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The Incidence of Cryptosporidial Infections in Nebraska Dairy Calves

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THE INCIDENCE OF CRYPTOSPORIDIAL INFECTIONS IN NEBRASKA DAIRY CALVES

by

Douglas Lee Varner

A THESIS

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THE INCIDENCE OF CRYPTOSPORIDIAL INFECTIONS IN NEBRASKA DAIRY CALVES

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Cryptosporidium is a protozoan parasite of the order Eucoccidiorida and is closely related to other coccidian parasites of economic importance such as Eimeria. It has only been within the last decade that Cryptosporidium has become recognized as an enteropathogen producing clinical signs of infection in animals and humans particularly immunocompromised individuals. The emergence of Cryptosporidium as a potential pathogen was due primarily to the advent of AIDS and its association with this syndrome. Cryptosporidium exhibits several unique biological properties that differentiate it from other coccidia. These include several differences in its life cycle which allow the organism to produce severe watery diarrhea lasting several weeks in immunocompetent individuals and chronic life-threatening diarrhea in immunocompromised individuals particularly those with AIDS. Evidence exists that support the role of Cryptosporidium as a zoonosis. Cryptosporidium also exhibits a low degree of host specificity with experimental infections being produced by the inoculation of human isolates into calves, lambs, mice and goats and calf isolates into similar species.

The purpose of the present study was to determine the incidence of cryptosporidial infections in Nebraska dairy calves,
whether infection was associated with other enteropathogens and
determine if an association exists between infection and the
production of scouring. Seventy-one dairy herd owners
participated in the study by sending fecal samples from five of
their calves when the animals were 5 and 12 days of age. A total
of 620 fecal samples from 334 dairy calves were examined for
cryptosporidial oocysts using the Sheather's sugar flotation
technique. Fifty-five of the 620 fecal samples from 52 of the
334 calves were positive for *Cryptosporidium*. The positive
samples were from 18 of the 71 herds. Forty-nine positive fecal
samples were examined for the following enteropathogens:
*Escherichia coli*, *Clostridium perfringens*, rotavirus, coronavirus
and *Salmonella*. Twenty of the calves were infected with
*Cryptosporidium* alone, 15 of which scoured and 1 of which
eventually died. One or more of the aforementioned
enteropathogens were observed in the remaining 29 samples.
Results of this study suggest an association between infection
with *Cryptosporidium* and scouring.
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Introduction

Diarrheal diseases in humans and domestic animals continue to represent a significant health problem in the world today, especially among the young.

The World Health Organization estimates the median mortality rate of diarrheal diseases in children 1 year of age and under to be 20 deaths per 1000 children per year and in the first 2 years of life 2-3 episodes of diarrhea occurred per year per child. (293). These data are based on 24 community-based surveillance studies carried out in 18 developing countries. Further analysis of these data revealed children less than 5 years of age in Africa, Asia and Latin America had approximately one billion episodes of diarrhea per year, resulting in 4.6 million deaths. Another study showed morbidity and mortality rates in the combined populations of Africa, Asia and Latin America estimated at 3 billion to be 3-5 billion and 5-10 million respectively (347).

Diarrheal diseases among neonatal food-producing animals has a similar impact and is a complex syndrome involving a variety of infectious agents interacting with various physical characteristics including age, sex, breed, strain, weight, stress factors such as environment, shipping and handling, and genetic factors contributing to heightened resistance or susceptibility and immunologic parameters. Acute diarrhea occurs commonly in neonatal food animals especially beef and dairy calves,
piglets and to a lesser extent in lambs and kids. Colibacillosis in piglets may account for 50% of all gastroenteropathies. Transmissible gastroenteritis may affect 100% of piglets a few days of age with a much lower morbidity rate as the piglets reach 3 to 6 weeks of age (263). In beef calves the population morbidity will vary from 10 to 50% in the majority of herds. In well managed herds the percentage affected can reach as low as 3%. (263).

Neonatal diarrhea in the dairy industry can be a devastating disease with an estimated incidence as high as 10 to 15% and the morbidity approaching 100% in severely affected herds (2). Economic losses due to calf diarrhea have been estimated to average about $9.00 for each calf born (2) or in excess of $95,000,000 annually in the United States alone (142).

Neonatal diarrhea in humans and animals can be multi-etiological. The identification of the different agents causing the diarrhea is very important in terms of treatment, control and the understanding of the epidemiology, pathology and the mechanism by which the enteritis and resulting diarrhea occur. Within the last decade a new etiologic agent has been identified as a possible cause of diarrhea in humans and domestic animals—that being the protozoan parasite Cryptosporidium. The purpose of this study was to determine the incidence and distribution of Cryptosporidium in the state of Nebraska, to determine if Cryptosporidium occurs more frequently in association with other enteropathogens and to determine about the relation of scouring in the calves to infection with Cryptosporidium.
**Literature Review**

**History and Taxonomic Status of the Organism**

**Cryptosporidium** is classified into the phylum Apicomplexa. Apicomplexa is a phylum of protozoa in which all members possess a structure termed the apical complex. A group of specialized structures observed only under the electron microscope comprise the apical complex. These structures include one or more electron dense polar rings; a conoid formed by electron-dense microtubules inside a polar ring, a number of rhoptries which are electron-dense tubular or saccular organelles often enlarged posteriorly extending back from the anterior region inside the conoid, a number of micronemes which are elongate electron dense organelles extending longitudinally in the anterior part of the body and/or a number of subpellicular microtubules which are slender, electron-dense hollow structures extending back just beneath the pellicle from a polar ring. One or more micropores are generally present into which food is taken (183). Further classification places **Cryptosporidium** in the class Sporozoasida in which oocysts or spores are formed, the subclass Coccidiasina in which organisms are typically intracellular, the order Eucoccidiorida in which merogony occurs, the suborder Eimeriorina in which the macrogamete and the microgametocyte develop independently with the microgametocyte producing many flagellated microgametes. **Cryptosporidium** is placed in the family Cryptosporidiidae because the oocysts contain 4 naked sporozoites with no sporocysts (183, 233). The order Eucoccidiorida in addition to containing **Cryptosporidium** also
contains other parasitic protozoa which are of significant economic importance to domestic animals as well as humans including *Eimeria, Isospora, Sarcocystis, Toxoplasma* and the blood parasites *Plasmodium* and *Babesia*.

*Cryptosporidium* was first recognized in 1907 by Tyzzer (312) from histological sections of the stomach glands of mice. The organism was named *Cryptosporidium muris* to signify a sporozoan in which spores were indistinguishable, absent or concealed in the oocysts. On the basis of the absence of spores *Cryptosporidium* was assigned to its own family in 1911 (177). In 1912 Tyzzer (314) proposed another species in the family named *Cryptosporidium parvum* which in contrast to *C. muris* infected only the intestine. From that point until the beginning of this decade *Cryptosporidium* was assumed to exhibit the same strict host (342) and site specificity (314) as other closely related coccidia. Therefore when *Cryptosporidium* was discovered in a new host a new species was named. Nineteen different named species of *Cryptosporidium* can be found in the literature (182). However many of the described species have been invalidated based on the original description in which they resemble *Sarcocystis* (181, 182, 184). A lack of host specificity was first demonstrated by Tzipori in 1980 (321) when feces from a 10-day-old calf caused oocyst shedding in lambs, calves, pigs, rats, mice, guinea pigs and a chicken when given orally. Based on these data *Cryptosporidium* was proposed as a single species genus. Subsequent investigations have shown *Cryptosporidium* to be transmissible between a wide range of host species (127, 217,
Levine (182) observed published reports which show 31 of 37 mammal-to-mammal transmissions have been successful while only one of five mammal-to-bird attempts have been successful. While experimental studies on reptilian or piscine isolates of Cryptosporidium have not been performed Levine, (182) made the assumption that those types of transmission studies would not be successful. With this information Levine (182) proposed the genus Cryptosporidium should be divided into 4 species: Cryptosporidium muris Tyzzer 1907 infecting mammals, Cryptosporidium crotali Triffit 1925 in reptiles, Cryptosporidium meleagridis Slavin 1955 in birds and Cryptosporidium nasorum Hoover, Hoerr, Carlton, Hensman and Ferguson 1981 in fish. In this particular classification system C. parvum is synonymized with C. muris.

Upton and Current (339) have criticized this lumping of these mammalian species of Cryptosporidium into 1 species because Tyzzer's original description of C. muris and C. parvum clearly demonstrates the 2 species are structurally and developmentally different. Also they occupy separate sites in the gastrointestinal tract of the murine host. These species are differentiated based on oocyst size with C. muris ranging in size from 6.6 to 7.9 um in length and C. parvum having a size range of 4.5 to 5.4 um. Upton and Current (339) believe C. parvum to be responsible for most reported cases of cryptosporidiosis in mammals and to be the cause of profuse watery diarrhea in calves less than 21 days of age. C. muris is
thought to be associated with mild diarrhea in cattle of all ages especially younger adult animals.

Further support for the validity of 2 mammalian species of Cryptosporidium is offered by Anderson (12) who recently identified *C. muris* from a 6-week-old calf and six feedlot steers not only by morphological identification of the oocysts but also by the demonstration of the organisms in the peptic glands of the abomasum—the same location in which Tyzzer identified the organism in 1907 (312).

While it appears as though the species of Cryptosporidium infecting mammalian hosts exhibit some degree of site specificity it should be remembered host factors especially the immunologic state seem to predispose the host to a disseminated infection in a range of host tissue sites (10).

