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THE NEWBORN CALF SEPTICEMIA SYNDROME - CLINICAL AND BACTERIOLOGIC STUDIES OF AFFECTED AND NORMAL INDIVIDUALS

by

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Hyderabad, India, 1959

A MASTER'S THESIS

submitted in partial fulfillment of the

requirement for the degree

MASTER OF SCIENCE

Department of Surgery and Medicine

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1966

Approved by:

F. W. Oehme

Major Professor

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1966
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INTRODUCTION

The newborn calf septicemia syndrome has been known for years. According to Jensen (38) the disease had been present for 100 years when first described in 1893. Calf diarrhea, colibacillosis, "calf scours," "infectious scours," "white scours," septicemia neonatorium, and Kälberruhr are other terms used to designate this disease.

High morbidity and mortality rates in newborn animals cause major economic loss and can seriously disrupt a planned breeding program. Half the deaths take place in the first week of life and three-quarters occur in the first month (45). A survey conducted by Withers (76) emphasized the importance of the disease in dairy calves, where 27.9% of the total deaths were reported to be caused by Escherichia coli (E. coli) infection. Forty-eight per cent of the deaths occurred in the first week, about 66% in the first fortnight, over 74% in the first 3 weeks, and over 80% in the first month (76). Jordan (40) reported a mortality of 25% in the spring and 8% in the fall in 26 dairy herds. Postmortem examination of 78 calves which died in the University of Connecticut herd during a 17 year period, indicated that calf diarrhea was responsible for 43.6% of the losses (74).

It is well established that numerous organisms are associated with calf diarrhea. Most of the bacteria are normally present in the intestinal tract of adults, i.e., E. coli, Proteus vulgaris, and Pseudomonas pyocyanea (aeruginosa). Of all, the first mentioned is most frequently incriminated. E. coli is regarded by some workers as non-pathogenic for man and animals (35). As remarked by Spence (70), however, fixed ideas as to what is pathogenic are apt to lead one astray, especially

when considering diseases of the newborn. Predisposing factors such as age, nutrition, management, and immunity may be of equal importance.

The purpose of this investigation was to study the newborn calf septicemia syndrome in an attempt to differentiate various forms of the disorder.

REVIEW OF LITERATURE

Disease Description

Fincher (26), in discussing calf septicemia, wrote: "Calves may be born at full term from apparently normal cattle and die from acute septicemia in 12 to 96 hours, may scour profusely for several days, or may have milder diarrhea for a variable period. Several of a group of affected calves may survive, only to terminate in unthriftiness with or without pneumonia." Blood and Henderson (7) observed that the commonest single disease entity and cause of death appeared to be E. coli infection, the disease leading to enteritis or toxemia-septicemia, with the former commonly progressing to a septicemia. Amstutz (2) remarked that all types and breeds of calves appear to be equally susceptible.

A mortality of 25% in the spring and 8% in the fall was reported in 26 herds in Ayrshire (40). Lovell and Hill (45) observed a 5% mortality among calves in England and an 8% mortality in Scotland. In England, 68% of the calves died during the first 2 weeks of life. Of 100 calves examined bacteriologically by Lovell and Hughes (43), 25 were less than 7 days old and 28 were from 7 to 14 days of age. Thirty-seven of the 100 calves examined had died from colibacillosis, 22 by the 7th day and another 11 by the 14th day of life. Lovell and Hill (45) recorded nearly half of the deaths in female calves during the first week of life and three-quarters in the first month.

Significance of Colostrum

The significance of colostrum in the nutrition of calves has long been appreciated. Smith and Little (65) directed attention to the association between calf diarrhea and the lack of colostrum. All 10 calves permitted to take colostrum after birth survived, whereas 8 of 12 which did not receive it died. Of the latter group, 7 were less than 1 week of age.

Comline et al. (14) introduced fat free colostrol whey into the small intestine of young calves and found that the unchanged globulins did not enter the portal circulation in appreciable amounts; but are instead carried in the lymph to the peripheral blood. It has been pointed out that calves deprived of colostrum lacked something which prevented the intestinal bacterial flora from invading the body and multiplying in various tissues (65). In most cases the disease developed into a rapidly fatal septicemia. According to Smith and Orcutt (66), a great increase in the number of E. coli in the lower part of the small intestine occurred in cases of calf diarrhea. They inferred that "scours" is the result of inadequate digestive function and increasing virulence of E. coli.

A higher concentration of antibodies was found by Smith (69) in the colostrum than in the serum. The colostrum of normal cows contained antibodies to an E. coli associated with the calf septicemia syndrome.

Orcutt and Howe (53) presented evidence associating absorption of agglutinins with the appearance of certain blood protein fractions of newborn animals. They observed that it may be possible for colostrum

to be of benefit in many ways and that this accounted for the difference of opinion as to how colostrum exerts its action. It is thought by some authors to confer a passive immunity and to benefit the calf because of its vitamin content or because it supplies the newborn with protein (46). Many calf problems are traceable to improper feeding. It has been suggested that all calves receive colostrum reinforced with vitamin A, riboflavin, and antibodies shortly after birth (2). Awad (4) remarked that the risk of enteritis and death is greatly increased when the young calf does not receive colostrum. In spite of feeding colostrum, however, outbreaks of calf diarrhea and death occurred.

McEwen (48) reported that calves deprived of colostrum survived and enjoyed good health depending on the quality of milk fed them. They suggested that cow's milk from the early stage of lactation may replace colostrum. Inglis (36) came to the conclusion that lack of colostrum feeding was not a common cause of enteritis in his area.

Calves born to a mother with less than 250 blue units (Moore's) of vitamin A were susceptible to infection (71). Fey and Lindt (22) reported that the mean vitamin A level in the livers of calves dying from septicemia was significantly lower than that for normal calves.

In a later paper, Fey and Lindt (23) reported that immunoelectrophoretic studies on the sera of 22 calves which received colostrum, but died from E. coli septicemia, showed that 21 had a deficiency or absence of gamma globulins in the blood. They considered it likely that calves deficient in gamma globulins developed septicemia when they came in contact with a pathogenic type of E. coli. Fey et al. (21) observed that

of 88 calves which died of colibacillosis, 80 had no gamma globulins in their blood, even though they had received colostrum. Bürki and Fey (9) reported that the absence or deficiency of gamma globulins in the blood was related to the occurrence of septicemia and that cytopathogenic entero-viruses did not influence colibacillosis in calves.

In studying the mutations of E. coli and their immunological significance, the rapid mutation of strains from the ileum of scouring calves were reported to give rise to forms without capsular substance having greatly reduced virulence (67). Lovell (44) confirmed these findings. Smith (68) felt that the capsular substance was the material carrying virulence or, expressed somewhat differently, was the factor which protected the microorganism against the host's defenses. Smith (64) reported that the capsular substance was probably significant for virulence only when functioning as a morphological capsule and was present in young culture filtrates in small amounts.

Etiology

One of the first workers to attempt to isolate the organism responsible for calf diarrhea was Jensen (38). Further observations were carried out in the United States by Smith and Little (65) in 1922 and by Smith and Orcutt (66) in 1925. In Great Britain, the first observations were made by Lovell and Hughes (43) in 1935 and Lovell (44) in 1937, although the significance of the disease had been recognized much earlier.

Nocard (51) in 1901, reported that attempts to isolate the causal

organism were not initially successful. Later, examinations of exudate from the joint of a calf suffering from a mild form of calf diarrhea revealed staphylococci, streptococci, bacilli resembling E. coli, and a small non-motile bacterium belonging to the fowl cholera type. This same organism was found in the heart blood and umbilical vessel. The disease was reproduced by inoculation with an artificial culture.

The same author (52) reported that "white scours" and "lung disease," in etiology, and clinical form came from the same infection. The specific "agent" sometimes killed the subject within a few hours due to a septicemia. It was Nocard who gave the name "white scours" to the disease (33).

Hagan (33) observed that the pneumonia which accompanies calf diarrhea was not like Nocard's type, but rather was confined to the lower part of one or both anterior lobes. Cultures made from these lungs revealed E. coli in the majority of cases. He further observed that in a large percentage of calves, E. coli and certain cocci were found in the meconium. The same organisms were found in the fetal fluids of apparently normal cows. The infection frequently existed in the intestines of newborn calves, inducing an acute toxic condition with diarrhea soon after birth. He described the signs in the acute type of calf scours as cold extremities, greatly accelerated pulse and respiration, body temperatures as high as 106°F, dull listless attitude, and inability to stand.

Williams et al. (75) criticized the work done by Jensen and observed that calf diarrhea was fundamentally an intrauterine infection of the fetus. On bacterial examination, the authors obtained a bacillus belonging to the colon group, accompanied by staphylococci, streptococci,

and micrococci. They failed to produce calf diarrhea by inoculations with cultures of the colon organism.

Bacteriologic examinations were carried out on 14 normal calves, from 1 to 98 days of age, and on 13 diseased calves whose ages varied from 2 to 67 days (13). The examinations of the 2 day old calves revealed that the colon-aerogenes organism was distributed in all the examined tissues. Normal calves examined the 3rd day after birth showed no E. coli organism in the duodenum or jejunum. The findings in calves 4 to 8 days of age were similar to those obtained from the 3 day old calves. E. coli was observed to multiply extensively in the small intestine.

Smith and Orcutt (66) investigated the bacteriology of the intestinal tract of young calves and reported E. coli in the small intestine in calves dying of scours. In an older group of normal calves, E. coli was either absent or present in very small numbers. A vibrionic enteritis was reported in calves over 2 weeks of age (39). It was indicated that infection might occur relatively early, the symptomology becoming more pronounced as the disease became chronic.

Jordan (40) felt that calf diarrhea appeared to be chiefly due to 1 of the strains of E. coli. In calves which had not taken food, the absence of intestinal juices and the presence of meconium afforded a favorable medium for bacterial growth. Their penetration through the mucous membrane thought to be facilitated by the intestinal epithelium not possessing a mucous covering at that age.

Lovell and Hughes (43) examined 100 calves dying within the first few months after birth. Pure infections with E. coli accounted for 37

cases, Corynebacterium pyogenes for 12, hemolytic coccobacilli for 6, Salmonella typhimurium for 2, and pasteurellae for 1. Mixed bacterial infections were encountered in 11 cases. Diarrhea was a common sign, in addition to inappetence, dullness, weakness, and in a few cases, respiratory distress. At necropsy, gross infection of the umbilicus and enteritis was found in less than half the animals. The lungs invariably showed some congestion. The authors pointed out the lack of any distinct pathological changes in E. coli infection. In 2 calves affected with Salmonella, symptoms included general weakness and diarrhea in 1, and shivering, coryza, and lacrimation in the other. In neither case were marked postmortem lesions present.

Thorp et al. (72) examined 125 cultures from 15 animals and reported that they were mostly of the coliform group. Hemolytic and non-hemolytic streptococci were also isolated. On necropsy, the organisms were isolated from the spleen, liver, kidney, heart blood, brain, and mesenteric lymph nodes. The four cases from which non-hemolytic streptococci were isolated from the brain exhibited definite nervous signs. A watery, sometimes bloody, diarrhea developed in calves 1 to 2 days old. On necropsy of the acutely affected animals, the small intestine was highly inflamed, with occasional large hemorrhagic areas present. Thrombosis of the intestinal blood vessels was observed in numerous areas.

