tryptic soy agar, with 5% bovine blood at days 2 and 5, and at any other time there was evidence of growth (cloudiness and opacity when looking through the bottle). The criteria used for identification of bacterial isolates were as described by Jang *et al* (16).

Antimicrobial susceptibility testing

In study 1, antimicrobial susceptibility was performed on 25 isolates, using a commercially prepared microdilution tray (Radiometer America, Westlake, Ohio, USA). The performance and interpretation of each test was done as previously described (17). In study 2, antimicrobial susceptibility testing was carried out on 14 isolates of *E. coli*, using the disk diffusion method according to the National Committee on Clinical Laboratory Standards (18). For ceftiofur, the zone interpretive criteria were those suggested by the manufacturer (Upjohn Animal Health, Orangeville, Ontario).

Serum immunoglobulin G determinations

One serum sample was collected from every calf at the time of first whole blood sampling (a.m.). After centrifugation, samples were frozen at −20°C until analyzed for immunoglobulin G (IgG) by radial immunodiffusion (ICN Immunoglobulins Bovine IgG Kit, ICN Biomedicals, Costa Mesa, California, USA).

Table 1. List of bacterial isolates recovered from blood cultures from critically ill calves in 2 different studies

Family Bacteria	Study 1 $n = 29$	Study 2 $n = 22$
Gram-negative enterics	**23**	**16**
Escherichia coli	15	11
Klebsiella pneumonia	4	0
Klebsiella oxytoca	1	0
Klebsiella spp.	0	2
Salmonella dublin	1	1
Salmonella typhimurium	2	0
Campylobacter fetus ssp. *fetus*	0	1
Enterobacter cloacae	0	1
Gram-negative anaerobes	**N/A**	**3**
Bacteroides eggerthii	N/A	1
Bacteroides thetaiomicron	N/A	1
Prevotella bivia	N/A	1
Gram-positive cocci	**4**	**2**
Aerococcus viridans	1	0
Staphylococcus aureus	1	0
Staphylococcus hyicus	1	0
Staphylococcus simulans	1	0
Staphylococcus spp.	0	1
Streptococcus spp.	0	1
Gram-positive rods	**2**	**1**
Bacillus spp.	2	0
Listeria spp.	0	1

N/A: no attempt to culture

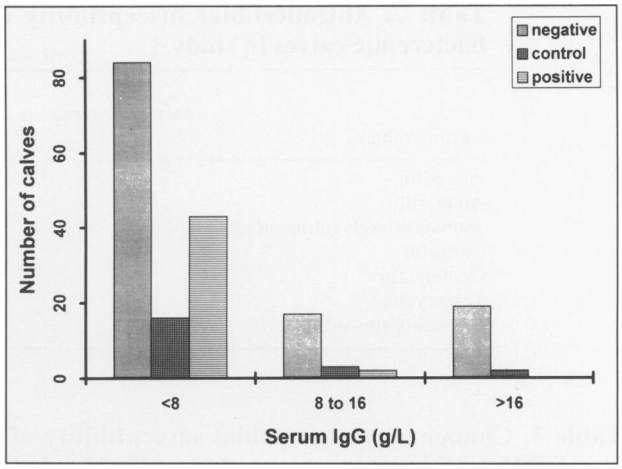

Figure 1. Distribution into low, medium, and high IgG concentration groups for blood culture-positive ill calves, blood culture-negative ill calves, and healthy control calves.

Definition of a positive animal

A calf was considered bacteremic if the same bacterium was isolated from both blood culture bottles, or at least 1 of the 2 blood culture bottles showed bacterial growth of a significant microorganism; bacteria were classified as significant or contaminant, based on the type of bacteria (probable pathogens or normal inhabitants of the skin), the rapidity of growth after sampling (less than or more than 2 d), and visual evidence of growth in the blood culture bottle.

Mortality

Records available on the ranch allowed for the follow-up of every calf for 60 d after sampling. Mortality dates were recorded.

Statistical analyses

Prevalence of bacteremia was computed separately for both studies as the number of blood culture-positive calves divided by the number of ill calves sampled. A 95% confidence interval (CI) was estimated using a normal approximation (19). For analytic purposes, data were classified into 3 groups: 1) Blood culture-positive ill calves, 2) blood culture-negative ill calves, and 3) control calves. Student's *t*-test was used to test the hypothesis of no difference in mean age and geometric mean IgG between blood culture-positive and blood culture-negative ill calves.