Reduker et al (273) recently undertook an ultrastructural examination of the oocyst wall of Cryptosporidium in which a suture was revealed which extended part way around the oocyst within the inner layer of the oocyst wall similar to what has been observed in the sporocyst wall of other coccidia namely Sarcocystis (49), Toxoplasma gondii (70), Isospora (103), and Eimeria funduli (235). A suture such as this has never been described on the oocyst wall of any other coccidian taxon. This fact lead the authors to speculate that Cryptosporidium like Sarcocystis is passed from the host gut as sporocysts not oocysts. The oocyst wall then either never forms, forms and then is discarded prior to or during release in the environment or the outer layer of the oocyst wall observed in electron
micrographs may be a vestigial oocyst wall with the thicker underlying areas representing the sporocyst wall. This fact would place Cryptosporidium in a taxonomic position more closely related to the sarcocystids and the calyptosporids.

**Life Cycle of the Organism**

The life cycle as described by Current (78) is similar to other organisms in the suborder Eimeriorina and can be divided into 6 major developmental cycles: excystation involving release of infective sporozoites, 2 generations of asexual multiplication termed merogony, gametogony in which gametes are formed, fertilization, oocysts wall formation and sporogony in which sporozoites are formed.

While Cryptosporidium follows the same basic life-cycle as other coccidia several important differences exist. One is the location of the organism in the host cell. Another is the observation that two distinct types of meronts exist, one of which can undergo cyclic development and the fact that two types of oocysts are produced as a result of sexual reproduction.

Because of the location of Cryptosporidium on the brush border of enterocytes it was unclear whether Cryptosporidium should be considered to occupy an intracellular location or an extracellular location. This point has been resolved and the term intracellular-extracytoplasmic has been coined (111).

Marcial and Madera (201) using high resolution thin sections and freeze fracture techniques demonstrated that Cryptosporidium invaginate the microvilli in which they colonize and the resulting redundant folds of membrane envelop the organism.
thereby internalizing it in a membrane sac of host cell origin termed a parasitophorous vacuole. This position of this vacuole confined to the microvillus region of the host cell differs from comparable forms of closely related forms *Eimeria* and *Isospora* which occupy a perinuclear position deep within the cytoplasm of the host cell (78).

Marcial and Madara (201) also identified *Cryptosporidium* in the cytoplasm of M cells which cover the lymphoid follicle or Peyer's Patches as they are called. This is the first example of a parasite within the cytoplasm of these cells. The authors postulate this mode of entry allows antigens to be processed and presented to the intestinal immune system in order to establish mucosal immunity. The inability of the intestinal immune system to respond to this type of antigenic stimulus presented by the M-cells may explain why *Cryptosporidium* infections become disseminated chronic infections in the immunodeficient individuals.

A detailed study of the endogenous development of *Cryptosporidium* in suckling mice using three different isolates: one from a naturally infected calf, one from an immunocompetent human with a short-term diarrheal disease and one from an AIDS patient with chronic life-threatening diarrhea showed all isolates producing indistinguishable infections in suckling mice (81). Two types of meronts were observed. Type I meronts were present 16 hours through 9 days post-infection and contained eight merozoites. Type II meronts were present in the enterocytes from 24 hours through 9 days post infection and
contained four merozoites. Type I meronts were always more numerous than Type II meronts and the authors concluded that merozoites from Type I meronts underwent cyclic development to produce more Type I meronts while the merozoites released from Type II meronts initiated gametogony. This same phenomenon of cyclic development of Type I meronts has been observed in human and calf isolates of Cryptosporidium grown in chicken embryos (80) and a human isolate grown in cell culture (79). Current and Reese (81) also observed an area of vacuolation in the anterior end of invading merozoites suggesting material is released from the rhoptries and/or micronemes which might aid in membrane invagination at the sites of entry of the merozoites.

The host parasite interface was shown to be composed of a feeder organelle which is speculated to be an adaption for nutrient transport into the parasite. No physiological data exist to date to substantiate this hypothesis. The feeder organelle is formed by the portion of the parasitophorous vacuole membrane in contact with the enterocyte cytoplasm losing its structural integrity and extensive folding of the associated plasma membrane of the developing parasite (81).

Experimental investigations examining factors influencing excystation of Cryptosporidium have been conducted by Reduker and Speer (272) and by Fayer and Leek (94). Excystation in Eimeria, Isospora, and Sarcocystis has been shown to require incubation under anaerobic or reducing conditions in solutions containing sodium dithionite, cysteine or an aerobic CO₂ atmosphere followed by exposure to pancreatic enzymes such as trypsin, alpha-
chymotrypsin, or lipase and whole bile or bile-salts such as taurocholate, cholate, deoxycholate, glycotauro-cholate or other surfactants (93, 135, 280). Fayer and Leek (94) found Cryptosporidium sporozoites were liberated from oocysts suspended in water, salt or saline solutions in the absence of reducing conditions or digestive enzymes. Liberation occurred at 20°C but was greatest at 37°C. The oocysts of Cryptosporidium appear to respond more like sporocysts because conditions liberating the sporozoites from the oocysts in Cryptosporidium mimic those conditions in which sporozoites of Eimeria tenella which are liberated from sporocysts while still inside the oocyst (145).

Reduker and Speer (272) showed excystation of sodium hypochlorite-treated Cryptosporidium oocysts was enhanced when sodium hypochlorite washing was followed by buffer washes such as 0.25% trypsin – 0.75% sodium taurocholate. No significant differences in excystation rates were detected between incubation in 5% CO₂:95% air or those incubated in 100% air. Reduker and Speer (272) suggested that since Cryptosporidium exhibits little host specificity different host bile types would induce excystation. These experiments have not been conducted as of yet.

The conclusion from these experiments is that sporulated oocysts can excyst endogenously in the intestine or other organs after exposure to bile or other body fluids. This can result in continuing invasion of new host cells by infective stages being released inside the host organs with no exogenous source of infection.
Current has made the observation that not all oocysts excyst inside the host--some oocysts pass out in the feces (77, 80). Approximately 20% of the sporozoites formed as a result of sexual reproduction are surrounded by only a single unit membrane making up the oocyst wall which upon being released from the host enterocyte ruptures to release the 4 sporozoites which penetrate additional host cells and the life cycle is reinitiated. The cell wall of remaining oocysts are composed of a multilayered, environmentally resistant thick wall similar to the oocysts formed by *Eimeria* and *Isospora* which are passed in the feces to infect new susceptible hosts by the fecal-oral route. The thin-walled oocysts in addition to the Type I meronts which recycle contribute to the autoinfective clinical course of cryptosporidiosis which is particularly serious in immunodeficient individuals (78).

**Caprine infections with Cryptosporidium**

The first case of cryptosporidial infection in goats occurred in a 2-week-old Angora goat in Tasmania (203). The predominant clinical sign of the infection was diarrhea. The kid died within 6 hours after showing signs of clinical illness which consisted primarily of diarrhea. Diagnosis was made post-mortem by the demonstration of the spherical bodies of *Cryptosporidium* on the brush border of the enterocytes. Pathological findings showed enteritis characterized by blunting of the intestinal villi, infiltration of leukocytes into the lamina propria and focal sloughing, and erosion and anaplasia of the enterocytes. Bacterial or viral pathogens were not detected in the intestinal
Since this time caprine cryptosporidial infections have occurred in 21 of 29 kids in an Australian herd, 3 of which died (330); 33 of 360 goat kids submitted to a diagnostic laboratory in New Zealand (302); a small herd in Belgium (90); two separate reports of diarrheic kids in Hungary in one report of which of which mortality reached 21% (224, 225); and in two goat kids from Tanzania (207). The age at which clinical signs became evident ranged from 3 days (225, 330) to 6 months of age (90). All infected goats showed mild enteritis characterized by atrophy and fusion of the villi with parasites demonstrated on the brush borders of the enterocytes of all cases except one.

_Cryptosporidium_ was the sole enteropathogen observed in 2 reports (203, 330) whereas concurrent infections with _Eimeria_ (90, 302), rotavirus (224, 225), coronavirus and K99 positive _Escherichia coli_ (225), and adenovirus (224) were reported in other cases.

Reproducing clinical infections of _Cryptosporidium_ has yielded mixed results. Inoculation of axenic, holoxenic, and gnotoxenic kids produced no clinical signs of the disease however oocysts were demonstrated in the feces of all groups (73). Kids fed colostrum exhibited fewer clinical signs and reached a higher body weight than kids fed reconstituted milk upon experimental infection (221).

_Ovine infections with Cryptosporidium_

Naturally occurring infections with _Cryptosporidium_ in lambs was first reported in a 3-week-old lamb in Australia (27).
Although at post-mortem examination the lamb was cachectic and histological examination revealed severe villus atrophy, low surface epithelium, dilated intestinal crypts and leukocytic and neutrophilic infiltration as well as endogenous stages of Cryptosporidium on the brush border of the epithelium the author was reluctant to attribute these pathological changes to cryptosporidial infection because of a concurrent Salmonella typhimurium infection.

The second report occurred in 2 lambs aged 6 days and 14 days in North Dakota (35). Clinical signs and histological examination revealed similar findings as the previous case with Cryptosporidium occurring in the microvillous border of the ileum. Again the authors were reluctant to attribute the observed pathological changes to Cryptosporidium because of a concurrent Escherichia coli infection.

Subsequent reports of ovine cryptosporidiosis occurred in Scotland where 40 of 48 artificially reared lambs scoured and 16 died of which 10 were infected with Cryptosporidium (320); another outbreak in Scotland 1 year later in which Cryptosporidium was found in scouring lambs born to 3 different groups of ewes (18); in a farm flock, an orphan-lamb rearing operation and in a hospitalized lamb in Idaho, USA (8); a mouflon sheep in Belgium exhibiting intermittent diarrhea (90); 12 out of 53 diarrheal lambs from 5 out of 14 sheep herds in Hungary (225); 125 out of 500 lambs of age 2 to 7 days in Italy (179); intestinal mucosal scrapings from 9 of 25 lambs of age 36 hours to 3.5 months in France (267); and 16 of 237 lambs in Iran
The clinical syndrome for cryptosporidial infections seemed to vary substantially between the different reported cases. The reports vary from severe diarrhea leading to death with no concurrent enteropathogen infections (4, 18, 320) to intermittent diarrhea complicated by infection with other coccidia and strongylid worms (90); to mild diarrhea associated with *Escherichia coli*, coronavirus, bovine viral diarrhea virus and *Clostridium perfringens* (8).