A highly fatal disease of very young calves was reported by Macrae et al. (47). The disease was characterized by a sudden onset, dullness, and diarrhea with subsequent coma and death. In all cases a bacillus resembling Clostridium welchii (type A) was demonstrated in the alimentary

tract, either as a pure or mixed infection. Griner and Bracken (32) described an apparently new, acute, infectious disease of baby calves, somewhat similar to enterotoxemia of lambs but caused by Clostridium perfringens (type C).

An infectious disease of calves caused by a filterable virus and characterized by fever, diarrhea, and pneumonia has been reported (5). On autopsy, a catarrhal enteritis and broncho-pneumonia was noted. Barr et al. (6) has drawn attention to a form of enzootic calf pneumonia, stating that the disease may be more common than realized and that outbreaks may be wrongly diagnosed as "white scours." Diarrhea was the main symptom and was complicated by pneumonia in young calves. Jennings and Glover (37) reported that a febrile reaction commencing the 5th day after exposure and constipation followed by diarrhea were features of enzootic pneumonia of calves. A non-specific diarrhea was described by Kendall (41) as occurring in white muscle disease areas of the United States. It was characterized by unthriftiness and diarrhea of varying degrees.

Despite the existence of many antigenic types of E. coli, a comparatively small number are reported to be regularly associated with colibacillosis (56). Rees assumed that the principal elements of the local coliform flora will be shared by adult and young animals in a herd.

Van Pelt et al. (74) recorded a low bacterial count during diarrhea; this was, however, preceded by a high count 1 to 2 days before the diarrhea began.

Wood (77) examined 153 calves affected with calf diarrhea. Of these, 144 had infections caused by coli-aerogenes organisms. Seventy-three

of the calves had septicemia. In 33 cases, the infection appeared to be limited to the intestinal tract. Pure cultures of E. coli were frequently isolated.

It was felt by some authors that less emphasis should be placed on calf diarrhea as being associated with a relatively few specific types of E. coli (58). Dunne et al. (19), however, presented evidence to emphasize the importance of E. coli in enteric conditions of newborn calves. They summarized that E. coli had been isolated more times than any other organism from calves dying of calf diarrhea, and that the disease was reproducible under controlled conditions. They suggested that the diarrhea caused by E. coli was a different entity from the pneumoenteritis of viral etiology.

It was observed that the infectious E. coli organism replaced the normal flora of the calf alimentary canal rapidly in disease states (31). Osborne et al. (54) isolated microorganisms of the genus Escherichia from the feces of scouring calves in 37 of 38 attempts. Smith (60) indicated that proliferation of E. coli in the upper regions of the intestinal tract was an important factor in the pathogenesis of natural cases of calf diarrhea.

Until recently, Salmonellosis in cattle was not considered a serious problem in the United States (27). Ingram and Lovell (35) observed that E. coli was still regarded by many workers as non-pathogenic, whereas salmonellae were considered pathogenic for man and animals. Salmonella dublin and Salmonella typhimurium were commonly reported in calves. Gibson (28) reported that cattle of all ages became infected with different salmonellae organisms, giving rise to clinical or subclinical

infections.

Moore et al. (50), while reviewing the recent literature, observed that acute Salmonellosis in calves was characterized by depression, anorexia, fever, fetid diarrhea, dehydration, and joint swellings. Death due to septicemia occurred in calves during their first week of life. Buxton (10, 11) observed that young calves were more susceptible to Salmonella infections than adults, and that the development of fatal Salmonella infections was dependent upon the ability of the organism to invade and multiply in the tissues. Field (25) noted the organism could be recovered from the feces and, during the early febrile stage of the disease, from the blood.

Smith (59) reported that salmonellae were present in the feces of only 3 of 184 cases of calf diarrhea. Shigellae were not found. Schofield (57) noted only persistent diarrhea, with rapid loss in condition in young calves affected with salmonellae.

It was noted that the principal fecal bacteria identified from healthy young animals were E. coli, Cl. welchii, streptococci, lactobacilli, bacteroides, and Staphylococcus aureus (61). Smith (62) observed that the alimentary bacterial content of mildly or moderately ill colostrum-fed calves differed very little from that of healthy calves. In severely ill animals, however, greater numbers of E. coli and lactobacilli were present. Serologic studies of E. coli, and generally negative transmission results, suggested that neither E. coli nor any other bacterium was primarily concerned in the etiology of the disease.

The application of immunofluorescent staining techniques to the

diagnosis of diarrhea due to enteropathogenic E. coli has been described (3).

Smith (63) reported that hemolytic E. coli were found in fecal samples from 76% of normal cattle, 63% of normal pigs, 53% of healthy sheep, and 18% of normal humans. Khera and Dhanda (42) described results from the examination of 182 buffaloe calves and 9 bovine calves dying within 3 months after birth. In addition to 74 salmonellae infections, pure septicemia infections were found in 67 individuals (E. coli 35, Bact. aerogenes 5, Proteus spp. 15, Pseudomonas aeroginosa 6, Bact. alcaligenes 3, Brucella abortus 2, and Vibrio sp. 1). Mixed infections occurred in 22 cases.

Botes (8) reported that on 1 farm an illness affected calves aged 3 to 8 days of age with a 10% mortality over a period of 3 years. It was characterized by diarrhea and dehydration and occasionally with nervous complications. Proteus mirabiles and E. coli were found to be the cause. Hibbs and Foltz (34) reported about a 2 week old calf presented for necropsy by an owner that had lost 10 of 150 calves. The blood count was hemoglobin 13.0, hematocrit 46, white blood cells 26,168, juvenile neutrophils 15, band neutrophils 10, segmented neutrophils 19, and lymphocytes 56. The body temperature was 96.0°F. E. coli and Salmonella typhimurium were isolated from the intestine.

Calves were experimentally infected with E. coli serotypes 078:B80 and 0115 (commonly associated with septicemic "calf scours"), 0141 (rare in calves), and 018 (isolated from healthy calves) (15, 17). Their virulence was found to decrease in the order 078:B80, 0115, 0141,

and 018. Dam (16) concluded that a fairly constant predominance of strains of E. coli belonging to the O groups (078, 0115, 015) was to be expected in calf diarrhea.

Ulendeev (73) typed 249 strains of E. coli (68 from healthy and 181 from sick calves) with the aid of 18 different O-group sera. They belonged to O-groups 9, 119, 115, 117, and 78--in order of decreasing frequency. Akhmedova and Agdami (1) reported the isolation of 661 strains of E. coli. Seventy-three per cent of those from calves and 74% of those from lambs were of serotypes O-86, O-55, or O-26. These strains were not very pathogenic for mice.

Fey et al. (24) produced E. coli septicemia by oral and intranasal infection with type 78:80B and indicated that intestinal infection occurred through the blood stream rather than by the digestive system. Glantz and Rothenbacher (29) suggested the possibility of the lymphogenous-hematogenous route of infection via the nasopharynx. Cameron (12) injected live enteropathogenic E. coli serotypes into mice and dead serotypes into mice, rabbits, and calves. They found that an immunity followed in 24 to 72 hours, depending upon the route of injection.

With regard to etiology, Fincher (26) says, "This defies accurate description, but the condition is assumed to be due to a great variety of known and, no doubt, several undiscovered factors."

MATERIALS AND METHODS

Selection of Normal Calves

Ten normal, apparently healthy calves from the Department of Dairy Science, Kansas State University, ranging in age from 3 to 26 days, were selected for study as control individuals. There were 6 female and 4 male calves composed of 5 Holsteins, 4 Ayrshires and 1 Jersey. The examination procedure included:

(1) History: (a) calving and breeding history; (b) history of recent vaccinations; (c) new additions to the barn; (d) mortality of calves in past; (e) management practices.

(2) Physical examination: (a) attitude of animal; (b) rectal temperature; (c) respiration and pulse; (d) auscultation of heart and lungs; (e) examination of visible mucous membranes; (f) condition of the skin and coat; (g) digestive tract function and character of feces.

After physical examination, 2 samples of blood and 1 of feces were collected for laboratory examination. The technique for collection of samples is given under "Collection of Samples."

Selection of Affected Calves

Nineteen calves, 1 day to 4 weeks of age, admitted to the Dykstra Veterinary Hospital and suspected of suffering from "calf septicemia," were selected for study. The tentative diagnosis of "calf septicemia" was based on history, character of feces, rectal temperature, dehydration, and general condition of the animal.

Of the 19 calves treated, 11 were subsequently discharged and 8 died. There were 9 males and 10 females (6 Holstein, 3 Hereford, 3 Shorthorn, 2 Ayrshire, 2 Angus, 1 Guernsey, 1 Jersey, and 1 mixed breed).

A record of the owner's observations, management practices, and previous treatment was maintained. A thorough physical and systemic examination was undertaken, including: rectal temperature, character and frequency of bowel movements, and general condition of the calf (dehydration, debility, anemia, etc.). Two samples of blood and 1 of feces were collected after admission of the animal into the clinic, but prior to starting treatment. The technique is described under "Collection of Samples."

Calves that died were subjected to necropsy within 24 hours. After necropsy, specimens of the kidney, liver, spleen, lungs, intestinal content, and mesenteric lymph nodes were subjected to bacterial examination.

Collection of Samples

Two blood samples and 1 fecal sample were collected from normal and affected calves for laboratory examination. Undue excitation was avoided at the time of sampling. Samples were collected only once before treatment was instituted.

Fecal specimens, freshly voided or removed from the rectum, were collected with care to prevent contamination by extraneous organisms.

Blood for bacteriologic examination was collected aseptically

from the jugular vein into "Bacto Blood Culture Bottles"* containing 50 ml Bacto-tryptic soy broth and carbon dioxide under vacuum, permitting aerobic and anaerobic cultivation. The direct procedure, using the blood culture bottle and the sterile blood collector tube, required no additional preparation of equipment.

In the control group, the jugular area was cleansed with 70% alcoholic Roccal and the needle inserted intravenously, after removing the protector shield. The blood was allowed to flow to the extreme end of the tubing, where the flow was stopped by pinching the tube above the needle at the far end. After removing the protector from this needle, the stopper of the culture bottle was punctured by inserting the needle. The hold was released on the tubing to allow the desired amount of blood to flow into the bottle. Five ml of blood was collected since large amounts were likely to contain sufficient antibacterial substances to inhibit small numbers of organisms.

In animals of the affected group, blood was collected by clamping the tubing with a hemostat and inserting 1 of the needles at the ends of the tubing through the bottle stopper. A 14 gauge needle was inserted through the prepared calves' skin into the jugular vein. The needle at the free end of the tubing was inserted into the 14 gauge needle and the clamp on the tubing released. Five ml of blood was collected in the collecting bottle.

The blood from all 10 control calves was cultured anaerobically and aerobically. Likewise, in 6 affected cases, the blood was cultured

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*Bacto Blood Culture Set, Difco. Cat. #B1100-2. Scientific Product Co., 12th and Gentry Streets, North Kansas City 16, Missouri.

anaerobically and aerobically by allowing air to pass through the sterile air-filtering unit into the bottle. In 2 affected cases, sterile syringes and needles were used for passing air into the bottle. The last 6 affected blood samples were cultured aerobically only.

Ten ml of blood was collected in separate collecting vials containing 2 drops of ethylenediamine tetraacetic acid (EDTA) anticoagulant for a complete blood count (CBC). All necessary precautions were taken to preserve the normal blood constituents. Hematologic examination were performed within a few hours of sampling.