Cumulative proportions of calves surviving to 60 d after sampling were computed for blood culture-positive and -negative ill calves, using the product limit method (20). To test the hypothesis of no difference in 60-day mortality between blood culture-positive and -negative calves, rates of survival in the 2 groups were compared using the Breslow method, stratified by age group (<1 wk and ≥1 wk) (21).

To test the hypothesis of no difference in the pattern of *E. coli* antimicrobial susceptibility between the 2 studies, an adjusted chi-square statistic was used for each antibiotic examined. A level of significance of 0.10 was used for all analyses.

Results

Bacteriological results

Bacteriological culture of blood was performed on 378 samples from 190 calves (2 calves died before the 2nd sampling). Blood cultures were positive in 43 of the 179 bottles in study 1, and in 33 of the 199 bottles in study 2. Of 51 bacterial isolates cultured from blood, 51% were *Escherichia coli*, 25.5% other gram-negative enterics, 5.9% gram-negative anaerobes, 11.8% gram-positive cocci, and 5.9% gram-positive rods (Table 1). Positive cultures considered to be due to contaminants occurred in 5 bottles (1.3%). Bacteria attributed to the contamination were 1 *Bacillus* sp, 1 unclassified gram-positive rod, 1 unclassified gram-negative rod, and 2 unidentified bacteria. Polymicrobial growth was observed in only 1 blood culture bottle in study 1, and 2 in study 2. Bacterial combinations isolated in this study were *E. coli* and anaerobic bacteria, *Staphylococcus hyicus* and *Aerococcus viridans*, and *Klebsiella* spp, and *Enterobacter cloacae*.

Among clinically ill calves, 31% ($n = 28/90$, 95% CI, 22.0%–41.9%) were bacteremic in study 1, and 24% ($n = 19/79$, 95% CI, 15.4%–35.2%) in study 2. None of the control calves ($n = 21$) were bacteremic. The same bacterial species was present in both blood samples from 15 calves (54% of positive) in study 1, and from 14 calves (74% of positive) in study 2.

Susceptibility of 25 bacterial isolates from study 1 to 7 antimicrobial agents is presented in Table 2. Comparison of susceptibility of *E. coli* to 6 different antibiotics indicated that susceptibility in the 1st study

Table 2. Antimicrobial susceptibility of 25 bacterial isolates from the blood of bacteremic calves in study 1

Antimicrobial	*Staphylococcus* ($n = 3$)	*Escherichia coli* ($n = 14$)	*Salmonella* spp. ($n = 3$)	*Klebsiella* spp. ($n = 5$)	Total ($n = 25$)
Penicillin	0	N/A	N/A	N/A	0/3
Ampicillin	1	9	1	0	11/25
Amoxicillin/clavulinic acid	0	13	2	5	20/25
Ceftiofur	0	13	1	5	19/25
Gentamicin	1	4	2	5	12/25
Tetracycline	1	1	1	0	3/25
Trimethoprim/sulfas	2	4	3	5	14/25

Table 3. Change in antimicrobial susceptibility of *Escherichia coli* isolates recovered from the blood of bacteremic neonatal calves between summer 1991 (study 1) and 1993 (study 2), and the statistical significance of changes in the proportion of susceptible isolates

Antimicrobial	Study 1 ($n = 14$)	Study 2 ($n = 11$)	Significance
Ampicillin	9	1	$P = 0.02$
Amoxicillin/clavulinic acid	13	1	$P < 0.0002$
Ceftiofur	13	6	$P = 0.08$
Gentamicin	4	8	$P = 0.07$
Tetracycline	1	0	$P = 0.89$
Trimethoprim/sulfas	4	7	$P = 0.18$

was significantly different from that of the 2nd study for 4 of the 6 antibiotics examined (Table 3).

Association with age

Among ill calves, the average age for the blood culture-negative group was 5.5 d, with a median of 4 d. The average age for the blood culture-positive calves, 7.5 d (median = 8 d), was significantly higher ($P = 0.004$) than that for blood culture-negative calves.