Experimental infections of specific pathogen free lambs with purified inoculum of human (322) and calf isolates (20, 325, 334) produced the same type of villous atrophy, epithelial cross bridging, infiltration with neutrophils and clinical signs of anorexia and severe watery diarrhea as natural infections. However experimental infections with a purified human isolate produced less severe lesions and clinical signs than did infections with calf isolates (322).

Experimental investigations in lambs seem to show there is a time span in the age of the lamb in which the animal is most likely to be showing clinical signs of *Cryptosporidium* infections. Lambs less than 4 days of age are more likely to exhibit clinical infections with rotavirus. In experimental infection of gnotobiotic and SPF lambs rotavirus and enterotoxigenic *Escherichia coli* induced clinical diarrhea in lambs under 4 days old while older animals became subclinically infected (320). Therefore *Cryptosporidium* appears to initiate a more severe disease in older lambs than does *E. coli* or rotavirus.
acting singly or in combination with each other and lambs become clinically resistant to \textit{E. coli} and/or rotavirus by 4 days after birth (334).

Newborn lambs experimentally infected with \textit{Cryptosporidium} became depressed, anorexic and developed diarrhea. Lambs infected at 5 to 20 days of age developed less severe clinical signs of the disease while lambs infected at 30 days of age excreted oocysts but did not develop clinical signs of the disease or growth retardation (325).

Lambs infected with \textit{Cryptosporidium}, euthanatized at specified time intervals and examined for the presence of \textit{Cryptosporidium} at specific sites in the small intestine, cecum and spiral colon by light and electron microscopy show establishment of infection in all sites of the small intestine examined at 48 hours, establishment of infection in all sites of the enteric tract and mucosal damage characterized by light cellular infiltration to severe villous atrophy and infiltration in all sites in the small intestine at 72 hours followed by mucosal damage at all intestinal sites from 144 hours post-infection to 288 hours post-infection which represent the last time interval examined. By transmission electron microscopy all life cycle stages were observed in the small intestine at 48 hours. Clinical signs of infection became apparent between 48 to 72 hours post-infection (290).

\textbf{Equine infections with Cryptosporidium}

Clinical signs of \textit{Cryptosporidium} infections in the equine have been reported in foals with severe combined immunodeficiency
in Colorado, USA (294) and in Australia (25, 109) and in immunocompetent foals in Canada (106). In all cases enteritis was produced however in one case a concurrent infection with adenovirus was noted which made the role of Cryptosporidium as a pathogen difficult to interpret (294).

Survey work on equine Cryptosporidium infections was performed in Australia in which Cryptosporidium was not found in 52 diarrheic foals sampled over a 5 year period (318); in Ohio, USA where 14 fecal samples from diarrheic foals were examined during the first 28 days of life were negative for Cryptosporidium (276) and in France where fecal samples from 13 or 82 foals aged 3 to 15 weeks were positive for Cryptosporidium but were not exhibiting signs of diarrhea (297).

The role of Cryptosporidium as an enteropathogen is unclear at this point and experimental infections of a bovine isolate of Cryptosporidium into colostrum fed and colostrum-deprived foals have produced only subclinical infections (318).

Avian infections with Cryptosporidium

Cryptosporidium infecting the respiratory tract has been reported in broiler and layer chickens from Japan (150, 231); Indiana, USA (86); and Scotland (269); in turkeys from Indiana, USA (136, 268), Georgia, USA (110), and Saskatchewan (304); and in the quail (308), peacock (202), and pheasant (353) all reported from Australia. The most common organ of the respiratory tract infected was the tracheal epithelium although other areas in which Cryptosporidium was found included the bronchi, sinuses, and the larynx. Respiratory distress occurred
from 2 to 11 weeks of age with clinical infection characterized by depression, sneezing, gurgling respiration, dyspnea and excessive exudation from the infected organs. The most common finding on histologic examination of infected organs revealed epithelial hyperplasia. Inflammatory cell infiltration and necrosis was also evident in some cases (86, 136, 304). Respiratory cryptosporidiosis is believed to be highly infectious with high mortality and morbidity.

_Cryptosporidium_ has been observed in the bursa of Fabricius of chickens, turkeys and ducklings (100, 150, 186, 231, 264, 269, 311). Clinical signs were not associated with these infections and histological sections of the tissue revealed epithelial hyperplasia and inflammatory cell infiltration.

Enteric infections of _Cryptosporidium_ have been reported in the villous epithelium of the terminal third of the small intestine in 10 to 14 day old turkey poults. The birds exhibited diarrhea with a low death rate (288). _Cryptosporidium_ has been shown on the cecal epithelium in chickens (150, 315) and in the large intestine of the domestic goose (262). These cases showed mild enteritis characterized by shortening or loss of villi.

Doster and co-workers (87) observed _Cryptosporidium_ in the cloacal coprodeum of a red-lobed parrot (*Amazona autumnalis*). Histological examination revealed epithelial hyperplasia with heterophil and lymphocyte infiltration of the lamina propria. Clinical signs of this infection were not evident.

 Cryptosporidial infection of the surface epithelium of the conjunctiva was observed in pheasants (270) and a peacock chick
Corneal opacity, protrusion of conjunctival folds and serous oculonasal discharge were observed grossly with histologic lesions characterized by epithelial hypertrophy, hyperplasia and infiltration by heterophil and mononuclear cells. The authors were hesitant to attribute these lesions and the clinical signs solely to infection by Cryptosporidium.

An unusual observation of Cryptosporidium infection from the kidneys of a black-throated finch was reported from the San Diego Zoological Gardens (108). At necropsy the kidneys were extremely large, firm, pale and uniform in appearance. Cryptosporidium was observed attached to the epithelial surface of the kidney tubules. This represents the first report of Cryptosporidium in the urinary system.

Cryptosporidium oocysts were isolated from the bursa of Fabricius of naturally infected broiler chickens inoculated orally into 28 2-day-old chicks, 7 of which were inoculated intratracheally and the remaining 21 inoculated orally to determine oocyst structure and tissue specificity (188). Oocysts were passed 4 to 5 days after inoculation and continued for 17 days. Both modes of inoculation produced infections within the digestive tract with the cloaca being the most common site of infection followed by the bursa of Fabricius, the terminal portion of the colon, and the cecum. Four of the 21 chicks inoculated orally had tracheal infections while 6 of the 7 chickens inoculated intratracheally had tracheal infections. Clinical signs of infection did not result from either infection mode.
Canine infections with Cryptosporidium

Canine Cryptosporidium infections were first recognized in a 1-week-old pup in Tennessee, USA with a history of acute onset of diarrhea and labored breathing. Diagnosis of Cryptosporidium was made histologically with a mild enteritis characterized by several blunted villi and a mild mononuclear cell infiltration in the lamina propria (356). The author was reluctant to attribute the disease state to Cryptosporidium.

A second report of canine cryptosporidial infection was reported in Tennessee, USA in a 3-month-old puppy with distemper. Histological examination of the jejunum revealed Cryptosporidium attached to villous epithelial cells and in the crypts of Lieberkuhn with associated blunting of the microvilli in the area. The author considered Cryptosporidium to be an incidental finding occurring as a result of immunosuppression brought about by infection with canine distemper virus (105).

Sisk (286) identified cryptosporidial stages in the villi of the ileum by light and electron microscopy in two 6-week-old pups from Georgia, USA. One died in a weak, semi-comatose condition and the other died after developing seizures. Neither had diarrhea and the authors were unable to correlate clinical findings as being a result of cryptosporidial infection.

Experimental inoculations of Cryptosporidium into dogs have been performed with three 8-week-old puppies with an isolate of Cryptosporidium of human origin (82) and 25 dogs aged 1 to 100 days with oocysts of calf origin (24). In both cases oocysts were shed in the feces 2 to 14 days after inoculation but
clinical signs were not observed.

Survey work on canine *Cryptosporidium* infections have been conducted in the Munich, Germany area of which the parasite was not observed in any of 200 canine fecal samples (24) and in Finland where 57 canine fecal samples were collected from 12 breeds of dogs, all of which were negative (255).

**Feline infections with Cryptosporidium**

*Cryptosporidium* infections in the feline were first reported in Japan in three 1-month-old litter-mates and 2 adults. Oocysts were demonstrated in the feces and various stages of the organism observed on histological sections of the small intestine. Clinical signs of infection were not observed (147).

Poonacha (259) observed *Cryptosporidium* histologically in a 5-year-old domestic cat from Kentucky, USA with clinical signs of anorexia, weight loss and persistent diarrhea. Lesions in the intestine included fusion of villi, increased goblet cells, and hyperplastic crypt epithelium. No other enteropathogens were detected. *Cryptosporidium* was diagnosed in a kitten in Czechoslovakia aged 50 days (243).

Chronic diarrhea and wasting was observed in two separate cases in 6-month-old cats in Great Britian (37). Diagnosis of *Cryptosporidium* infection was made both histologically and from fecal smears. In one case the animal was serologically positive for feline calicivirus and was euthanatized. In the other case an untyped *Campylobacter* was observed and the cat recovered from clinical signs after hospitalization.

A survey conducted in the Munich, GFR area revealed 4 out of
300 cats shedding Cryptosporidium oocysts in the feces. Experimental inoculations of Cryptosporidium into kittens produced no clinical signs but oocyst shedding in the feces did occur (24, 82).