Laboratory Examinations

Blood Samples. After collecting the blood in the "Bacto Blood Culture Bottles," the bottles were immediately transferred to an incubator at 37°C. They were kept there until growth appeared, or for a minimum of 14 days before reporting a negative blood culture. The preliminary detection of growth in the culture bottles was based upon:

a) appearance of turbidity in the broth,

b) change in color of the blood,

c) hemolysis of blood, and

d) appearance of gas bubbles in the broth.

Examinations were made daily for the appearance of growth. It was noted that prolonged incubation of the blood culture resulted in the disintegration of red cells, which caused turbidity and a change of color even in the absence of bacterial growth. Microscopic examinations were made regularly for additional checks on possible growth by withdrawing a small portion of broth with a sterile 20 gauge needle

and syringe.

Most of the positive blood cultures contained a pure culture of the etiological agent and was readily identified by a gram stain followed by minimal cultural procedures. For subsequent culturing and identification of the organisms, a small portion of the broth was streaked on differential media, following similar procedures as for fecal culturing. This will be described in detail on the following pages.

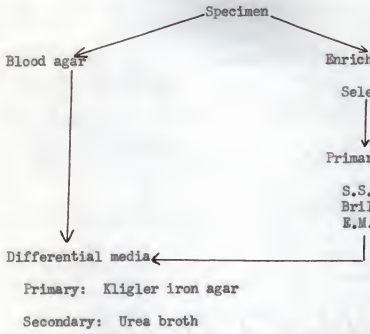
Blood collected in separate collecting vials with EDTA was subjected to the following hematologic examinations comprising the CBC:

1. Total white blood cell count--using Coulter counter or hemocytometer.
2. Hematocrit--using microhematocrit.
3. Hemoglobin determination--using spectrophotometer.
4. Differential count--using Wright's stain in the usual procedure.

Fecal Samples. After collection, fecal samples were subjected to immediate examination. Physical examination of the feces included color, consistency, odor, and presence of blood, mucus, or undigested food materials. The direct method and the flotation technique, utilizing zinc sulphate solution, were used for the detection of parasite ova.

Bacteriologic examinations were performed aerobically using enrichment media, primary plating media, and differential media as indicated in Table 1.

Table 1. Scheme followed for the examination of stool for bacteria.*



*Procedure as given in Difco Manual (18), with slight alterations.

A selective medium, S.S. agar, was used for isolation of Shigella and Salmonella strains. Brilliant green agar was utilized for isolating Salmonella, since it is more inhibitive than the preparations recommended for Shigella. Petri plates were incubated in an inverted position. The plates were examined after 24 hours and the organisms present identified. Plates negative at that time were incubated for another 24 hours and reexamined.

The preliminary and basic study of a bacterium lies in the determination of its cultural characters. The most important of those considered were:

1. Gross morphology--size, texture, color, and shape of colonies of the organism.

2. Microscopic morphology--(a) size, shape, and grouping of organisms; (b) presence or absence of spores; (c) motility; (d) presence or absence of capsule; (e) staining reactions.

3. Biochemical reactions--(a) fermentation of sugars; (b) production of hydrogen sulfide.

Motility of the organisms was determined by the hanging drop method, using overnight cultures.

Gram's stain was used to identify staining reactions, and methylene blue was used to determine bipolar staining characteristics. Slides were grease freed by cleaning in soap and water, thoroughly rinsing, and then cleaning with xylol. Before use, the slides were passed through the Bunsen burner flame 2 or 3 times. A drop of distilled water was placed on the cooled slide, and a carefully picked small amount of bacterial growth was suspended in it, spread, and allowed to air dry. The film was then fixed by passing through the burner flame 2 or 3 times, or, for the methylene blue stain, fixed with methyl alcohol.

Cultural Media and Procedures

Blood Agar Plates. Blood agar plates were streaked with a small inoculum of stool specimen. Plates were incubated at 37°C for 24 hours and hemolysis and colony characteristics noted. The isolated colonies were then transferred to Kligler iron agar and urea broth for further identification.

Selenite Broth. Selenite broth was used as an enrichment media for the salmonellae and shigellae group of organisms. It does not inhibit the fecal coli and enterococci completely. The colon bacilli, however, are reduced in numbers during the first 8-12 hours. Proteus and Pseudomonas are not inhibited. Selenite broth was inoculated by adding about 1 gram of feces. Tubes were incubated at 37°C for 18 to 24 hours. After incubation, a loopful of the enrichment culture was streaked on S. S. agar, and a similar amount was streaked on brilliant green agar. In a few instances E.M.B. media was also used.

S.S. Agar. S.S. agar is a plating medium that is not only highly selective, but also differential. It is highly selective for the isolation of shigellae and salmonellae and provides excellent differentiation of lactose fermenters from non-lactose fermenters. The plates were incubated at 37°C for 24 hours, at which time 3 or 4 of each type of suspected colony were picked from the plate and transferred to Kligler iron agar. The hydrolysis of urea, as demonstrated in urea broth, was used to identify Proteus organisms.

Brilliant Green Agar. Brilliant green agar is a highly selective medium for the isolation of salmonellae. Its selectivity permits the use of moderately heavy inocula. Usually pure cultures of salmonellae result. Following incubation at 37°C for 24 hours, the plates were examined for typical colonies. Kligler iron agar and urea broth were used for further identification.

Kligler Iron Agar. Kligler iron agar permits a differentiation of the gram-negative rods on the basis of their ability to ferment

dextrose or lactose and on their ability to produce hydrogen sulfide. It differentiates between the lactose-splitting organisms and lactose non-fermenters. Kligler iron agar is used to identify pure cultures of colonies picked from primary plating media. The medium, so tubed that it has a deep butt and slant, is stabbed to the bottom with a slightly curved needle. The needle is then drawn back over the slant so as to produce sufficient surface growth with which to work. The tubes were incubated at 37°C for 18 hours, keeping the screwcap loose.

Urea Broth. Urea broth is recommended for the identification of the Proteus group of organisms from other gram-negative intestinal bacteria. Inoculation is made from 24 hour agar cultures with a straight needle and the broth incubated at 37°C. Reactions were recorded after 24 and 48 hours of incubation.

E.M.B. Agar. E.M.B. agar is recommended for the detection and isolation of the gram-negative intestinal pathogenic bacteria. It differentiates between colonies of lactose fermenting organisms and those which do not ferment lactose. Kligler iron agar and urea broth were used for further identification.

RESULTS

Normal Calves

The individual results obtained for the 10 normal calves are as follows:

Calf No. A185 (1): This was a female Holstein, 26 days old. It was slightly depressed on the day it was born, but was treated with oral and parenteral antibiotics and recovered in a short time. On the day of examination she was defecating normally and appeared active and alert. The calf's temperature was 101.4°F, respirations were 15/minute, and the pulse rate was 100/minute. Auscultation of heart and lungs revealed no abnormality. The conjunctiva were bright pink and moist, and the muzzle was wet. A thorough systemic examination revealed no abnormality. Hemotologic examination revealed a total leukocyte count of 10450/cmm. The hematocrit was 37, hemoglobin 10.6 gms/100 ml of blood, segmented neutrophils 53, lymphocytes 40, monocytes 4, and eosinophils 3. The blood was negative for microorganisms. The feces was soft, light green in color, and negative for parasite ova. Non-hemolytic streptococci and E. coli were isolated from the feces.

Calf No. 015C (2): This was a female Holstein, 26 days old. The history revealed that she was sick on the day of birth but responded to treatment with antibiotics. She appeared to be in perfect health the day she was examined. Her temperature was 100.8°F, respirations were 23/minute, and her pulse returns 120/minute. No abnormality was noted on auscultation of the heart and lungs. The muzzle was moist, and the conjunctiva were pink. Systemic examination showed no abnormality. The

blood leukocyte count was 6,500/cmm, the hematocrit was 34, hemoglobin 8 gms/100 ml, segmented neutrophils 27, lymphocytes 68, monocytes 4, and eosinophils 1. The blood was negative for bacterial growth. Feces was soft, green-yellow in color, and no parasite ova observed. Non-hemolytic streptococci and E. coli were isolated on culture.

Calf No. 025C (3): This was a male Holstein, 21 days old, with no history of any previous illness. He was active and alert on the day of examination, with a rectal temperature of 101.8°F, respirations of 17/minute, and pulse rate of 100/minute. The heart and lungs were normal on auscultation. The blood leukocyte count was 6,350/cmm. The hematocrit were 33, hemoglobin 12.2 gms, segmented neutrophils 42, lymphocytes 50, monocytes 4, and eosinophils 4. Blood culturing resulted in negative findings. The feces was firm, dark green in color, and negative for parasite ova. Non-hemolytic streptococci, E. coli, and Proteus were isolated.

Calf No. 291C (4): This was a female, 19 day old, Ayrshire calf with no history of previous illness. She was as active as the other calves examined, with a rectal temperature of 102.4°F, respirations of 15/minute, and a pulse of 105/minute. Auscultation of the heart and lungs revealed no abnormalities. The conjunctiva were pink and the muzzle moist. Systemic examination showed no pathology. Hematologic findings were a total leukocyte count of 11,750/cmm, a hematocrit of 43, hemoglobin of 13.4 gms, segmented neutrophils 44, lymphocytes 52, monocytes 3, and eosinophils 1. The blood was negative for any bacterial growth. Feces was soft, light green in color, and contained no parasite

ova. Fecal samples were positive for non-hemolytic streptococci and E. coli.

Calf No. 19D (5): This was a female Holstein, 25 days old with no history of previous illness. She was active, bright, and apparently in good health with 102.0°F rectal temperature, 16 respirations and 110 pulse rate/minute. The heart and lungs showed no abnormality on auscultation. The blood leukocyte count was 7,100/cmm, the hematocrit was 25, hemoglobin 7.5 gms, segmented neutrophils 38, lymphocytes 65, monocytes 5, and eosinophils 2. The blood was negative for bacterial growth. Feces was soft and light grey in color, revealing no parasite ova on microscopic examination. Fecal culturing revealed non-hemolytic streptococci and E. coli.

Calf No. 187D (6): This was a 9 day old Ayrshire in good health with no history of any previous illness or treatment. The rectal temperature was 101.4°F, respirations were 16 and pulse rate 120/minute. The heart and lungs showed no abnormalities on auscultation. The conjunctiva and other mucus membranes were normal. Total blood leukocyte count was 6,050/cmm, the hematocrit was 30, hemoglobin 9.5 gms, segmented neutrophils 28, lymphocytes 64, monocytes 6, and eosinophils 2. Blood culture revealed no growth. The feces was soft and yellow-brown in color, with negative microscopic findings. Only non-hemolytic E. coli was isolated from feces.

Calf No. 0136E (7): This was a 12 day old male Ayrshire which was sick 8 days previous to the day of examination. It was treated with antibiotics and was healthy at the time of the examination. The calf's

temperature was 100.0°F, respirations were 22/minute, and the pulse rate was 72/minute. Auscultation of the heart and lungs, and examination of the other body systems, revealed no abnormalities. Hematologic examination revealed a total leucocyte count of 10,250/cmm, a hematocrit of 30, hemoglobin of 9.2, segmented neutrophils 29, lymphocytes 68, monocytes 1, and eosinophils 2. Blood culture revealed negative results. Feces was soft, light brown in color, and negative for parasite ova. Non-hemolytic streptococci and E. coli were isolated on fecal culture.