Association with serum immunoglobulin concentrations

Overall, the mean and median immunoglobulin concentrations were 2.373 g/L and 2.154 g/L, respectively. Mean IgG concentration was lower in the blood culture-positive, ill calves (1.146 g/L, median = 0.96 g/L) than in the blood culture-negative, ill calves (3.077 g/L, median = 3.248 g/L) ($P = 0.0001$). Using previously described IgG categories to indicate low, moderate, and good passive transfer (22), the distributions for blood culture-positive, -negative, and control calves are presented in Figure 1. None of the calves with a serum IgG > 16.0 g/L was identified as bacteremic.

Association with mortality

Follow-up records were available for all calves sampled, except for 3 blood culture-negative calves. Forty-six calves died during the 60 d following sampling. Postmortem examination was not performed on any of the dead calves. The mortality rate was 57.4% (27/47) among blood culture-positive, ill calves and 15.1% (18/119) among blood culture-negative, ill calves. In the control group, the mortality rate was 4.8% (1/21).

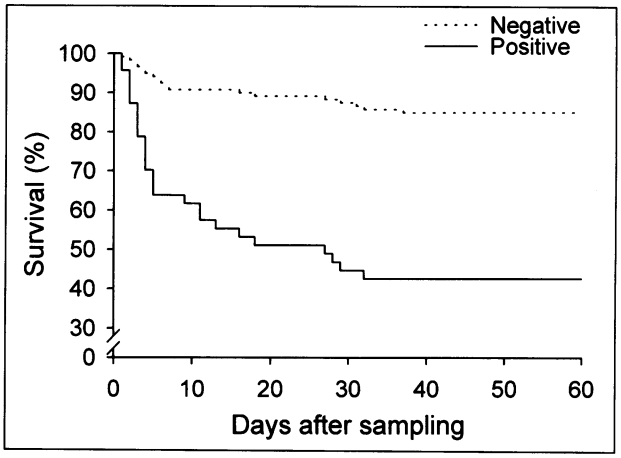

Figure 2. Survival to 60 d postsampling for blood culture-positive ($n = 47$) and blood culture-negative ($n = 119$) ill calves.

Among ill calves, the survival rate, stratified by age, was significantly higher in blood culture-negative calves than in blood culture-positive calves ($P < 0.0001$) (Figure 2). In blood-culture positive calves, 25% were dead within 4 d. In calves > 1 wk, 50% died within 17 d after sampling (Figure 2).

Discussion

The definition of a bacteremic animal is variable. For the purpose of this study, it was based mainly on the types of bacteria isolated. In a case by case situation, the microbiologist and the attending clinician should discuss and decide upon the significance of a particular bacterium in the blood of a patient.

Bacteria isolated from critically ill, neonatal calves in the present study were similar to those isolated from humans and foals (3,10). Compared with the findings of Aldrige *et al* (1), in which 28% of calves had polymicrobial infection, very few bacteremic calves had a polymicrobial infection. However, differences between the 2 populations studied must be considered. In the Aldrige study, the population consisted of 25 neonatal calves presented to a veterinary hospital in a moribund state (1). The mortality rate in bacteremic calves in that study (88%) was also different from the one in this study (57%). One explanation could be that it is not uncommon for critically ill calves to develop polymicrobial infection in the final stage of disease. Another

explanation could be differences between the sampling techniques.

In the present study, gram-negative enteric bacteria were the most commonly isolated microorganisms, which supports the hypothesis that the early environment of the calf may be the source of infection. The route of entry of bacteria could be the alimentary tract, the umbilicus, or the respiratory tract. Infection could enter through the gut due to the permeability of the enterocytes during the first 24 h of life. If this were the case, one would expect a higher frequency of bacteremia in younger calves than in older calves. The opposite was observed. The significantly higher prevalence of bacteremia in older calves appears contradictory to current literature, which indicates that colisepticemic calves usually die between 24 and 48 h of age (2,4). Four theories serve as possible explanations: (1) calves used the small amount of passive antibody available in the 1st wk of life, which made them more susceptible in the 2nd wk (the running out of defense theory), (2) severely ill calves died quickly and thus were not represented in this study (the missing calf theory), (3) calves fed milk replacer at only 10% of their bodyweight are energy deficient until they eat enough concentrate to complete their needs: during their 1st wk of life, they use the small amount of brown fat that they are born with, so that in the 2nd wk they are more susceptible to translocation of bacteria from the gut to the circulation (23) (the running out of fuel theory), and (4) older calves have had a greater opportunity for exposure to viral and bacterial agents that facilitate entry of pathogens through damaged gut mucosa.