**Porcine infections with Cryptosporidium**

Naturally occurring cases of Cryptosporidium in the pig have been reported in Canada (220); Kansas, USA (159); Australia (189); and Germany (282). Although enteritis and diarrhea (159, 189, 282) were observed in the piglets aged 2 to 14 weeks of age the authors did not attribute the clinical signs to infection with Cryptosporidium.

Experimental infections of pigs with Cryptosporidium have been established using isolates from humans (218) and calves (129, 217, 333, 337). All pigs infected during the first week of life exhibited clinical signs of enteritis with associated diarrhea and histological lesions characterized by atrophic villi, immaturity of villous epithelial cells and edema with increased cellularity in both the small and large intestine (217, 333, 337).

Pigs experimentally infected at 7 days of age experienced moderate diarrhea and subclinical infections were observed in pigs at 15 days of age. In pigs aged 7 days and older the upper intestine was sparsely populated but the ileum and the large bowel were heavily infected with associated mucosal damage (337).

Inoculation of eight hysterectomy-derived, colostrum deprived pigs at one day of age with a human isolate of Cryptosporidium resulted in oocyst shedding at 4 days post-
infection and continued for 22 days. On histological examination diffuse villous atrophy and an irregular flattened surface in the ileum and an irregular flattened surface and crypt epithelium in the cecum and the colon was noted (218).

Inoculation of Cryptosporidium into the trachea and conjunctival sacs resulted in tracheal and conjunctival infections. All life cycle stages of Cryptosporidium were observed attached to epithelial cells by a folded vacuolated feeder organelle and surrounded by a parasitophorus vacuole. Affected areas of the epithelium were irregular, low and stratified with no goblet cells and evidence of sloughed cells from the epithelial surface. Intraepithelial lymphocytes and infiltrations with lymphocytes, monocytes and macrophages were observed (129).

Cryptosporidium infections in wild animals

Cryptosporidium has been found in a variety of wild mammals including several species of the order Artiodactyla (52, 95, 166, 234, 310, 323, 340), non-human primates (71, 167, 355), the Indian jungle cat (89), a gray squirrel (301), rabbits (146, 275 279), a raccoon (59), a fox (352), the Australian dingo (33), mice (120, 312, 313, 314) and a guinea pig (152).

Diarrhea was evident only in the Artiodactyla species and the non-human primates. The other mammalian species showed mild enteritis characterized by blunting of the intestinal villi, epithelial hyperplasia in other infected organs and mild inflammatory infiltration.

Three reports of cryptosporidial infections among deer
species have been reported in the literature to occur in red deer in New Zealand, England and Scotland (52, 234, 323) and one report in the Roe deer (166). *Cryptosporidium* appears to be a significant pathogen among red deer calves particularly if they are artificially reared. The outbreak in New Zealand resulted in death of all scouring hand-reared calves. A subsequent outbreak occurred in the same area among calves that had suckled from the dam for two days and were then hand-reared. Only one calf survived from this group after undergoing prolonged treatment with electrolytes, antibiotics and adult deer serum. Bovine colostrum and adult deer serum were then administered as a prophylactic measure to succeeding groups of deer calves and clinical signs of illness did not develop (234). Colostral protection was insufficient to protect against cryptosporidial infections in Scotland in which an outbreak of diarrhea occurred among 82 artificially reared red deer calves of which 56 developed diarrhea and 20 died. During the outbreak 80% of diarrheal and 50% of apparently healthy deer calves excreted oocysts in the feces suggesting a causal relationship particularly as no other significant pathogens were detected in the outbreak. It was believed that colostrum did not offer protection because the animals had no prior exposure to the organism and were introduced into an area where diarrhea had occurred six months earlier in suckled beef calves (323).

Severe diarrhea and anorexia resulted in the death of a 2-week-old Roe deer in Denmark (166). In this case moderately severe subacute enteritis was observed upon histological
examination with fusion, swelling, and atrophy of the villi and infiltration of the lamina propria with macrophages and neutrophils. Various stages of Cryptosporidium were observed throughout the large and small intestine.

Two 1-week-old blackbucks from the San Diego Zoo recently experienced diarrhea. Cryptosporidium was observed upon examination of histologic sections in the duodenum, jejunum, ileum, cecum, spiral colon and colon. Previous cases of diarrhea in other young artiodactyls were reviewed with Cryptosporidium being observed in 10 blackbuck, 2 samitas-horned oryx, 2 fringe-eared oryx, 2 addax and 1 sable antelope. The authors observed young animals moved to a confinement center and deprived of colostrum frequently developed diarrhea within 1 week and died within 1 to 2 weeks after onset of the illness. Stress brought on by over-crowding and colostrum-deprivation increased the animals susceptibility to infection with Cryptosporidium and other enteropathogens particularly Salmonella typhimurium which was found in frequent association with Cryptosporidium (340).

Cryptosporidium has been reported in a male Gazella subgutturosa which died 24 hours after birth. Histologic sections revealed Cryptosporidium throughout the intestine colonizing the microvillus border of the mucosal epithelium. Infection was most severe in the ileum and the proximal portion of the large intestine. While clinical signs of infection were not present in the animal it is remarkable that the organism could establish an infection in an animal so young. In experimental infections development to oocyst shedding occurs 2
days post-infection in mice and 5 to 8 days post-infection in goats, calves and lambs (82). Therefore the possibility exists that the infection appears to have been acquired in utero (95).

Cases of Cryptosporidium infections in non-human primates have occurred in rhesus monkeys (71, 167) and in macaques (355). A total of 10 cases of Cryptosporidium infection have been reported in rhesus monkeys from 2 separate outbreaks. In 1 case Cryptosporidium was observed infecting the epithelial cells of the common bile, intrahepatic and pancreatic ducts and the gall bladder (167). In all remaining cases Cryptosporidium was found in the large and small intestine. In two of the ten cases enteritis characterized by villus blunting and atrophy, epithelial hyperplasia and neutrophil infiltration was observed.

The cases of Cryptosporidium infection reported in 4 macaques aged 3 to 10 months with clinical signs of depression, dehydration, weight loss and persistent diarrhea resulted in death of 2 animals and euthanasia of the other 2 animals because no response to treatment was elicited despite intensive fluid therapy. Lesions in the small intestine were characterized by mild to moderate blunting and fusion of villi, necrosis of enterocytes and increased numbers of mitotic figures. Ultrastructural changes in Cryptosporidium-infected enterocytes were consistent with alterations in absorption and resulting loss of fluid and support the role of Cryptosporidium as an enteropathogen.

The first reports of Cryptosporidium infections in reptiles occurred in snakes and a lizard of the following species:
Crotalus confluentus, Ctenosaura similis and Lampropeltis calligaster. The infection in these cases was subclinical with diagnosis made by demonstration of the oocysts from feces (16, 91, 309). Subsequent reports of Cryptosporidium occurred in Pseudechis porphyriacus, Elaphe obsoleta, and several species of the genus Elaphe, Crotalus, and Sansinia (54, 209, 303). In all these cases a syndrome presented itself as persistant emesis and hypertrophic gastritis with Cryptosporidium observed on the epithelial surface of the gastric mucosa of snakes held in confinement in zoos or privately owned. In more severe cases mucosal necrosis occurred possibly as a result of an inability to resist invasion by normal bacterial flora of the snake alimentary canal. Unlike the clinical course of Cryptosporidium infections in higher animal species which is primarily a disease of the young with an acute clinical course the infection in reptiles occurs in mature snakes with a chronic, protracted insidious clinical course (54).

The first report of Cryptosporidium infections in fish occurred in the marine tropical fish Naso lituratus in Indiana, USA (140). Clinical signs of the infection consisted of a 2-month progressive illness characterized by severe emaciation, regurgitation of food and passage of feces containing undigested food. Cryptosporidium was observed in the intestine causing morphologic changes characterized by displacement of microvilli and focal indentation at sites of attachment. A later report of Cryptosporidium occurred in the mid-section of the intestine in Cyprinus carpio collected in Czechoslovakia. Morphologic
alteration of the infected tissue was not noted in this report (240).

**Bovine infection with Cryptosporidium**

The first report of a bovine Cryptosporidium infection occurred in Oklahoma in 1971 (237). An 8-month-old Santa Gertrudis calf presented with emaciation, dehydration and chronic diarrhea. Histologic examination of the small intestine revealed villus atrophy and marked alteration of glandular structures. Subsequent reports of Cryptosporidium infection has shown the distribution of the parasite to be world-wide as can be seen in Table 1.

Numerous surveys have been conducted to determine the prevalence of Cryptosporidium in different areas of the world. Heine (127) conducted a survey in the German Federal Republic and found of 322 calves without diarrhea 44 were infected with Cryptosporidium as were 88 of 222 with diarrhea. Anderson (15) showed 41 of 73 herds had one or more calves infected with Cryptosporidium in Idaho by demonstration of oocysts in the feces. Forty-two of 161 neonatal calves with diarrhea were positive for Cryptosporidium in a study conducted by Sanford in Ontario, Canada (281). A random sampling of calves aged 1 to 4 weeks of age from 20 dairy farms in Ohio revealed Cryptosporidium oocysts in the feces from calves in all 20 farms (121). Jungman (157) detected Cryptosporidium in the feces of 51% of 172 calves in the German Democratic Republic. Forty-five percent of the infected calves had acute diarrhea. A survey in Maryland showed 36 of 136 calves from 12 farms were excreting Cryptosporidium
oocysts in the feces. Sixteen calves had diarrhea of which 8 were excreting Cryptosporidium oocysts (174). Leeuw (175) reported Cryptosporidium present in 11 dairy herds with 19 to 85% of the calves infected. Subclinical infections were observed in 15% of the infected calves. Fiedler (96) reported an infection rate of 44% of 284 calves received for post-mortem examination in the German Federal Republic.