Calf No. 0284C (8): This was a male Jersey 10 days old, which was sick 8 days previous to examination. It was treated with antibiotics and recovered fully. It was active and healthy on the examination day. The rectal temperature was 101.0°F, respirations were 17/minute, and the pulse rate was 70/minute. The heart and lungs were normal on auscultation. Hematologic examination revealed a total leukocyte county of 6,305/cmm, a hematocrit of 33, a hemoglobin of 9.2, segmented neutrophils 30, lymphocytes 64, monocytes 4, and eosinophils 2. The blood was negative for bacterial growth. Feces was firm, light brown in color, and negative for parasite ova. Non-hemolytic streptococci, E. coli, and Proteus were isolated.

Calf No. A184 (9): This was a 3 day old female Ayrshire, had no history of any previous illness. A thorough physical examination revealed no abnormality. The rectal temperature was 100.0°F, respirations 15/minute, and pulse rate of 90/minute. The total leukocyte count was 7,300/cmm, the hematocrit was 44, hemoglobin 12.2, segmented neutrophils 34, lymphocytes 61, monocytes 4, and eosinophils 1. The blood was negative for microorganisms. The feces was soft, yellow-white in color,

and negative for parasite ova. Non-hemolytic streptococci and E. coli were isolated.

Calf No. 20D (10): This was a 3 day old female Holstein calf with no history of illness. Systemic examination showed no abnormalities. The body temperature was 101.0°F, the respirations were 22/minute, and the pulse was 90/minute. Blood values were: total leukocyte count 6,500/cmm, hematocrit 32, hemoglobin 9.6, segmented neutrophils 38, lymphocytes 59, monocytes 2, and eosinophils 1. Blood culture was negative for bacteria. The feces was soft, yellow-white in color, and negative for parasite ova. Non-hemolytic streptococci and E. coli were isolated from the fecal sample.

Tables 2 through 5 summarize the results of the physical examinations, the blood examinations, and the physical and bacteriologic examinations of the feces from the normal calves.

Table 2. Summary of physical examination of normal calves.

Calf No.	A185 (1)	015C (2)	025C (3)	291C (4)	19D (5)	187D (6)	0136E (7)	0284C (8)	A184 (9)	20D (10)	Average (Range)
Age (days)	26	26	21	19	25	9	12	10	3	3	
Temperature (°F)	101.4	100.8	101.8	102.4	102.0	101.4	100.0	101.0	100.0	101.0	101.2 (100-102.4)
Pulse/minute	100	120	100	105	110	120	72	70	90	90	97.7 (70-120)
Respirations/ minute	15	23	17	15	16	16	22	17	15	22	17.8 (15-23)

Table 3. Summary of hematologic findings in normal calves.

Calf No.	Age (days)	Hemoglobin gms/100 ml of blood	Hematocrit %	Leukocytes/ cmm	Seg. Neut. %	Lympho-cytes %	Mono-cytes %	Eosino-phils %
A185 (1)	26	10.6	37	10,450	53	40	4	3
015C (2)	26	8.0	34	6,500	27	68	4	1
025C (3)	21	12.2	33	6,350	42	50	4	4
291C (4)	19	13.4	43	11,750	44	52	3	1
19D (5)	25	7.5	25	7,100	38	65	5	2
187D (6)	9	9.5	30	6,050	28	64	6	2
0136E(7)	12	9.2	30	10,250	29	68	1	2
028AC(8)	10	9.2	33	6,350	30	64	4	2
A184 (9)	3	12.2	44	7,300	34	61	4	1
20D (10)	3	9.6	32	6,500	38	59	2	1
Approximate age group				Mean Values and Range				
One week old (Nos. 9 and 10)		10.9 (9.6-12.2)	38 (32-44)	6,900 (6,500-7,300)	36 (34-38)	60 (59-61)	3 (2-4)	1
Two weeks old (Nos. 6,7,8)		9.3 (9.2-9.5)	31 (30-33)	7,550 (6,050-10,250)	27 (28-30)	65.3 (64-68)	3.6 (1-6)	2
Three to four weeks old (Nos. 1,2,3,4,5)		10.3 (7.5-13.4)	34.4 (25-43)	8,430 (6,350-11,750)	40.8 (27-53)	55 (40-68)	4 (3-5)	2.2 (1-4)
Under one month (Nos. 1 to 10)		10.2 (7.5-13.4)	34.5 (25-44)	7,626.6 (6,050-11,750)	34.6 (27-53)	60.1 (40-68)	3.5 (1-6)	1.7 (1-4)

Table 4. Summary of physical examination of feces of normal calves.

Calf No.	Sex	Age (days)	Consistency	Color	Blood	Mucus	Parasite Ova
A185 (1)	F	26	Soft	Light green	-	-	-
015C (2)	M	26	Soft	Green-yellow	-	-	-
025C (3)	M	21	Firm	Dark green	-	-	-
291C (4)	F	19	Soft	Light green	-	-	-
19D (5)	F	25	Soft	Yellow	-	-	-
187D (6)	F	9	Soft	Yellow-brown	-	-	-
0136E (7)	M	12	Soft	Light brown	-	-	-
028hC (8)	M	10	Firm	Light brown	-	-	-
A18h (9)	F	3	Soft	Yellow-white	-	-	-
20D (10)	F	3	Soft	Yellow-white	-	-	-

Table 5. Summary of bacteriologic findings in normal calves.

	Non-hemolytic *E. coli*	Hemolytic *E. coli*	Non-hemolytic streptococci	Hemolytic streptococci	Staphylococci	*Proteus* spp.	*Salmonella* spp.	Organisms isolated (% of attempts)
				Feces				
A185 (1)	+*	-**	+	-	-	-	-	Non-hemolytic *E. coli* 100%
015C (2)	+	-	+	-	-	-	-	
025C (3)	+	-	+	-	-	+	-	Non-hemolytic streptococci 90%
291C (4)	+	-	+	-	-	-	-	
19D (5)	+	-	+	-	-	-	-	*Proteus* 20%
187D (6)	+	-	+	-	-	-	-	
0136E (7)	+	-	+	-	-	-	-	
028L4C (8)	+	-	+	-	-	+	-	
A184 (9)	+	-	+	-	-	-	-	
20D (10)	+	-	+	-	-	-	-	
Total	10	0	9	0	0	2	0	

Blood

No organisms were found in the blood of the normal calves.

*+ organism isolated

**- no organism isolated

Affected Calves

Of the 19 calves affected with calf septicemia, 8 died. Necropsy examinations were conducted on these 8 calves, and specimens were collected for bacterial study. The individual results for the 19 animals are listed below.

Case No. 14513: This calf, a day old female Holstein, was admitted with diarrhea. She was weak, depressed, and slightly dehydrated. Some respiratory difficulty was present. The rectal temperature was 103.0°F, and anorexia was present. Hematology revealed an increase in the number of leukocytes with a shift to the left. There was an increase in the percentage of segmented neutrophils and a decrease in the lymphocytes. The hematocrit and hemoglobin were high (see Table 7). Non-hemolytic streptococci and E. coli were isolated from the feces. The blood was negative for microorganisms. The calf died after two days. Necropsy examination revealed a severe subcutaneous edema of the ventral body surfaces. The lungs were congested and edematous. The mesenteric lymph nodes were congested and hyperemic. A submucosal hyperemia and congestion was present in the digestive system. Most of the posterior portion of intestine was inflamed. E. coli was isolated from the liver and intestine. Alpha streptococci were isolated from the intestine.

Case No. 14785: A 2 day old Ayrshire heifer was admitted with diarrhea. The feces was mucus coated, semi-solid, and gray-white in color. The calf was dehydrated and anorexic. The temperature was 103.0°F. There were no signs of respiratory involvement. The CBC revealed an increase in total leukocyte count, an increase in the percent

of segmented neutrophils, and a decrease in the per cent of lymphocytes. The hematocrit and hemoglobin values were high (see Table 7). Feces and blood were cultured, and only non-hemolytic E. coli was isolated from the feces. No organisms could be isolated from the blood. The calf responded to fluid and antibiotic treatment and was discharged after 2 days.

Case No. 5470: This was a 2 day old Holstein heifer. The animal had a profuse watery diarrhea, yellow-white in color with an offensive odor. The calf was dehydrated and anorexic. The body temperature was 104.4°F. No signs of respiratory involvement were detected. A CBC was not taken. Feces was positive for E. coli, Proteus, and Salmonella spp. Blood was negative for organisms. The calf died the next day. Necropsy revealed yellow-white liquid feces the entire length of the intestinal tract. E. coli and Proteus were isolated from the liver, kidney, and intestines. Salmonellae were isolated from the intestines.

Case No. 10300: This 2 day old female Holstein calf was admitted with a diarrhea. Feces was watery and yellow in color. The calf was slightly dehydrated, anorexic, and had a 101.5°F rectal temperature. There were no abnormal respiratory signs. Blood examination revealed an increase in the total leukocyte count with a shift to the left. The hematocrit and hemoglobin values were high (see Table 7). Streptococci and E. coli were isolated from the feces. The blood was negative for bacteria. The calf died the day of admittance, and a necropsy was performed. Foam was present in the trachea. The mesenteric lymph nodes, liver, and intestines were congested. E. coli was isolated from the liver, spleen, intestines, and mesenteric lymph nodes. Alpha streptococci were isolated from the intestine.

Case No. 10652: A 3 day old male Hereford calf was admitted with a profuse, watery, yellow diarrhea. It had sunken eyes, was depressed and unable to stand. There was severe dehydration and anorexia. The rectal temperature was 99.0°F. Respiratory disease signs were exhibited. The total leukocyte count was in the normal range, but there was an increased percentage of segmented neutrophils. Slight anisocytosis and poikilocytosis was noted. The feces was positive for streptococci, E. coli, and Proteus on culture. No organisms could be isolated from the blood. There was an increase in the hematocrit (see Table 7). The calf died the day of admittance. Necropsy examination showed a reddened area in the abomasum, a catarrhal enteritis with watery feces in the small intestines, and a mild enteritis in the large intestine. E. coli was isolated from the liver, kidney, and intestines. Alpha streptococci were isolated from the kidney and intestines, and Proteus was cultured from the intestines.

Case No. 11355: This was a 4 day old bull Hereford calf. It had a profuse diarrhea that was yellow-white in color with mucus present. The calf's temperature was 102.5°F, and anorexia and dehydration were present. There was no respiratory involvement. CBC revealed a normal leukocyte count with lymphocytosis. The hematocrit was elevated, and there was hypochromia (see Table 7). On bacterial examination, the feces was found to be positive for E. coli and streptococci. The blood was positive for hemolytic streptococci and non-hemolytic E. coli. The calf was discharged after two days of supportive and antibiotic treatment.

Case No. 12392: This 4 day old bull Shorthorn calf was admitted with enteritis. The feces was watery and yellow in color with blood

present. There was anorexia and slight dehydration. A body temperature of 103.0°F was present. The total leukocyte count was in the normal range, but the per cent of segmented neutrophils was greatly increased. There was hypochromia and an increase in the hematocrit (see Table 7). Feces was positive for E. coli and streptococci upon culture. The animal was discharged after 3 days of antibiotic and fluid therapy.

Case No. 12333: A 4 day old bull Shorthorn calf with profuse yellow diarrhea was admitted. The calf was depressed and dehydrated, with a body temperature of 101.6°F and anorexia. No respiratory disease signs were noted. CBC revealed a normal leukocyte count, but the percentage of segmented neutrophils was increased. The hematocrit and hemoglobin levels were in the normal range (see Table 7). Feces was cultured and was positive for hemolytic streptococci and E. coli. The animal was discharged after 4 days of treatment with antibiotics and electrolytes.