Antimicrobial susceptibility patterns of bacteria isolated in the present study were interesting, but the small number of isolates hinders drawing strong conclusions. However, the general resistance to gentamicin (20/36), a drug that was commonly used on this farm in 1991, should generate some concerns. Surprisingly, there was less resistance to gentamicin in 1993 than there had been in 1991 ($P = 0.05$). Ceftiofur appeared to be an excellent choice according to the results of study 1, but due to an increase in bacterial resistance, it became a 2nd choice 2 y later. However, it is important to note that ceftiofur was not commonly used on this ranch in 1991, but it was used as a 1st line antibiotic in 1993. The susceptibility pattern of *E. coli* observed in the present study indicates that resistance patterns can change quite drastically in a relatively short period of time. Monitoring drug resistance should be carried out on a regular basis; this could be a valuable tool for clinicians for the evaluation of the impact of the use of antimicrobial agents in food animals. Some human hospitals have such a monitoring system, and it allows infectious disease committees to control the use of some antibiotics when increased resistance is observed (Pierre Turgeon, St-Luc Hospital, Montreal, personal communication). This finding also raises the question of the transfer of resistance to humans through food-borne, antimicrobial residues. Public perceptions that food-borne residues pose a major health risk are not supported by actual case reports (24).

Serum IgG concentrations of the majority of the calves sampled were below those described as acceptable for the bovine neonate (22,25). Despite this fact, there was a significant difference between blood culture-positive and blood culture-negative calves. Higher frequency of bacteremia in colostrum-deprived calves may be explained by an increased susceptibility to disease. However, it could be hypothesized that it is technically easier to isolate bacteria from a sample containing a lower concentration of antibodies, because the antibacterial effect in blood would be diminished. Theoretically, however, the addition of sodium polyethanol sulphonate and the dilution of blood by the medium contained in the blood culture bottle should have neutralized the antibacterial effect of blood (26).

The mortality rate was significantly higher in the blood culture-positive group than in the blood culture-negative group, although the exact cause of death was not investigated. Possibly the same risk factors that contributed to an increased risk of a calf being bacteremic were responsible for the increased risk of death. Early deaths following identification of blood culture-positive calves suggests a causal effect between positive blood culture and death.

Surprisingly, the rate of contamination with fortuitous bacteria was low (1.3%), considering that the sampling was performed on the farm. Preparation techniques were therefore deemed appropriate. Veterinarians in private practice could submit blood culture samples when judged appropriate. Human medical literature reports contamination rates to be as high as 14% in some studies (26). However, 1.5% to 4.8% contamination rates are more commonly considered as acceptable (27).

High prevalences of bacteremia were identified among a nonrandom sample of ill neonatal calves in the studies presented here, with the most frequently cultured bacteria being *Escherichia coli*. However, other gram-negative enterics, gram-negative anaerobes, gram-positive cocci, and gram-positive rods could also be cultured. Serum IgG concentration plays an important role in the development of bacteremia and these results support the need to address failure of passive transfer of maternal antibodies in the control and prevention of bacteremia in calves. In contradiction to reports in the current literature, older calves seem to be more frequently identified as bacteremic. As one would expect, development of bacteremia increased the risk of death, so early recognition of the problem, as well as appropriate treatment, may be beneficial in increasing survival rates. Bacteremia and its consequences appeared to be a frequent entity in this particular calf-rearing operation. Colisepticemia should be included in the differential list when neonatal mortality is being investigated in large veal calf operation. CVJ

References

1. Aldridge BM, Garry FB, Adams R. Neonatal septicemia in calves: 25 cases (1985–1990). J Am Vet Med Assoc 1993; 203: 1324–1329.
2. Blood DC, Radostits OM. Veterinary Medicine, 7th ed. London: Baillière Tindall, 1989: 47–48, 95–121, 619–621.
3. Wilson WD, Madigan JE. Comparison of bacteriological culture of blood and necropsy specimens for determining the cause of foal septicemia: 47 cases (1978–87). J Am Vet Med Assoc 1989; 195: 1759–1763.