Diarrhea in calves in which Cryptosporidium was the only organism isolated have been reported (13, 36, 143, 212, 223, 229, 239, 258, 328). Cryptosporidium has frequently been reported to occur with other enteropathogens of the neonatal calf scours complex such as rotavirus, coronavirus and/or enterotoxigenic Escherichia coli (74, 97, 151, 168, 198, 222, 226, 257, 261, 281, 283, 291).

The clinical picture from outbreaks in the field show a syndrome of mild to severe diarrhea occurring in calves aged 1 to 4 weeks with low to moderate mortality and moderate to high morbidity (78, 316).

Histologic examination of naturally infected calves has revealed Cryptosporidium most commonly found in the distal regions of the small intestine specifically the ileum, jejunum and occasionally the cecum (250, 281, 296, 345). An unusual case of Cryptosporidium was recently found in the abomasal peptic glandular mucosa associated with the lumenal border of gastric cells (12). Cryptosporidium was found in an intracellular, extracytoplasmic area along the microvillous brush border of epithelial cells (249). Attached parasites were detected
primarily at villous tips and all stages were present on a single villus. The stages observed included merozoites, trophozoites, schizonts, gametes and oocysts. Attachment sites of the parasite stages were characterized by absence or disintegration of microvilli and disorganization of the terminal web (256). Villi infected with Cryptosporidium were shortened, atrophied, and distended at the apex. The enterocytes comprising the infected villi lost their cylindrical form and became cuboid or flat as squamous metaplasia developed. In the cecum the organism was found at the outlets of the crypts of Lieberkuhn often on the walls but rarely deep within the crypts (346). Infected areas showed hyperemia and inflammatory infiltration of the lamina propria with lymphocytes, macrophages, plasma cells, a few neutrophils and numerous eosinophils (98, 346).

Experimental infection of specific pathogen free or gnotobiotic calves inoculated with purified inoculum of Cryptosporidium oocysts obtained directly from diarrheic calves or gut contents from experimentally infected mice or piglets have been conducted (130, 336). The incubation period before oocyst shedding in the feces was slightly longer in colostrum fed calves (3 to 4 days) as compared with specific pathogen free calves (2 to 3 days). Clinical signs included depression, anorexia, weakness and diarrhea. Cryptosporidium was observed in the distal small intestine in calves necropsied at 5 days post-infection in which the organism was confined to the villi. Cryptosporidium was observed in the large intestine in animals necropsied 5 to 9 days post-infection where the organism was
present in the crypts and the mucosal surfaces.

Histologic lesions in the small intestine were similar to those observed in naturally infected calves with villous atrophy and fusion and were more severe in animals in which the infection was allowed to continue. Lesions were not observed in the large intestine. Enterocyte membrane-bound lactase activity was measured in experimentally infected calves and was shown to decrease during clinical illness but returned to normal after recovery. There appeared to be no difference in the clinical course of the disease or pathological findings in any experimentally infected calves. The results of these experiments indicate Cryptosporidium can destroy intestinal epithelial cells and cause diarrhea in calves experimentally infected with a purified inoculum of Cryptosporidium (130, 336).

Experiments were recently conducted by Fayer and others (92) to determine the factors necessary to produce clinical illness in calves experimentally infected with a bovine isolate of Cryptosporidium. Varying the dosage levels of Cryptosporidium in the inocula, differences in susceptibility between colostrum fed and colostrum deprived calves and the interaction between Cryptosporidium and other enteropathogens were all examined in order to make statements about factors necessary to produce a clinical infection in calves. Three experiments were conducted in which 2 dosage levels of Cryptosporidium, $5 \times 10^6$ versus $30 \times 10^6$ oocysts were inoculated into colostrum fed and colostrum deprived animals. A large variation existed in response to the infection ranging from no to severe diarrhea, none to numerous
oocysts shed, none to moderate fever and complete recovery to death.

In subsequent experiments oocysts stored in water were used for inoculation since the effects of storage of the oocysts used in previous experiments in 2.5% potassium dichromate were not known. In this experiment a calf inoculated with an aqueous solution of *Cryptosporidium* developed severe diarrhea, shed large numbers of oocysts and died. *Clostridium perfringens* was isolated from the calf.

Experiments were then conducted in which colostrum derived (CD) and colostrum fed (CF) calves were inoculated either with a centrifuged pellet containing *Cryptosporidium* or the supernatant. CD calves died after receiving either treatment with *C. perfringens* being isolated from the intestinal contents of 3 calves and rotavirus antigen detected in one of the 3 calves. One CF calf died after exhibiting clinical signs of severe diarrhea. Large numbers of yeast were found in the intestinal contents but other pathogens were not evident.

The last experiment consisted of removing fecal debris from inoculum containing *Cryptosporidium* oocysts and treating with antibiotics. Both CF and CD calves received this inocula. None of the calves became ill although rotavirus and *C. perfringens* were isolated in all cases.

The results of this experiment present a rather confusing picture of the relationship between dosage level, the ingestion of colostrum and the interaction with other pathogens in the clinical course of infection with *Cryptosporidium*. Variability
in the production of clinical signs of disease appears to be related to experimental manipulations of the inocula and variation in different strains. Experimental manipulations performed on the inoculum as well as strain variations were thought to play a role in the production of clinical disease.

**Human infections with Cryptosporidium**

Cryptosporidium infections in humans can be divided into infections occurring in immunocompetent individuals and those occurring among immunocompromised individuals. The forms of the infection present with similar symptoms but differ in the severity and duration of these symptoms.

The first case of human infection of Cryptosporidium occurred in an immunocompetent 3-year-old female presenting with vomiting and severe watery diarrhea (230). Electron microscopic examination of rectal biopsy revealed Cryptosporidium attached to the microvillus border of the epithelial cells.

After the first report of a human infection with Cryptosporidium in 1976 only 6 other cases of Cryptosporidium were reported (171, 211, 324, 350). Human infections with Cryptosporidium were then considered a rare occurrence until the advent of the Acquired Immune Deficiency Syndrome (AIDS). The association of Cryptosporidium with AIDS was first reported in 1982 (67). Its frequent association with AIDS has lead the CDC to include chronic enterocolitis due to Cryptosporidium as one of the hallmarks of the disease along with the presence of several other infectious agents and neoplasms (170). Due to the organisms association with AIDS and the surrounding publicity and
intense research emphasis associated with this syndrome detection and diagnosis of Cryptosporidium infections have improved to the point where it has been shown to be prevalent as a cause of previously undiagnosed cases of enteritis in immunocompetent individuals. Reports of immunocompetent individuals harboring Cryptosporidium have been shown in California (26) and the United Kingdom (99, 324).

Numerous studies have been conducted in an effort to determine the incidence of Cryptosporidium infections in the immunocompetent population. A study in Newfoundland and Labrador Canada showed an incidence of 1.2% out of 2,252 fecal samples submitted for analysis. The majority of the patients with positive stools had gastroenteritis with Cryptosporidium being observed as the only enteropathogen. Although Cryptosporidium was one of the common enteropathogens identified and Cryptosporidium was found in patients of all ages, they occurred slightly more frequently in infants and children (271). In the United Kingdom oocysts were identified in the feces of 7 out of 213 children with acute or chronic diarrhea and in one of 112 healthy controls (149). Cryptosporidium oocysts were observed in 46 fecal samples out of 7,300 patients with diarrhea in Canada (216). A study conducted in Boston, Massachusetts showed 43 patients were observed with Cryptosporidium oocysts in the feces. Nineteen of the 43 patients were under 4 years of age and 14 were 30 to 39 years of age. Fifteen of the 43 patients had other gastrointestinal pathogens (Giardia lamblia and Entameba histolytica) and in 28 patients with diarrhea Cryptosporidium was
the only pathogen observed (358). Fecal samples from 1,967 of 2,369 children with diarrhea were examined in the United Kingdom for Cryptosporidium and the organism was seen in the feces of 27 patients making it the fourth commonest pathogen detected (123). An Australian study revealed 36 out of 884 hospital patients with gastroenteritis excreting Cryptosporidium oocysts in the feces. In 31 of these patients Cryptosporidium was the only pathogen isolated with an incidence higher in children (4.8%) than in adults (1.6%). None of 320 hospital patients without gastroenteritis were excreting oocysts (335). In an urban center in the United Kingdom Cryptosporidium was identified in 43 of 867 patients with gastrointestinal symptoms. Twenty-four of the 43 cases occurred in children (144).

Several cases exist in which Cryptosporidium was contracted through contact by humans with infected animals in a research setting. At Auburn University 12 of 18 immunocompetent individuals who had been in direct contact with Cryptosporidium-infected calves excreted Cryptosporidium oocysts in their feces. Nine of the 12 individuals experienced diarrhea and abdominal cramps (82). Cryptosporidium oocysts were detected in the feces of a veterinary student who had cared for calves infected with Cryptosporidium. Clinical signs included nausea, vomiting, diarrhea, fever, sweating, chills, abdominal pain, bloating, headache, and general weakness (14).

A 35-year-old research worker acquired an infection due to Cryptosporidium after trying to infect a rabbit through a stomach tube which when removed caused the animal to cough several
droplets of the inoculum into the researcher's face (42).

The immunological status of the human host dictates in most cases the clinical course of *Cryptosporidium* infections. Infections in immunocompetent individuals present as a short-term cholera-like diarrheal illness often associated with flu-like symptoms of vomiting, nausea, fever and weight loss. Symptoms are similar in immunocompromised individuals except the diarrhea becomes chronic, protracted and life-threatening (78, 82) with fluid loss of 3 to 6 liters per day common and as much as 17 liters of watery feces being excreted daily in certain cases (67). The clinical course of *Cryptosporidium* does not always fall into either of these two categories based on the immune status of the host. Immunocompetent individuals who excrete oocysts with no diarrhea have been reported (82, 149). Immunocompetent individuals with diarrhea lasting over four months with a failure to thrive have also been reported (149). Asymptomatic carriage of *Cryptosporidium* in the stool of a patient with AIDS has been reported (360) as well as a spontaneous resolution of a *Cryptosporidium* infection in a child with AIDS (38).