Case No. 6733. A 5 day old female Guernsey calf was sick with a profuse watery diarrhea that was yellow in color and contained mucus. The calf was weak, dehydrated, and had anorexia. The rectal temperature was 102.0°F. CBC revealed a leukocytosis, neutrophilia, lymphopenia, and shift to the left. The hematocrit was high (see Table 7). Fecal and blood samples were cultured. Both were positive for streptococci and E. coli. The patient was treated with antibiotic and electrolytes and was discharged after 4 days.

Case No. 5445: This was a 7 day old Ayrshire heifer presented with a slight diarrhea. The feces was semisolid, yellow-white in color and mucus coated with an offensive odor. The rectal temperature was 103.6°F.

The calf showed anorexia and slight dehydration. There was no respiratory involvement. CBC revealed a normal total leukocyte count. The total per cent of neutrophils was in the normal range, although the percentage of bands was considerably increased. The hematocrit was normal, but there was a slight anemia (see Table 7). Fecal and blood samples were cultured, revealing the presence of Salmonella, Proteus, and E. coli in the feces, and E. coli and Proteus in the blood. The calf died after 6 days and a necropsy was performed. The abomasum and small intestine were distended with gas, and contained small amounts of liquid feces. The liver was slightly yellow with fatty changes. E. coli, Proteus, and Salmonella were isolated from the liver and intestine of this calf.

Case No. 11849: A 7 day old Jersey heifer was admitted with profuse diarrhea. The feces was watery in consistency and gray in color. The rectal temperature was 102.5°F, and the calf was slightly dehydrated. E. coli and streptococci were isolated from the feces. The calf was discharged after 3 days of antibiotic treatment.

Case No. 7084: An 8 day old bull Holstein calf was admitted with a history of having a profuse watery diarrhea. The owner reported that his calves became comatose the 2nd day after birth. Seven calves had exhibited this syndrome in the past 2 months, all but 1 recovering without medication in 2 to 3 weeks. The rectal temperature was 104.0°F, with slight dehydration, weakness, and anorexia present. There were signs of respiratory involvement. The feces at the time of collection was like sheep pellets and yellow in color with a mucus coating. The blood cell count was not significant because of clot formation (see

Table 7). Cultural results revealed Salmonella, E. coli, and streptococci in the feces, and streptococci and E. coli in the blood. The calf died after 2 days and a necropsy was conducted. The respiratory system had white foam in the trachea, interlobular edema, and scattered atelectasis. There were patchy areas of hemorrhage on the epicardium, and hyperemia and edema of the mesenteric lymph nodes. There was cream colored exudate in the oral cavity, and serosanguinous fluid in the abdominal cavity. The liver was swollen with fatty changes present. There was subcapsular hemorrhage and congestion of the medulla of the kidney. Streptococci were isolated from the kidney, lung, and intestine. E. coli were isolated from the kidney and lung.

Case No. 5521: A 10 day old Holstein bull calf arrived at the clinic with a profuse diarrhea. The stool was fetid, watery, and yellow in color. The calf was weak, depressed, and lethargic. The rectal temperature was 106.2°F. There was anorexia and slight dehydration. Bacterial examinations revealed Salmonella, E. coli, and Proteus in the feces, and Salmonella and Proteus in the blood. The calf was treated with antibiotics and fluids and was discharged after 4 days.

Case No. 12225: This was a 10 day old Angus bull calf. The owner had lost 10 of 45 calves in 2 years time. The calf had a mild diarrhea, the feces being semisolid in consistency and white-gray in color. The rectal temperature was 101.5°F. Slight dehydration was present. Hematologic examinations showed a leukocytosis with neutrophilia (see Table 7). The feces was positive for staphylococci. Blood was not collected for bacterial culture. The calf was discharged after 3 days of electrolyte and antibiotic treatment.

Case No. 11471: A 14 day old Angus heifer was admitted with a mild diarrhea. The feces was semisolid and gray-white. The calf had a rectal temperature of 104.8°F and was slightly dehydrated. There was no anorexia. Hemtalogic examinations revealed a leukocytosis with lymphocytosis. Atypical lymphocytes were demonstrated. Hematocrit and hemoglobin readings were considerably elevated, showing hemoconcentration (see Table 7). On bacterial culture, the feces was found to be positive for hemolytic E. coli and hemolytic streptococci. The blood was negative for microorganisms. The calf was discharged the same day after antibiotic treatment.

Case No. 7043: This 17 day old male Shorthorn calf was purchased 2 weeks previous. It was admitted with a slight diarrhea. The feces was semisolid and white-yellow in color with mucus present. Appetite was good and the rectal temperature was 101.0°F. There was slight dehydration, but no respiratory disease signs. Hematologic examinations revealed a normal leukocyte count with neutrophilia. There was no shift to the left. The hematocrit was high (see Table 7). Bacterial examination of the blood was negative, while the feces contained E. coli and streptococci. The calf was discharged after 4 days of antibiotic and fluid treatment.

Case No. 14049: A 21 day old Hereford female was admitted with a history of having diarrhea for 2 weeks. The calf was depressed, weak, and severely dehydrated. There was no diarrhea at the time of admittance and the rectal temperature was 100.4°F. The feces was firm and black in color. There was leukocytosis with neutrophilia and lymphopenia. There was no shift to the left. The hematocrit and hemoglobin were

normal (see Table 7). Bacterial examinations revealed non-hemolytic streptococci and E. coli in the feces, and hemolytic streptococci in the blood. The calf was discharged after 5 days of antibiotic and electrolyte treatment.

Case No. 9734: This was a 28 day old Holstein female. The owner had previously lost 7 calves. It had a mild diarrhea and dehydration when admitted. The feces was semisolid and yellow-white in color. Rectal temperature was 101.0°F. Anorexia was present and the animal exhibited abnormal respiratory signs. There was lymphocytosis, with a slight leukopenia and shift to the left. The hematocrit and hemoglobin were in the normal range (see Table 7). On bacterial examination, the feces was found to contain hemolytic E. coli. The blood was negative for bacteria. The calf died the next day, and a necropsy was performed. The lungs had several dark areas and blood oozed from the cut surface. The large intestine had dark yellow fluid feces, and the small intestine contained scant yellow material with mucus. The gall bladder was extremely distended and was full of thick yellow-green bile. The bladder contained dark yellow urine. Hemolytic E. coli was isolated from intestine and lung.

Case No. 11804: This was a 30 day old Angus-Hereford male. There was no diarrhea, and the feces was firm and gray in color. The calf was anorexic. The rectal temperature was 99.1°F, and there were signs of respiratory involvement. Upon hematologic examination, leukocytosis was noted with neutrophilia. There was no shift to the left. Hematocrit and hemoglobin values were high (see Table 7). Streptococci were isolated from the feces. Blood was not collected for bacterial

culture. The calf died the next day. At necropsy, yellow tenacious mucus was found in the trachea. The lungs were congested. Fibrinous strands were noted on the pericardium. There was abomasitis, enteritis, and colitis. Organs were not collected for bacterial examination.

The results of the examinations performed on 19 affected calves are summarized in Tables 6 through 12. Included are listings of the clinical signs, blood cell counts, physical characteristics of the feces, bacteriologic results of blood and fecal examinations, age incidence of calves from which organisms were isolated, the organisms isolated from necropsied cases, and a comparison of the results of bacterial culture of the feces, blood, and organs from the 7 necropsied calves.

Table 6. Summary of the signs exhibited by affected calves in relation to the organisms isolated.

Case No.	Age (days)	Organisms isolated	Initial temperature (°F)	Diarrhea	Anorexia	Dehydration	Respiratory signs	Termination (days)
14513	1	E. coli + Strep	103.0	++	+	+	+	2 D*
14785	2	E. coli	103.0	+	+	+	-	2 WH**
5470	2	Salm + E. coli + Proteus	104.4	++	+	++	-	1 D
10300	2	Strep + E. coli	101.5	++	+	+	-	Same day D
10652	3	Strep + E. coli + Proteus	99.0	++	+	++	+	Same day D
11355	4	Strep + E. coli	102.5	++	+	+	-	2 WH
12392	4	Strep + E. coli	103.0	++	+	+	-	3 WH
12333	4	Strep + E. coli	101.6	++	+	+	-	4 WH
6733	5	Strep + E. coli	102.0	++	+	+	-	4 WH
5445	7	Salm + E. coli + Proteus	103.6	+	+	+	-	6 D
11849	7	Strep + E. coli	102.5	++	-	+	-	3 WH
7084	8	Salm + E. coli + Strep	104.0	+	+	+	+	2 D
5521	10	Salm + E. coli + Proteus	106.2	++	+	+	-	4 WH
12225	10	Staph	101.5	+	Not fed	+	-	3 WH
11471	14	Strep + E. coli	104.8	+	-	+	-	Same day WH
7043	17	Strep + E. coli	101.0	+	-	+	-	4 WH
14049	21	Strep + E. coli	100.4	-	+	++	-	5 WH
9734	28	E. coli	101.0	+	+	+	+	1 D
11804	30	Strep	99.1	-	+	-	+	1 D

*D = died, **WH = went home

+ = slight, ++ = severe, - = absent

Salm = Salmonella spp, Strep = Streptococci, Staph = Staphylococci

Table 7. Summary of hemotologic findings in affected calves in relation to the organisms isolated.

Case No.	Age (days)	Organisms isolated	Hemoglobin gms/100 ml	Hemotocrit %	Leukocytes /cmm	Seg Neut %	Band Neut %	Lymphocytes %	Monocytes %	Eosinphils %	Other findings
11513	1	F*-E. coli, Strep B**-Neg	16.6	56	13,800	60	—	28	—	2	Myel 6
11785	2	F-E. coli B-Neg	16.6	54	13,550	75	1	21	3	—	—
5470	2	F-Salm, E.coli, Proteus B-Neg	—	—	—	—	—	—	—	—	—
10300	2	F-Strep, E. coli B-Neg	15.2	44	36,150	28	3	64	—	1	Juv 1, Promyel 1, Myel 1
10652	3	F-Strep, E.coli, Proteus B-Neg	12.0	39	9,500	69	—	31	—	—	Slight aniso, poik
11355	4	F-Strep, E. coli B-Hemolytic Strep, Non-hemolytic, E. coli	11.6	40	7,750	23	1	76	—	—	Slight hypochromia
12392	4	F-Strep, E. coli B-Not collected	13.5	45	8,300	81	—	18	—	1	Slight hypochromia
12333	4	F-Strep, E. coli B-Not collected	12.2	35	8,700	65	—	32	2	—	—
6733	5	F-Strep, E. coli B-Strep, E. coli	13.2	42	15,680	59	21	15	1	1	Juv 3
5445	7	F-Salm, E. coli, Proteus B-E. coli, Proteus	9.5	34	8,700	12	23	62	3	—	—
11849	7	F-Strep, E. coli B-Not collected	—	—	—	—	—	—	—	—	—
7084	8	F-Salm, E.coli, Strep B-Strep, E. coli	8.9	28	8,517	8	—	50	42	—	Slight hypochromia
5521	10	F-Salm, Proteus, E. coli B-Salm, Proteus	—	—	—	—	—	—	—	—	—
12225	10	F-Staph B-Not collected	8.2	27	11,700	67	—	30	3	—	—
11471	14	F-Hemolytic Strep, Hemolytic E. coli B-Neg	17.0	58	12,100	20	—	76	4	—	Atypical lymph 4
7043	17	F-Strep, E. coli B-Neg	13.0	43	8,100	60	—	37	3	—	—
11049	21	F-E. coli, Strep B-Hemolytic Strep	10.1	32	15,013	58	—	42	—	—	—
9734	28	F-Hemolytic E. coli B-Neg	10.2	35	6,473	27	—	72	—	—	Myel 1
11804	30	F-Strep B-Not collected	16.8	57	14,900	59	—	38	3	—	—

*F = feces, **B = blood
Salm = Salmonella spp., Strep = Streptococci, Staph = Staphylococci

Table 8. Summary of physical examinations of feces collected from affected calves in relation to the organisms isolated.