4. Roy JHB. The Calf, vol 1. Management of Health, 5th ed. Toronto: Butterworths, 1990: 38, 58.
5. Waltner-Toews D, Martin SW, Meek AH, McMillan I. Dairy calf management, morbidity and mortality in Ontario Holstein herds. I. The data. Prev Vet Med 1986; 4: 103–124.
6. Curtis CR, Scarlett JM, Hollis EN, White ME. Path model of individual-calf risk factors for calfhood morbidity and mortality in New York Holstein herds. Prev Vet Med 1988; 6: 43–62.
7. Koterba A, Madigan JE. Manifestation of disease in the neonate. In: Smith BP, ed. Large Animal Internal Medicine. 1st ed. St Louis: Mosby, 1990: 316–384.
8. Guay CC. Failure of passive transfer of colostral immunoglobulins and neonatal disease in calves: A review. Proc 4th Int Symp on Neonatal Diarrhea 1983: 346–364.
9. Hancock D. Epidemiologic diagnosis of neonatal diarrhea in dairy calves. Proc Annu Conv Am Assoc Bovine Pract 1983; 15: 16–22.
10. Weinstein MP, Reller LB, Murphy JR, Lichtenstein KA. The clinical significance of positive blood cultures: A comprehensive analysis of 500 episodes of bacteremia and fungemia in adults. I. Laboratory and epidemiologic observations. Rev Infect Dis 1983; 5: 35–53.
11. Dow SW, Curtis CR, Jones RL, Wingfield WE. Bacterial culture of blood from critically ill dogs and cats: 100 cases (1985–1987). J Am Vet Med Assoc 1989; 195: 113–117.
12. Hirsh DC, Jang SS, Biberstein E. Blood culture of the canine patient. J Am Vet Med Assoc 1984; 184: 175–178.
13. Fecteau G, Paré J, Van Metre DC, *et al.* Use of a clinical sepsis score for predicting bacteremia in neonatal dairy calves on a calf rearing farm. Can Vet J 1997; 38: 101–104.
14. Holdeman LV, Cato EP, Moore WEC. Anaerobe Laboratory Manual, 4th ed. Blacksburg: Virginia Polytechnic Institute and State Univ, 1977.
15. Sutter VL, Citron DM, Edelstein MAC, Finegold SM. Anaerobic Bacteriology Manual, 4th ed. Toronto: Star Publ, 1985.
16. Jang SS, Biberstein EL, Hirsh DC. A Diagnostic Manual of Veterinary Clinical Bacteriology and Mycology. Davis, California: Univ California, 1982.
17. Barry AL. The Antimicrobial Susceptibility Test: Principles and Practices, 1st ed. Toronto: Lea & Febiger, 1973: 73.
18. National Committee on Clinical Laboratory Standards. Performance Standards for Antimicrobial Disk Susceptibility Tests. Approved Standards, 4th ed. Villanova, Pennsylvania: National Committee on Clinical Laboratory Standards, Document M2-A4, 1990.
19. Fleiss JL. Statistical Methods for Rates and Proportions, 2nd ed. New York: John Wiley, 1981: 14–15.
20. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958; 53: 457–481.
21. Breslow N. A generalized Kruskal-Wallis test for comparing k samples subject to unequal patterns of censorship. Biometrika 1970; 57: 579–594.
22. McGuire TC, Adams DS. Failure of colostral immunoglobulin transfer to calves: Prevalence and diagnosis. Compend Contin Educ Pract Vet 1982; 4: S35–S39.
23. Deitch EA, Winterton J, Li M, Berg R. The gut as a portal of entry for bacteremia. Role of protein malnutrition. Ann Surg 1987; 6: 681–692.
24. Sundlof SF. Antimicrobial drug residues in food-producing animals. In: JF Prescott, JD Baggot, eds. Antimicrobial Therapy in Veterinary Medicine, 2nd ed. Ames: Iowa State Univ Pr, 1993: 569–591.
25. Parish SM. Ruminant Immunodeficiency Diseases. In: Smith BP, ed. Large Animal Internal Medicine. St-Louis: Mosby, 1990: 1610–1614.
26. Gould JC, Duerden BI. Blood culture: Current state and future prospects. J Clin Pathol 1983; 36: 963–977.
27. Courcol R, Broutin M, Martin G. Aspects récents des techniques d'hémoculture. Ann Biol Clin 1987; 45: 1–14.