While AIDS is probably the most common immunodeficient condition predisposing an individual to infection with *Cryptosporidium* other immunodeficient states have been associated with *Cryptosporidium* infections. These include hypogammaglobulinemia (22, 48, 171), primary immunoglobulin deficiency (289), IgA deficiency (351), bone marrow transplantation (72, 200), malnutrition and altered T cell function.
(299), severe combined immune deficiency (165), acute lymphoblastic leukemia (185, 213), and administration of immunosuppressive drugs (124, 211).

Cryptosporidium is considered to be one of the less frequent pathogens reported from AIDS patients (180). However, the organism is isolated from AIDS patients with enough frequency to be considered as a component in the clinical definition of AIDS. A study examining the clinical diagnoses of 87 patients with AIDS in Colorado and 359 other AIDS cases from the literature reports have shown that among persons native to developed areas Cryptosporidium was diagnosed in homosexual men at a rate of 8%. In non-homosexual populations the incidence was 2%. Cryptosporidium was diagnosed in 4% of the AIDS cases in patients native to the tropics (43).

Chronic diarrhea is a common clinical presentation in AIDS and its prevalence has been shown to be present in up to 90% of AIDS patients. However enteric pathogens are found in only a minority of these cases which may reflect either an insensitivity of culture methods, causation by infectious agents which are as yet unknown, factors unrelated to an infectious agent or process such as diet, etc., or factors related to the immune status of the individual contributing to the diarrhea (236).

Intestinal infections in AIDS are frequent possibly because of continuing exposure to infectious agents such as Giardia lamblia, various amoebas, Salmonella, Shigella, cytomegalovirus, Herpes virus, Hepatitis B virus, Mycobacterium intracellulare, or Isospora belli which are acquired through the environment or from
previously asymptomatic endogenous infections (115).

Cryptosporidium infections in AIDS patients are not thought to be the direct cause of death, but the diarrhea resulting in massive fluid loss and associated dehydration and malnutrition requiring prolonged hospitalization and multiple invasive procedures was often though to be a contributing factor in death (227).

Endogenous life cycle stages of Cryptosporidium are found adherent to the microvillus border throughout the ileum, duodenum and jejunum of the small and large intestine in both immunocompetent and immunocompromised hosts (10, 39, 40). However, in immunocompromised individuals the organisms disseminate to other organs and tissues. Cryptosporidium has been demonstrated in the gallbladder (252), tonsil (10), and the trachea and the bronchi (50, 102, 122, 165, 196). Pulmonary Cryptosporidium infections are characterized by clinical signs of persistant sore throat, dyspnea and diffuse rales associated with lung marking in chest X-rays (317). It is difficult to assess the role Cryptosporidium is contributing to the manifestations of these signs because often the infection is associated with other respiratory pathogens such as cytomegalovirus (50, 102), Mycoplasma sp. (165), and Pneumocystis carinii (196). The primary site of infection is not believed to be the lung. Rather the infection is believed to have originated in the gut and spread to the pulmonary system by sputum or by aspiration of vomit both of which have been shown to contain Cryptosporidium oocysts (62, 214). Parasite stages have been observed on the epithelium of
the trachea (196), bronchioles (165), in alveoli exudates and on or inside macrophages (196).

Examination of electron micrographs of the parasite life cycle stages at the attachment site in the small intestine have shown a phenomenon occurring referred to as "peaking" in which there is elongation and elevation at the apex of the microvilli. Also an abnormal accumulation of multiple, dense lysosome-like bodies were observed in the epithelium which may reflect an ineffective host phagocytic response (176).

Cryptosporidium infections have been shown statistically to be associated with Giardia infections (155, 357). However, it is not known if this association is a synergistic effect between the two parasites, infection with one leading to greater susceptibility to the other or similar modes of transmission. Other studies have shown an association between Cryptosporidium and Giardia cannot be demonstrated statistically (154, 287).

An association between Cryptosporidium and viral infections has been noted in the literature particularly in association with adenovirus (39, 40), cytomegalovirus (350), and the measles virus (85). It was speculated that infection with these viruses induced an immunosuppressive state and predisposed the individuals to infection with Cryptosporidium.

Epidemiology of the infection

Experimental studies performed on animals and electron microscopic examination have shown Cryptosporidium oocysts which are passed in the feces are fully sporulated and infective (17, 77, 82, 316). In addition, oocysts have been shown to be
resistant to most laboratory disinfectants. The ubiquitous nature of the parasite and its ability to cross host species barriers contribute to the potential for a reservoir of infective stages being shed into the environment and increase the ability of the infection to be transmitted to new susceptible hosts.

The evidence that Cryptosporidium may have a zoonotic potential first surfaced when cryptosporidial infections were established in 26 individuals who had direct contact with feces of infected calves (14, 82). These infections demonstrated a clear association between Cryptosporidium being transmitted between calves and humans. An earlier report of a possible bovine source was suspected in a human case of cryptosporidiosis which occurred in a child who was raised on a cattle rearing farm (230). A more recent case of bovine-to-man transmission of Cryptosporidium occurred in Bangladesh where Cryptosporidium was detected in 32 of 410 calves and 14 of 28 calf-handlers (265, 266). Three other cases of possible transmission from animals other than calves to humans have been reported. One case occurred when a 13-month-old boy possibly became infected by contact with a pet cat shown to be excreting Cryptosporidium oocysts (123). Cats shedding Cryptosporidium oocysts were implicated in another case in which a 36-year-old male with hemophilia, common variable hypogammaglobulinemia and the Acquired Immune Deficiency Syndrome became infected with Cryptosporidium and eventually died from complications of his depressed immune state (164). An association was also hypothesized between a Cryptosporidium infection in a healthy
professional athlete in which the source of infection was thought to be from cleaning horse barns (26). However, this association is rather poor because the patient presented with clinical symptoms of the infection several months after the suspected exposure to the infective stages. The period between exposure and onset of symptoms is fairly rapid--usually 5 days (14).

Since subclinical infections can occur in animals commonly kept as pets such as cats, dogs, guinea pigs, rabbits and monkeys the potential exists for a reservoir in animals becoming a potential source of infection for humans.

While there is good evidence for humans acquiring Cryptosporidium infections from animals epidemiologic studies have shown infections can occur in urban settings (6, 63, 144). The epidemiologic picture which seems to be unfolding from current studies (64, 137) is one in which the vast majority of human acquired Cryptosporidium infections appear to be acquired by person-to-person transmission in settings such as day care centers (5, 6, 68, 306), hospitals (32, 88, 163) and households (72, 277). Water, raw milk and foods have all been proposed as sources of infection but are difficult to substantiate because no enrichment media exists in order to propigate oocysts to detectable levels and the difficulty in distinguishing Cryptosporidium oocysts from artifacts (62, 359). A common waterborne source has been implicated in an outbreak of cryptosporidiosis infection in Texas (84) and accounts of infection among individuals traveling abroad have led to the characterization of Cryptosporidium as one of the etiologic
agents of traveler's diarrhea and support the contention that infection can occur by ingestion of contaminated water (139, 153, 155, 194, 295, 300). The association of Cryptosporidium with Campylobacter (65) and Giardia (155, 357) indicate that the epidemiology of Cryptosporidium may be similar to these other organisms.

Fecal-oral transmission is believed to be the way Cryptosporidium is passed among the homosexual population (348) and does occur independent of AIDS. Henkel (131) reported Cryptosporidium in 1 of 148 homosexual men in the German Federal Republic and was reported in 2 of 363 patients in another study (348). It was assumed that all individuals in these studies had a normal functioning immune system. Fecal-oral contact appears to contribute to the transmission of Cryptosporidium as it occurs in the homosexual population irrespective of AIDS at a rate similar to that reported in the general population. However, AIDS is a major predisposing factor that increases the prevalence of this disease among the homosexual population.

Patterns of shedding of oocysts in both humans and animals seems to indicate a chronic carrier state and relapses are rare. A study conducted of 33 immunocompetent patients infected with Cryptosporidium showed 20 of 33 individuals ceased to shed oocysts in the week following cessation of diarrhea, 5 continued to shed oocysts for 2 weeks or more and 1 patient was still shedding oocysts 3 weeks after diarrhea ceased (31). Studies with lambs and calves have shown shedding to coincide with the clinical signs and cease shortly thereafter (7, 8, 336).
Therefore the contamination of the environment from chronic shedding after recovery from clinical signs appears to be negligible. However, since subclinical infections exist in a variety of animals species (59, 92, 301) and humans (82, 149, 360) these types of infections could contribute to environmental contamination and play a role in infection where its source was not determined. Studies have not been conducted which would specifically address the role of subclinical infections in the transmission of the disease in either animals or humans.

Infections in adult animals is not an epidemiological consideration since unlike adult humans, Cryptosporidium has not been detected in adult animals. A study by Anderson (9) did not reveal Cryptosporidium oocysts in 1600 adult cows. It appears as though unlike humans animals acquire a resistant to Cryptosporidium similar to what occurs with enterotoxigenic Escherichia coli. Adult humans remain susceptible to ETEC throughout life (317).

A tendency towards a seasonal occurrence of infection has been noted in which Cryptosporidium was diagnosed at a higher rate during the warmer summer months in Canada, Australia and Brazil (216, 335, 349).

The distribution of Cryptosporidium infections is believed to be cosmopolitan, similar to the distribution shown in cattle. The organism has been found throughout the U.S. and in the Canadian provinces of British Columbia, Newfoundland and Labrador (216, 271). The distribution in the United States closely parallels that of AIDS but with the increasing awareness
among the medical community that cryptosporidial infections in immunocompetent individuals exist the distribution will most likely expand outside coastal urban areas where AIDS is considered endemic.