Case No.	Age (days)	Organisms isolated	Color	Consistency	Blood	Mucus	Parasite Ova
14513	1	E. coli, Strep	Yellow-white	Fluid	-	-	-
14785	2	E. coli	Gray-white	Semisolid	-	+	-
5470	2	Salm, E. coli, Proteus	Yellow-white	Fluid	-	-	-
10300	2	Strep, E. coli	Yellow	Fluid	-	-	-
10652	3	Strep, E. coli, Proteus	Yellow	Fluid	-	-	-
11355	4	Strep, E. coli	Yellow-white	Fluid	-	+	-
12392	4	Strep, E. coli	Yellow	Fluid	+	-	-
12333	4	Strep, E. coli	Yellow	Fluid	-	-	-
6733	5	Strep, E. coli	Yellow	Fluid	-	+	-
5445	7	Salm, E. coli, Proteus	Gray-white	Semisolid	-	+	-
11849	7	Strep, E. coli	Gray	Fluid	-	-	-
7084	8	Salm, E. coli	Yellow	Pellet-like	-	+	-
5521	10	Salm, E. coli, Proteus	Yellow	Fluid	-	-	-
12225	10	Staph	Gray-white	Semisolid	-	-	-
11471	14	Strep, E. coli	Gray	Semisolid	-	-	-
7043	17	Strep, E. coli	Yellow-white	Semisolid	-	+	-
14049	21	Strep, E. coli	Black	Firm	-	-	-
9734	28	E. coli	Gray-white	Semisolid	-	-	-
11804	30	Strep	Gray	Firm	-	-	-

+ = present, - = absent,
Salm = Salmonella spp., Strep = Streptococci, Staph = Staphylococci

Table 9. Summary of the bacteriologic findings in the blood and feces of the affected calves.

Case No.	Non-hemolytic *E. coli*		Hemolytic *E. coli*		Non-hemolytic Streptococci		Hemolytic Streptococci		Staphylococci		*Proteus* spp.		*Salmonella* spp.	
	Blood	Feces	Blood	Feces	Blood	Feces	Blood	Feces	Blood	Feces	Blood	Feces	Blood	Feces
5445	+	+	-	-	-	-	-	-	-	-	+	+	-	+
5470	-	+	-	-	-	-	-	-	-	-	-	+	-	+
5521	-	+	-	-	-	-	-	-	-	-	+	+	+	+
6733	+	+	-	-	+	+	-	-	-	-	-	-	-	-
7084	+	+	-	-	+	+	-	-	-	-	-	-	-	-
7043	-	+	-	-	-	+	-	-	-	-	-	-	-	-
9734	-	-	-	+	-	-	-	-	-	-	-	-	-	-
10300	-	+	-	-	-	+	-	-	-	-	-	-	-	-
10652	-	+	-	-	-	+	-	-	-	-	-	+	-	-
11355	+	+	-	-	-	+	+	-	-	-	-	-	-	-
11471	-	-	-	+	-	-	-	+	-	-	-	-	-	-
11804	NC*	-	NC	-	NC	+	NC	-	NC	-	NC	-	NC	-
11849	NC	+	NC	-	NC	+	NC	-	NC	-	NC	-	NC	-
12225	NC	-	NC	-	NC	-	NC	-	NC	+	NC	-	NC	-
12333	NC	+	NC	-	NC	+	NC	-	NC	-	NC	-	NC	-
12392	NC	+	NC	-	NC	+	NC	-	NC	-	NC	-	NC	-
14049	-	+	-	-	-	+	+	-	-	-	-	-	-	-
14513	-	+	-	-	-	+	-	-	-	-	-	-	-	-
14785	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Total	4	15	0	2	2	12	2	1	0	1	2	4	1	4

*NC = not collected
+ = organism isolated
- = organism not isolated

Table 10. Age incidence of affected calves related to the organism isolated from blood and feces.

Age (days)	Number of calves examined	Number of *Salmonella* isolated	Number of *E. coli* isolated	Number of Strep. isolated	Number of *Proteus* isolated	Number of Staph. isolated	Number of mixed infections
1 to 7	11	2	11	8	3	--	10
8 to 14	4	2	3	2	1	1	3
15 to 21	2	--	2	2	--	--	2
22 to 30	2	--	1	1	--	--	--
Total	19	4	17	13	4	1	15

Table 11. Distribution of the organisms isolated from various organs on necropsy examination conducted on 7 affected calves.

Bacteria isolated	Spleen	Liver	Kidney	Lungs	Intestines	Mesenteric lymph gland
E. coli (Hemolytic)	—	—	—	1	1	—
E. coli (Non-hemolytic)	1	5	3	1	5	1
Salmonella	—	1	—	—	2	—
Proteus	—	2	1	—	3	—
Non-hemolytic Streptococci	—	—	1	1	1	—
Alpha Streptococci	—	—	1	—	3	—

Table 12. Comparison of organisms isolated antemortem and postmortem from 7 affected calves.

	Antemortem		Postmortem					
Case No.	Blood	Feces	Liver	Kidney	Intestine	Spleen	Lungs	Mesenteric lymph gland
11513	—	E. coli Streptococci	E. coli	—	E. coli Alpha Streptococci	—	—	—
5470	—	Salmonella E. coli Proteus	E. coli Proteus	E. coli Proteus	E. coli Proteus Salmonella	—	—	—
10300	—	E. coli Streptococci	E. coli	—	E. coli Alpha Streptococci	E. coli	—	E. coli
10652	—	E. coli Streptococci Proteus	E. coli	Alpha Streptococci E. coli	Alpha Streptococci E. coli Proteus	—	—	—
5445	E. coli Proteus	E. coli Proteus Salmonella	E. coli Proteus Salmonella	—	E. coli Proteus Salmonella	—	—	—
7084	E. coli Strepto- cocci	E. coli Streptococci Salmonella	—	Streptococci E. coli	Streptococci	—	Streptococci E. coli	—
9734	—	Hemolytic E. coli	—	—	Hemolytic E. coli	—	Hemolytic E. coli	—

DISCUSSION

Normal Calves

Data from 10 normal calves, ranging from 3 to 26 days of age, showed certain similarities. The rectal temperatures ranged from 100.0°F to 102.4°F, with an average of 101.1°F. Pulse rates ranged from 70 to 120, with an average of 97.7/minute. Respirations ranged from 15 to 23, with an average of 17.8/minute.

In no instance was diarrhea encountered. The color of the feces was yellow-white to light brown in 1 and 2 week old calves, respectively. As the calves' age advanced, the color tended to become more green. Pounden et al. (55) examined the fecal color of 24 newborn calves and recorded brown or yellow for the first week of life, followed by shades of gray. At about 2 weeks of age, darker shades of gray-brown or green occurred. They suggested this was due to the type and quantity of dry feed consumed, whereas Carpenter (75) believed that the color of the feces was largely determined by bile.

In this study, the organisms isolated from the stool of healthy calves had no bearing on the color and consistency of the feces. In no case were parasite ova detected in the fecal samples.

Hematologic Findings. The hematologic findings were somewhat confusing when correlations were attempted between the blood values of different age groups. A mean of the values from the age group of 3 to 26 days (referred as "under 1 month" in Table 3) was considered significant.

The total leukocyte count/cmm ranged from 6,050 to 11,750, with a mean of 7,627. Segmented neutrophil cells ranged from 27% to 53%, with a mean of 34.6%. No bands or immature cells were recorded. Lymphocytes ranged from 40% to 68%, with a mean of 60.1%; monocytes from 1 to 6%, with a mean of 3.5%; and eosinophils from 1 to 4%, with a mean of 1.7%. Hemoglobin values ranged from 7.5 to 13.4 gms, with a mean of 10.2 gms/100 ml of blood, and hematocrit levels varied from 25 to 44, with a mean of 34.

Bacteriologic Findings. Blood samples from all the normal calves were found to be negative on bacterial examination. The fecal samples were all positive (see Table 5). Non-hemolytic E. coli was isolated from all 10 fecal samples, non-hemolytic streptococci from 9, and Proteus from 2. Hemolytic E. coli, hemolytic streptococci, or staphylococci were not isolated. Salmonella were also not found, as was suggested by Amstutz (2). Van Pelt et al. (74) studied the calf's fecal flora and reported that it increased rapidly after birth. Within 24 hours, gram-negative bacteria predominated. The ratio of gram-negative organisms to the total bacteria count tended to become constant by the 14th day. No attempt was made to undertake the counting of bacterial colonies in this study, but it was observed on the plates that calves under 10 days of age showed a greater number of gram-negative colonies than calves over this age.

Since non-hemolytic E. coli was isolated from all the 10 fecal samples, it is apparent that this organism is present in the intestines of normal calves, as suggested by Smith and Orcult (66). They reported

that E. coli is present in small numbers at all levels of the normal calves' small intestine. In the disease state, the number of bacilli increase locally and the infection spreads towards the duodenum. Non-hemolytic streptococci, isolated from 9 calves, and Proteus found in the feces from 2 calves are also apparent normal intestinal bacteria. Smith and Crabb (61) reported E. coli, Clostridium welchii, and certain types of streptococci in the normal intestinal flora. They became less numerous as the animals grew older. Bacteroides and lactobacilli colonized the intestines later, but Staphylococcus was never isolated.

Affected Calves

The 19 affected calves ranged from 1 to 30 days old. Sex and breed did not seem to play an important role in the incidence of the calf septicemia syndrome.

Clinical Findings. A very thin, liquid diarrhea developed in most of the cases in the age group of 1 to 10 days. The feces had a peculiar putrid odor. Calves in a later age group had primarily semi-solid or firm feces. Diarrhea was an outstanding feature of all the affected calves except 2, in which case 1 calf had the history of diarrhea and the other had respiratory signs without diarrhea. Both calves were more than 3 weeks old.

It was not possible to establish the effect of the isolated microorganisms on the consistency and color of the feces (see Table 8), as the organisms isolated from different age calves had different fecal consistency and color. In cases of pure infections, the color of the

feces was usually gray, except in 1 case where it was yellow-white with a semisolid or firm consistency. Profuse liquid diarrhea was characteristic of mixed infections. The majority of the cases had feces of a liquid consistency with yellow or yellow-white coloration. This could suggest an aid in diagnosis.

Blood was found in the feces in only 1, 4 day old calf, whereas mucus was noted in 6 cases. Of these 6 cases, E. coli was isolated in all. Salmonella was found in 2 instances. Although Salmonella and E. coli were isolated from other cases, mucus and blood were not present in the fecal samples. Parasite ova were not found in any of the fecal samples.