In Europe a number of outbreaks have occurred in the United Kingdom (31, 32, 63, 99, 123, 144, 149, 190, 324); in both immunocompetent and immunocomprised patients in France (22, 173, 215), Finland (154), Denmark (139), Spain (192) and the German Federal Republic (131); and six children in Greece (158).

In Africa Cryptosporidium has been found in AIDS patients from Zaire (134, 156), 10.4% of children and 3% of adults in a study conducted in Rwanda (85), in Liberian children (138) and in children with diarrhea from Ghana (3).

In Asia Cryptosporidium has been found in children with diarrhea in India (206), Thailand (305) and the Philippines (76); and in calf handlers and children in Bangladesh (266, 284).

In Central and South America Cryptosporidium infections have been reported in immunocompetent individuals in Brazil aged 2 months to 23 years (349), in AIDS patients from Haiti (199), in children in Costa Rica (204, 205), and in Venezuela (251).

In Australia Cryptosporidium infections have been diagnosed in an AIDS patient (75), in 9 of 94 Aboriginal children (193), in 4.1% of hospital patients with gastroenteritis in a hospital (335) and in a 23-year-old woman whose family had gastrointestinal upsets in Tasmania (208).

Pathogenic Mechanisms of Infection

The mechanism by which Cryptosporidium produces diarrhea is
believed to be similar to organisms such as *Vibrio cholerae*, rotavirus, *Escherichia coli*, Norwalk virus and *Giardia*. These organisms colonize and multiply in the intestinal lumen, usually the upper small bowel, and cause net secretion in the gut and often watery diarrhea by elaboration of an enterotoxin or colonization of the microorganism itself. This mechanism of diarrhea production is in contrast to organisms such as *Shigella* sp., *Salmonella enteritidis*, and *Clostridium difficile* in which the microorganism or their cytotoxic products cause an invasive process resulting in dysenteric diarrhea with polymorphonuclear neutrophils associated with blood and pus in the feces (117).

The contribution of parasite products such as enterotoxins or hormone-like substances to the production of diarrhea have yet to be investigated. Parasite antigens or metabolites are thought to induce a hypersensitivity reaction which could lead to an inflammatory response resulting in mucosal damage (317).

Diarrhea is believed to occur due to mechanical damage to the brush border of the enterocytes by colonization of the organism, disruption of the microvillus and release of parasite products (117). Extracellular organelles or colonization factors have not been shown to mediate attachment of the parasite to the mucosal surface (66). Electron microscopic studies have shown thin filaments extending from the parasite glycocalyx to the host cell glycocalyx (256). The union of electronegatively charged surfaces facilitated by sugar-sugar binding proteins (66) in a mechanism similar to what occurs with *Entamoeba histolytica* (162) is thought to be the mechanism by which *Cryptosporidium* attaches
to the epithelial surface of the intestine.

The main enteropathogenic effect of Cryptosporidium infections appears to be in its attachment to villi and the particular location in the enteric tract where colonization of the organism occurs (317).

Heavy infections with Cryptosporidium produce depressions or craters in the intestine as observed by SEM (290). As mentioned previously the most common gross changes that occur are in the architecture of the villi which include crypt hyperplasia, replacement of mature epithelial cells with immature cells, stunting and fusion of the villi, and degeneration of the enterocytes as noted by various researchers (40, 171, 211, 289, 328, 350, 351). This type of proliferation particularly in the immunocompromised host is thought to lead to impaired digestion, malabsorption, and profuse watery diarrhea which is the major symptomatic expression of Cryptosporidium infection. The malabsorption is thought to be caused by large numbers of organisms adhering to the villi which causes the stunting and fusion of the villi thereby leading to a reduction in the absorptive surface area of the mucosa. Also disruption of the surface mucosa can lead to a decrease in membrane-bound enzymes which can also be a contributing factor in the production of osmotic diarrhea.

Malabsorption of water-soluble nutrients leads to a drawing out of water into the lumen (66). A deficiency in lactase similar to what has been shown to occur in animals infected with Cryptosporidium (316) causes the disaccharide lactose to remain
in the lumen until it reaches the colon at which time it is split by bacteria increasing the number of solutes (66). Fermentation of this split product to fatty acids stimulates additional withdrawal of water (46, 46). This type of carbohydrate malabsorption has been shown in viral enteritis (41), cholera (187), shigellosis (1) Isospora belli (51), and giardiasis (307).

Another major factor in the enteropathogenicity of Cryptosporidium is the predilection of the organism to infect the lower small intestine before spreading to the rest of the gut (290). In immunodeficient patients the proximal small intestine is predominantly involved with only a mild mucosal reaction and inflammation except in terminal stages of the disease (215). The posterior small intestine has been shown to be particularly efficient at net fluid absorption (56) and consequently the distribution of Cryptosporidium to these areas of the intestine may be crucial in producing symptoms of the disease (66).

Studies in nude mice which have depressed regulatory and effector T-cell activities but intact natural killer cell activity showed persistent infection with Cryptosporidium characterized by diarrhea and occasional death when experimentally inoculated at 11 days of age (128). Both nude and white mice appeared to be relatively more resistant to infection when inoculated at 42 days of age. These results suggested that T cells are required for recovery from the Cryptosporidium infection, but do not prevent epithelial cell loss in cryptosporidiosis. In a 6-month-old infant with severe combined immune deficiency a severe, disseminated infection developed
involving the epithelium of the bronchial tree in addition to the pancreatic duct and the entire small bowel eventually resulting in a fatal outcome (165). Therefore it appears that both branches of the immune system are required for complete recovery from infection (317).

The role of Cryptosporidium as a significant enteropathogen has been established experimentally (130, 328) and in field outbreaks of scours in which Cryptosporidium was the only enteropathogen isolated (13, 36, 143, 212, 223, 229, 239, 258, 324). While Cryptosporidium has frequently been reported to occur in association with other enteropathogens (74, 97, 151, 168, 197, 222, 223, 257, 261, 281, 283, 291) it should be remembered that associations often exist between enteropathogens and these associations may have a synergistic effect on the severity of clinical signs and the outcome of outbreaks of diarrhea in the field (142, 220). Epidemiological surveys have shown rotavirus infection alone can cause enteritis and diarrhea of varying degrees of severity (210). However, an interaction between rotavirus and enterotoxigenic Escherichia coli have been demonstrated in calves (116, 278, 331), pigs (329), and foals (332). Subclinical infections with Cryptosporidium are no more or less common than what has been observed with other enteropathogens (78, 305). Subclinical infections of Cryptosporidium may indicate differences in virulence between isolates from different areas as what has been suggested by other researchers (92, 316).
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Treatment and Control of the Infection

A large number of compounds have been tested in attempts to treat Cryptosporidium infections in humans and experimentally in calves and mice. These compounds include broad-spectrum antibiotics, antimalarials, other anti-protozoal drugs and anthelmintics (67, 219, 289, 299, 327, 341, 350). A summary of the compounds tested are listed in Table 2. None have been shown to be effective against clinical infections. Lasalocid was shown to prevent discharge of Cryptosporidium oocysts in calves at a dosage level which was toxic (219).

Spiramycin has been shown to be effective in the treatment of cryptosporidiosis but mixed results have been reported (28). Barriga (28) reported treatment using 1 gram of spiramycin given orally 3 or 4 times daily to 13 AIDS patients and 1 immunologically normal individual. Four were clinically and parasitologically cured, 3 showed clinical improvement but oocysts continued to be excreted and 7 patients did not respond to treatment. Portnoy (260) reported 6 of 9 AIDS patients were cured. Collier (72) reported resolution of diarrhea and negative stool samples after the treatment in a bone marrow transplant patient who was administered immunosuppressive drugs. Cure could not be attributed solely to spiramycin since immunocompetence in the patient was restored by the successful bone marrow transplantation and discontinuation of the immunosuppressive drugs.

Spiramycin is a macrolide antibiotic with activity similar to erythromycin and clindamycin. Administration of the drug
rarely causes serious side effects. Experimental work in animals has not been performed and the mechanism by which the drug exhibits its antiprotozoal activity is not known (69).

Lasalocid has been shown to interfere with the developmental stages of Cryptosporidium upon light and electron microscopic examination of the ileal mucosa in experimentally infected mice. Schizonts showed functional damage of the intracellular membrane system leading to vacuolization. The ultrastructure of other developmental stages appeared to deviate from a normal appearance upon examination (112).

Aggressive drug regimes do not appear to be indicated in immunocompetent individuals since spontaneous recovery is the usual outcome of clinical infections (233). Fluid replacement therapy and possible antibiotic therapy to reduce the possibility of secondary bacterial infections are important in both immunocompetent and immunocompromized humans and animals. A high calorie, low residue enteral feeding regime is recommended for AIDS patients but these patients continue to lose weight even on total parenteral nutrition (348).

Efforts to find disinfectant compounds which will inactivate oocysts of Cryptosporidium have yielded only a few compounds which are effective. Compounds such as 2.5% sodium dichloroisocyanurate, 5% formaldehyde, 3% chloramine B, 0.33% iodophore, 5% cresylic acid and commercial compounds such as 3% Dikenit, 3% Jodonol A, 0.2% Lastanox Q, 0.2% Mycolastanox, Tegoder and Formula-H were shown to be ineffective in inactivating oocysts by demonstration of continued viability
after 24 hour exposure to the compounds or continued infectivity to mice after treatment with the compound (19, 242, 244). Only 10% formal saline or 5% ammonia have been shown to completely destroy oocyst infectivity (57). It is believed transmission of Cryptosporidium can occur by contaminated endoscopes which are disinfected only with glutaraldehyde as glutaraldehyde will not kill the oocysts (348).