The rectal temperatures of the affected calves varied from 99.0 to 106.2°F. Temperatures were below normal in 2 calves. Both had septicemias, but in calf no. 10652 the lesions were confined to the intestinal tract, although organisms were isolated from liver, kidney, and intestine. In 6 calves, rectal temperatures were found to be in the normal range (100.0 to 101.6°F). E. coli was isolated from all, streptococci from 4, and staphylococci from 1 calf. Of these 6 calves, only 3 were affected with septicemia.

In 11 cases the temperature was above normal (102.5 to 106.2°F). E. coli was isolated from all of these calves. In 4 cases where Salmonella, E. coli, and Proteus or streptococci were isolated, temperatures were always found to be higher than 103.5° (ranging from 103.6° to 106.2°F). In the others, where E. coli was isolated alone or together with streptococci, rectal temperatures were usually below 103.5°. In

1 case (no. 11471), the body temperature was 104.8°, and hemolytic E. coli and hemolytic streptococci were isolated from the feces. Of the 11 calves with elevated temperatures, 7 were in a septicemic state, including the 4 calves from which Salmonella was isolated.

Rectal temperatures were found in the normal range in 4 of 7 over 10 day old affected calves, in spite of their carrying active infections. Two of the 7 had fevers, and 1 had a subnormal temperature. Calves below 10 days of age (12 cases) as a rule carried high body temperatures. Calves no. 1065, 10300, and 12333 were exceptions. The former calf had a subnormal temperature, while the latter two had normal readings.

In calf no. 11355, where hemolytic streptococci and non-hemolytic E. coli were isolated from the blood, together with non-hemolytic streptococci and E. coli from the feces, the rectal temperature was moderately elevated (102.5°F). In calf no. 9734, however, the temperature was in the normal range, although the intestine and lungs were positive for hemolytic E. coli. In another calf (no. 11471), where hemolytic streptococci and hemolytic E. coli were isolated from feces, the rectal temperature was considerably elevated (104.8°F). This suggests that hemolytic strains of E. coli and streptococci, when independently present may cause the temperature to be normal or slightly elevated. If present together, however, they may cause the temperature to be considerably elevated--similar to that found in Salmonella infections. In pure infections the body temperature varied considerably, ranging from subnormal to elevated, depending upon the organisms present.

Anorexia was present in all cases except 3. Dehydration of varying

intensity was noted in all the calves except 1, in which diarrhea was absent and respiratory difficulty predominated. Respiratory involvement was observed in 5 calves, of which 4 also had enteric signs. E. coli was isolated from 4 calves, streptococci from 4, and Salmonella from 1. There were mixed infections in 3, all of which produced septicemia and death within 2 days. The respiratory afflictions didn't seem to have much bearing on the body temperature.

The digestive and respiratory systems appeared to be the most vulnerable as diarrhea and respiratory signs were frequently encountered, either independently or together. Nervous involvement was not observed in any of the affected calves. Osborne et al. (54) remarked that the most commonly incriminated organism is E. coli. They divided the calf diarrhea syndrome into three groups: a) an acute, rapidly fatal diarrhea, b) a subacute debilitating diarrhea, and c) a chronic localized joint or middle ear infection. Joint or middle ear infections were not observed in this study.

The clinical syndrome seen in this study could be divided in 4 entities: 1) enteritis, 2) pneumonia, 3) enteritis and pneumonia, and 4) septicemia. Each entity was clinically distinguishable by the signs, results of hematologic examinations, and postmortem findings. A high mortality was noted in the calves with septicemia, especially when respiratory involvement occurred.

It seemed difficult to discriminate clinically between salmonellosis, colibacillosis, and other infections, as Buxton (11) also observed. A few affected calves are reported to remain carriers of the salmonellae

organism and in later adult life may show typical symptoms of the disease. This seemed more likely in calves with mild or subclinical infections since they were not likely to succumb to the initial infections (28).

Hematologic Findings. The hematologic examinations revealed leukocytosis in 8 cases, leukopenia in 1 case, and a shift to the left in 4 cases (see Table 7). The total leukocyte count was normal in 6 cases. In most calves there was an absolute neutrophilia and lymphopenia, and a high hematocrit and hemoglobin reading. No eosinophilia or basophilia was found. Monocyte levels were usually in the normal range. No correlations could be made between the CBC, the organisms isolated, the calves' ages, and the occurrence of septicemia. In the calf septicemia entity involving a mixed infection, there was leukocytosis, neutrophilia, lymphopenia, hemoconcentration, and at times a shift to the left.

Bacteriologic Findings. It is not easy to obtain convincing evidence indicating that bacteria which are normal inhabitants of the alimentary tract are also, under certain conditions, able to produce disease. This is particularly so in the case of a clinical syndrome as complex as that of calf septicemia.

Non-hemolytic E. coli were isolated from the feces of 15 calves and from the blood of 4 calves. Hemolytic E. coli were isolated from the feces of 2 calves. The organisms were not found in the blood. Non-hemolytic streptococci were isolated from the feces of 12 calves and the blood of 2 calves. Hemolytic streptococci was isolated from the feces of 1 calf and blood of 2 calves. Staphylococci were isolated from the

feces of 1 calf. The organism was not found in the blood. Proteus was isolated from the feces of 4 calves and blood of 2 calves. Salmonella was isolated from the feces of 4 calves and blood of 1 calf. There were mixed infections in 15 calves. In 4 cases only 1 organism was isolated. In 5 cases, the calves' blood were initially negative for organisms; the calves died of later developing septicemias and organisms were isolated from various body organs. Distribution of the organisms isolated following necropsy is given in Tables 11, 12, and 13.

A total of 12 calves were affected with a septicemia. Seven died within 2 days, while 1 died after 6 days. The number of deaths was 8. This suggests that if death is to supervene, it usually occurs within 2 days in calves with septicemia. "In the absence of any specific protection, the organisms usually induce a septicemia that is often rapidly fatal" (Ingram and Lovell, 35). Septicemia was a common finding. This seems to be consistent with the reports of Wood (77).

There still is much to be said on behalf of E. coli, however, for its part in this complex picture. Whether a primary or secondary factor, E. coli was isolated in 17 of 19 cases. Osborne et al. (54) isolated E. coli from the feces of "scouring" calves in 37 of 38 attempts. Another worker (19) presented evidence strengthening the theory that E. coli is a primary etiological agent in calf scours. It could as well, however, be considered a most important causative organism.

Since streptococci, Proteus, and Salmonella were isolated, as well as E. coli, it is likely that other organisms play an important role in the calf septicemia syndrome. The same organisms, with the exception

of salmonellae were isolated from normal calves. It thus becomes apparent that there may be other factors responsible for these organisms becoming pathogenic. The answer to this problem is somewhat nebulous in its interpretation. Synergism between E. coli and other organisms appears to be one possibility. The present concepts of mixed infections, synergism, phage systems, and virus stains suggest numerous alternatives for combinations of etiologic agents causing newborn enteritis (29). In addition to this, season of the year, climate, sunshine, lack of Vitamin A, overfeeding, synthetic diets, lack of colostrum, poor management, weak animals, and over concentration of stock may be equally important.

It is becoming evident that Salmonella and E. coli serotypes are not confined to one species of animal. "This outlook should be linked with the view that adaptation and mutation of bacteria occur more frequently than at one time supposed" (35).

Necropsy Findings. Necropsy examinations conducted on 8 calves showed lesions in the digestive system, respiratory system, lymphatic glands, cardiovascular system, and urinary system. Different calves demonstrated different lesions.

The digestive system had lesions most of the time. Submucosal hyperemia, congestion with hemorrhages, congestion of the intestines, reddened areas in the abomasum, abomasitis, colitis, catarrhal or mild enteritis with watery contents, or an absomasum and small intestines distended with liquid feces were seen. Serosanguinous fluid was occasionally present in the abdominal cavity together with fibrin strands.

The liver was congested, had fatty changes, and was swollen. The gall bladder was distended and full of thick yellow-green bile in 2 calves. In 3 cases, the lungs were congested and edematous, often with atelectasis. White foam was found in the trachea or yellow tenacious mucus was present. Hyperemia and congestion of the mesenteric lymph nodes was found in a few cases. Cardiac lesions varied from fibrinous strands on the pericardium to patchy hemorrhagic areas on the epicardium. The urinary system had subcapsular hemorrhages on the kidney, congestion of the kidney medulla, and a full bladder containing dark yellow urine.

The above mentioned lesions were never all observed in 1 calf. In a few cases in which organisms were isolated from different organs, lesions were not present. In some cases no evidence of septicemia was present at postmortem. This was also observed by Ingram and Lovell (35). Lovell and Hughes (43) had earlier indicated the lack, rather than the presence, of definite pathologic changes in calves dead from E. coli infection. This suggests, as does the present study, that postmortem lesions may be varied--showing extensive, few, or no gross changes.

One might wonder what would cause death of the host, and what part E. coli played in the disease process, in the absence of lesions. Though organisms may be present in various organs without causing gross damage, it is difficult to determine the disease mechanism when infection leads to disease or death of the host in the absence of pathology. Ingram and Lovell (35) remarked that "emphasis in outlook is shifting from toxicity to the relationship of enzymes and their substrates." These and other relationships could aid in the future understanding of the pathogenesis

of calf septicemia. In explaining the absence of marked gastrointestinal lesions, Glantz and Rothenbacher (29) suggested a lymphogenous-hematogenous route of infection via the nasopharynx.

Seasonal Incidence. From October to May, 19 calves were examined. A high percentage of the calves were examined during the February to April months, suggesting a higher incidence of the disease during this period. There was also a high incidence of E. coli and streptococci infections, mostly as mixed infections, during this same period. Salmonella was not isolated during this time. From October to January, the incidence of the disease, and E. coli and streptococci isolations, were low. Salmonella and Proteus were isolated during this time. All the calves examined had mixed infections. In the month of May, only 3 calves were examined. E. coli and streptococci were isolated in mixed infections from 2 of these.

The above data suggests that the incidence of calf septicemia is highest from February to April (early spring) and next highest from October to January (late fall and winter).

Age Incidence. The incidence of colibacillosis, salmonellosis, and mixed infections, when related to age (Table 10), revealed that E. coli, streptococci, and Proteus were isolated most often from 1 to 7 day old calves. Salmonella were isolated in equal numbers from calves 1 to 7 days and 8 to 14 days of age. This suggests that E. coli infection is predominant in very young calves and decreases as the calf's age increases. Although Salmonella was isolated from a comparatively small number of calves, it might be suggested that Salmonella may be

isolated from any group. Ingram and Lovell (35) imply that most calves die of "diarrhea" when less than 3 weeks old, while Salmonella infections affect young and adult alike. Other workers (49) suggest an upper age limit of 8 to 14 days for calf diarrhea.

Table 13 summarizes the physical and hematologic changes, and the bacteriologic and necropsy results, from the normal and affected calves.

Table 13. Comparison of physical changes, blood changes, cultural results, and necropsy lesions in normal calves and calves affected with the calf septicemia syndrome.

Case No.	Physical changes	Blood changes	Positive cultures	Necropsy
Normal (Nos. 1 to 10)	None	None	F[1]-E.coli, Streptococci, Proteus B[2]-Negative	--
Affected				
11513	T*, E**, D+ R++	Leukocytosis, neutrophilia, lymphopenia, shift to left, hemoconcenteration	F-E.coli, Strep B-Negative	Lungs congested, edematous; mesenteric lymph node congested; submucosal intestinal hyperemia and congestion
11785	T,E,D	Leukocytosis, neutrophilia, lymphopenia, hemoconcenteration	F-E.coli B-Negative	--
5470	T,E,D	CBC not taken	F-Salm, E.coli, Proteus B-Negative	Liquid feces in intestines.
10300	NT#, E,D	Leukocytosis, shift to left, hemoconcenteration	F-E.coli, Strep B-Negative	Foam in trachea; mesenteric lymph nodes, liver, intestine congested
10652	ST##, E,D,R	Neutrophilia, anisocytosis,	F-E.coli, Strep, Proteus B-Negative	Catarrhal enteritis
11355	T,E,D	Lymphocytosis, hypochromia	F-E.coli, Strep B-Hemolytic Strep, non-hemolytic E. coli	--
12392	T,E,D	Neutrophilia, lymphopenia; hypochromia, high Ht	F-E.coli, Strep B-Not cultured	--

Table 13 (cont.)

Case No.	Physical changes	Blood changes	Positive cultures	Necropsy
12333	NT,E,D	Neutrophilia, lymphopenia	F-E.coli,Strep B-Not cultured	—
6733	T,E,D	Leukocytosis, neutrophilia, lymphopenia, shift to left, high Ht	F-E.coli,Strep B-E.coli,Strep	
5445	T,E,D	Anemia	F-Salm,E.coli,Proteus B-E.coli,Proteus	Abomasum and s. intestine distended; liver showed fatty changes
11849	T,E,D	CBC not taken	F-E.coli,Strep B-Not cultured	—
7084	T,E,D,R	Clot found in blood	F-Salm,E.coli,Strep B-E.coli,Strep	White foam in trachea, inter lobular edema, scattered atelectasis; hemorrhages on epicardium; hyperemia and edema of mesenteric nodes; serasanginous fluid in abdominal cavity; liver swollen; subcapsular hemorrhage, congestion of medulla of kidney
5521	T,E,D,	CBC not taken	F-Salm,E.coli,Proteus B-Salm,Proteus	
12225	NT,E,D,	Leukocytosis, neutrophilia, lymphopenia, anemia	F-Staph B-Not cultured	

Table 13 (cont.)

Case No.	Physical changes	Blood changes	Positive cultures	Necropsy
11471	T,E,D	Leukocytosis, lymphocytosis, high Ht and Hb	F-Hemolytic Strep, hemolytic E. coli B-Negative	—
7043	NT,E,D	Neutrophilia, lymphopenia	F-E.coli,Strep B-Negative	—
14049	NT,D	Leukocytosis, neutrophilia, lymphopenia	F-E.coli,Strep B-Hemolytic Strep	—
9734	NT,E,D,R	Leukopenia, lymphocytosis, shift to left	F-Hemolytic E.coli B-Negative	Lungs congested; intestines contained fluid feces; gall bladder distended
11804	ST,R	Leukocytosis, neutrophilia	F-Strep B-Not cultured	Yellow tenacious mucus in trachea, lungs congested; fibrous strands on pericardium; abomasitis, enteritis, colitis

*T = elevated temperature, **E = enteritis,
+D = dehydration, ++R = respiratory symptoms,
#NT = normal temperature ##ST = subnormal temperature,
1F = feces, 2B = blood

CONCLUSIONS

The clinical signs of calves affected with calf septicemia were not highly diagnostic. Diarrhea was characteristic of mixed infections and often associated with dehydration. Rectal temperatures of affected calves varied from subnormal to normal and elevated. Fevers could be anticipated in mixed infections. A temperature above 103.5 was suggestive of Salmonella infection. High mortality was noted in calves with septicemia, especially those having respiratory involvement.

Hematologic values in the affected calves were not diagnostic in themselves. Leukocytosis, neutrophilia, lymphopenia, a shift to the left, and high hematocrit and hemoglobin levels were suggestive of the calf septicemia syndrome.

The fecal microflora of the affected and normal calves showed certain similarities. Non-hemolytic E. coli, streptococci, and Proteus were isolated from the fecal samples of normal calves. Blood and fecal samples from affected calves were positive for non-hemolytic and hemolytic strains of E. coli and streptococci, staphylococci, Proteus, and Salmonella.

Postmortem lesions were not diagnostic. Some affected calves had no lesions, while others had few or more lesions. Digestive tract pathology was common.

The clinical syndrome may be divided in 4 phases symptomatically: 1) enteritis, 2) pneumonia, 3) enteritis and pneumonia, and 4) septicemia. It was not possible to discriminate clinically between Salmonellosis, colibacillosis, and other infections. The disease could be generally designated as "calf septicemia." The syndrome involved different

etiologic agents with various manifestations depending on many factors. Diagnosis should be based upon history, symptomatology, hematology, and bacteriology. Necropsy findings may be of value in fatal cases.

Further research is needed illustrating the possible etiologic factors in the syndrome, by exploring not only the organisms involved but also the many other possible influences on the disorder.

SUMMARY

1. An attempt has been made to differentiate the various forms of the calf septicemia syndrome, giving special attention to clinical and bacteriologic studies of affected and normal calves.

2. Nineteen clinically affected calves and 10 normal calves, ranging from 1 to 30 days of age and irrespective of breed and sex, were used in this study.

3. Blood was collected from both affected and normal animals for hematologic and bacteriologic studies. Fecal samples from both groups were collected for parasite and bacterial examinations.

4. Clinical signs were varied. The feces of the affected calves was either loose, semisolid, or firm in consistency. Profuse liquid diarrhea was a common feature in animals suffering from mixed infections. The rectal temperatures varied considerably. In 2 affected calves the temperature was subnormal, in 6 normal, and in 11 elevated. Of the latter group, all 4 animals suffering from Salmonella infection, along with streptococci, E. coli, and Proteus, had temperatures of more than 103.5°F. In the 6 cases where Salmonella infection was not found, the temperatures were less than 103.5°F. Even though Salmonella was absent, the rectal temperature was 104.8°F in 1 case.

5. Five of the affected calves showed respiratory involvement. Twelve of the affected calves had a septicemia, with a high rate of mortality in those suffering from respiratory involvement.

6. The syndrome was clinically differentiated into 4 categories: 1) enteritis, 2) pneumonia, 3) enteritis and pneumonia, and 4) septicemia.

7. Blood cell examinations from both normal and affected calves indicated that the affected calves had leukocytosis, neutrophilia, lymphopenia, and high hematocrit and hemoglobin values. Four affected calves showed a shift to the left.

8. Fecal examinations did not reveal any parasite ova.

9. Bacterial cultures made from fecal samples of healthy calves revealed the presence of non-hemolytic E. coli, non-hemolytic streptococci, and Proteus. Blood cultures were negative. Similar samples from affected calves showed hemolytic and non-hemolytic E. coli and streptococci, and staphylococci, Proteus, and Salmonella.

10. Postmortem lesions were not diagnostic. Some affected animals did not have gross lesions, while others had few or extensive changes. The digestive tract was most commonly affected, with a lesser degree of respiratory, urinary, and cardiovascular involvement.

ACKNOWLEDGMENTS

The author wishes to express his sincere gratitude and appreciation to Dr. F. W. Oehme, assistant professor, Department of Medicine and Surgery, Kansas State University, for his valuable guidance, assistance and encouragement at every step in this work.

Dr. Harry C. Mussman and Dr. C. M. Hibbs gave much help and guidance in the hemotologic and bacteriologic studies, and valuable aid in the use of laboratory facilities. The author is very grateful for their efforts.

Sincere thanks and appreciation are also due to Dr. J. E. Mosier, Head, Department of Medicine and Surgery, for his help and advice.

Special thanks are due to the Department of Pathology, Parasitology, and Public Health for undertaking the necropsy examination and some of the clinical pathologic determinations, and to the Department of Dairy Science, Kansas State University, for providing the healthy calves.

To Dr. M. Ahmed Hussain, retired dean, University College of Veterinary Science and A. H., Hyderabad, for his untiring encouragement, guidance and, above all, help to the younger generation of pupils, the author feels highly indebted and wishes to express his deep sense of gratitude.

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THE NEWBORN CALF SEPTICEMIA SYNDROME - CLINICAL AND BACTERIOLOGIC STUDIES OF AFFECTED AND NORMAL INDIVIDUALS

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AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirement for the degree

MASTER OF SCIENCE

Department of Surgery and Medicine

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1966

The newborn calf septicemia syndrome has taken a heavy toll of the cattle population for at least two centuries. Much work has been done to isolate the causal organisms and to call attention to the pathogenesis. The purpose of this study was to differentiate various forms of the disorder, with special regard to clinical and bacteriologic studies in neonatal calves.

Nineteen calves, clinically affected with the calf septicemia syndrome and ranging from 1 to 30 days of age, formed the subjects for this study. They were compared with 10 normal calves of the same age.

Samples of blood and feces were collected from the normal and affected calves for hematologic and bacteriologic examinations. Specimens for bacteriologic examination were collected from the internal organs of affected calves submitted for necropsy. The blood was examined by performing complete blood counts and cultured using "Bacto Blood Culture Bottles" containing tryptic soy broth. Feces were cultured using selenite broth, blood agar, S.S. agar, brilliant green agar, Kligler iron agar, urea broth, and E.M.B. agar. Fecal examinations for parasite ova were performed.

The clinical findings of the affected calves were varied. The feces were either loose, semisolid, or firm. Profuse diarrhea was a characteristic feature in calves suffering from mixed infections. The body temperatures varied from subnormal in 2 cases, to normal in 6, and above normal in 11 cases. In these latter 11 cases, 4 from which Salmonella, E. coli, Proteus, and streptococci were isolated, the temperatures were always more than 103.5°F. In 6 cases where Salmonella was not isolated, the temperatures were always less than 103.5°F. In 1 case

the temperature was 104.8°F, even in the absence of Salmonella infection. Five calves had respiratory involvement. There were 12 systemically affected individuals. A high rate of mortality was noted in these calves, especially in those having respiratory involvement.

The blood examination results of the affected and normal calves were compared. The affected calves presented varied values, exhibiting primarily leucocytosis, neutrophilia, lymphopenia, and high hematocrit and hemoglobin readings. Blood from 4 calves revealed a shift to the left.

Blood taken for bacteriologic examination from the normal calves was negative for microorganisms in all cases. Fecal bacterial examination of the 10 normal animals revealed the presence of non-hemolytic E. coli. Non-hemolytic streptococci were found in 9 instances, and in 2 calves Proteus was isolated.

Blood bacterial examination of the 19 affected calves revealed non-hemolytic E. coli in 4 cases. Two animals carried non-hemolytic streptococci. Hemolytic streptococci were present in 2 calves, Proteus was isolated from 2, and Salmonella from 1 calf. Fecal bacterial examination showed the presence of non-hemolytic E. coli in 15 cases. Twelve cases had non-hemolytic streptococci. In 1 case hemolytic streptococci was found, in 2 cases hemolytic E. coli, in 1 case staphylococci, and in 4 cases Proteus and Salmonella were cultured. Fifteen cases had mixed infections and 4 carried pure infections.

The postmortem lesions were varied. Some animals had no lesions, while others had extensive pathology. The digestive tract was the most commonly affected system.

It appeared difficult to establish a definite line of demarcation between the various etiologic forms of the disorder, except to clinically differentiate them as enteritis, pneumonia, enteritis-pneumonia, and septicemia. These different manifestations may be caused by a single agent or by a group of organisms. They may be designated as 1 entity, i.e., "calf septicemia." It should be borne in mind that not 1, but a variety of organisms are capable of causing this syndrome, depending upon the susceptibility of the calves and other factors.