Other treatment regimes shown to be effective include 18-24 hour exposure to 10% formaldehyde, 5% ammonium hydroxide solution or 12% ethylene oxide (44). Warming inocula containing oocysts from calf feces, cecal contents or ileal scraping under moist heat from 9°C to 55°C over a period of 15 to 20 minutes or holding inocula at 45°C for 5 to 20 minutes neutralized the infectivity of the oocysts for mice (11). These experiments were performed to mimic conditions of pasteurization of raw milk to determine if these conditions are effective at eliminating the infectivity of Cryptosporidium oocysts since contaminated milk has been incriminated as a possible source of infection in several outbreaks (62, 359). Steam heat has been proposed by other researchers as an effective way of decontaminating animal pens (44, 119).

Housing methods seem to have little effect on reducing Cryptosporidium infections as one study showed scouring in all newborn calves regardless of whether they were housed with their dams for 10 to 16 days after birth, on litter in a special area of the farm or in individual stalls (247).

As with other infectious agents that are shed in the feces
and transmitted by the fecal-oral route the best prospect for controlling Cryptosporidium infection is by hygienic measures and the proper disposal of fecal material (118).

**Diagnosis of the Infection**

*Cryptosporidium* can be diagnosed in humans or animals by either direct or indirect methods. The direct methods involve demonstration of various stages of the life cycle of the organism by histological sections of biopsy material or demonstration of the oocyst stage from fecal material. Indirect methods involve diagnosis by symptomology, inoculation of new animal hosts, and immunoserology.

Diagnosis of the infection by indirect methods has proved to be difficult and unreliable at times. Clinical signs of infection are relatively non-specific therefore upon clinical presentation it would be difficult to differentiate between the various etiologic agents causing similar clinical signs. Inoculation of fecal material containing infectious stages of the parasite into other animals often produces inconsistent results due to variation in host specificity and differences in pathogenicity of various isolates (233).

Antibodies to intestinal stages of Cryptosporidium were observed by an indirect immunofluorescent test in the serum of 12 immunocompetent individuals, 8 of 15 AIDS patients, none of 2 individuals with hypogammaglobulinaemia, 2 of 10 individuals with no known exposure to the organism and 3 of 4 individuals who were exposed but in which clinical signs were not exhibited (58). Indirect immunofluorescence was also used by Tzipori and Campbell
(326) to detect antibodies to Cryptosporidium in 18 of 21 randomly selected blood donors, 16 of 20 dogs, 20 of 23 cats, all of 25 cattle, all of 23 sheep, 41 of 43 pigs, all of 12 deer, 20 of 22 horses, 22 of 25 chickens and in none of 11 specific pathogen free mice. No correlations were made in this study between seropositivity and clinical signs of infection. Indirect immunofluorescence was used to demonstrate person-to-person transmission of Cryptosporidium among hospital workers (163). Instead of using mouse intestine as in the previous studies oocysts were used for the antigen which results in a less sensitive test.

An ELISA test has been developed using oocysts of Cryptosporidium as the antigen for detection of serum IgG or IgM (338). For IgG 13 of 15 patients with cryptosporidiosis and 26 of 26 patients with cryptosporidiosis and AIDS were positive. Fifty-seven of sixty individuals with no clinical signs of infection were negative. The three positive individuals in this group had been potentially exposed. Patients without AIDS showed an early rise and fall of IgM and later elevation of IgG; some patients with AIDS produced IgM and all produced IgG.

Pathognomonic lesions as a result of infection can not be observed grossly and it is therefore necessary to examine the affected organs microscopically to demonstrate the various life cycle stages. Microscopic examinations must be performed at high magnification because of the small size of the endogenous stages. Biopsy and autopsy material being prepared for light and electron microscopy must be fixed very shortly after death of the host due
to sloughing and autolysis of the microvilli.

The diagnostic procedures producing the most consistent results are the various staining and concentration techniques used to demonstrate oocysts in fecal material. Several staining techniques have been proposed. Direct staining of the oocysts has been described using Giemsa (127, 354) or Safranin (29). Yeast cells can be present in fecal samples and are often difficult to differentiate using these direct stains. *Cryptosporidium* oocysts have been shown to be acid fast and retain the stain upon decolorization with an acid-alcohol solution whereas yeast cells do not retain stain upon decolorization. Modified Ziehl-Neelsen (61, 107, 132), rapid dimethyl sulphoxide (53) and modified acid fast (195) staining procedures have all been described for use to differentiate *Cryptosporidium* oocysts from yeast. The techniques all use carbol-fuchsin as the primary stain at various concentrations and staining times and a variety of counterstains are used.

Examination of fecal smears by fluorescent microscopy have been developed by Casemore and co-workers (61) and Nichols and Thom (228) using an auramine stain. Negative staining techniques using nigrosin (254), periodic acid-schiff (141) and carbol-fuchsin (126) have also been devised. Cryptosporidial oocysts have been detected using negative staining techniques followed by examination under the electron microscope as what is used to diagnose enteropathogenic viruses (30). Concentration techniques using various high density salt and sugar solutions (82, 127, 354) are widely used in veterinary diagnostic
laboratories and are considered more sensitive than staining of fecal smears because the oocysts are concentrated. The oocysts are differentiated from yeast in these flotation techniques by examination under phase contrast microscopy. The oocysts are highly refractile under these conditions with a halo appearing around the periphery of the oocyst.

Monoclonal antibodies have recently been developed which bind to the sporozoite surface and the oocyst wall of Cryptosporidium (23). A monoclonal antibody is being employed to detect Cryptosporidium infections by demonstrating oocysts in air-dried fecal smears. The development of monoclonal antibodies to various stages of Cryptosporidium could prove to be a useful diagnostic tool in the future.
Dairy Herd Improvement Association records were obtained for Nebraska and herds were selected from this roster. The selected herds were contacted by letter and given information about the organism Cryptosporidium, the study proposed, and soliciting their cooperation.

Dairy herd owners agreeing to participate in the study were sent a collecting kit containing the following materials:
- a set of instructions explaining to the owner that fecal samples should be collected from the next 5 calves born on their farm when they reach the age of 5 and 12 days.
- 10 6 ounce Whirl-Paks (Cole-Parmer Instrument Company) or 10 plastic centrifuge tubes which the fecal samples were placed into after collection.
- 10 addressed and postage paid mailing cannisters to be used to send the fecal samples to the Department of Veterinary Science at the University of Nebraska-Lincoln East Campus for laboratory analysis.
- plastic gloves and tongue depressors for use in collecting the fecal samples.
- a questionnaire requesting information about the management and operation of the participating dairy herd (Appendix 1).

Fecal samples received at the parasitology laboratory were placed in water with a small portion of the sample placed in 10% buffered formalin for storage purposes. The formalin fixed samples were used for the laboratory analysis.

The presence of Cryptosporidium oocysts in the fecal samples
were demonstrated using the sugar flotation method as described by Sheather (285). The flotation solution used to prepare the sample for microscopic examination had a specific gravity of 1.27.

Approximately 1-2 milliliters of the formalin fixed fecal sample was placed in a 15 milliliter centrifuge tube. The sugar solution was then added to within approximately 1 milliliter of the rim of the test tube. The tube was placed in the centrifuge holder and a meniscus of the sugar solution was formed over the rim using a pipette. A cover-slip was placed on the meniscus and the tubes were centrifuged at 1500 rpms for 5 minutes using an IEC model K centrifuge (Damon/IEC Division; 300 Second Avenue; Needham Heights, Massachusetts 02194). Fecal preparations were examined under oil immersion with phase contrast as described by Current (78) using a Leitz Wetzlar Dialux 20 microscope (Ernst Leitz Wetzlar GMBH; D-6330 Wetzlar; West Germany). Figure 1 demonstrates the appearance of the oocysts.

All owners were informed of the results of the laboratory analysis performed on the fecal samples. Permission slips were sent to owners whose calves were positive for Cryptosporidium oocysts requesting that these samples be submitted to the Veterinary Diagnostic Center, University of Nebraska-Lincoln East Campus to test for the presence of bacterial or viral pathogens. A second questionnaire (Appendix 2) was sent to the owners of Cryptosporidium-infected calves attempting to obtain information about the clinical course of the infection, if the owners had attempted to treat signs of scouring and if any calves born subsequent to the calves from which fecal samples were collected.
showed signs of scouring.

The portion of the fecal sample stored in water was submitted to the Veterinary Diagnostic Center from positive calves whose owners signed and returned the permission slip. Examination for bacterial pathogens was conducted by plating swabs from the fecal samples onto blood agar (178) incubated anaerobically to test for beta-hemolysis, onto tergitol 7 agar (60) to test for lactose fermentation and into tetrathionate enrichment broth (178) followed by plating onto MacConkey's agar (178) and BG-Sulfa agar (178) after incubation in the tetrathionate enrichment broth for 24 hours to test for lactose fermentation. Gram stains were also performed on smears from each sample (292). Pilus typing for Escherichia coli was performed using anti-sera to K88, K99, and 987P pili.

A negative staining technique was used to demonstrate viral particles in the feces as described by Flewett (101). A Philips 201 transmission electron microscope operated at an accelerating voltage of 60 Kv was used to examine the grids on which the fecal samples were stained.

Data on the incidence of Cryptosporidium as related to age, sex, breed, month in which the sample was collected and association with bacterial and viral organisms were compiled in tabular form. A 2 X 2 contingency table was constructed and a chi-square analysis performed to determine if scouring in calves is dependent on infection with Cryptosporidium. Information from the 2 questionnaires were also compiled and presented in tabular form.
Results

Three hundred eight owners were contacted by letter asking if they would be willing to participate in the study. Ninety-eight owners returned the postcard through the mail indicating they would participate. All 98 were sent collecting kits and of these 71 herd owners eventually sent at least 1 fecal sample from calves born on their farm for laboratory analysis.

A total of 620 samples were received from 334 calves. Two samples were received from 286 calves and only 1 sample was received from 48 calves. Although 90% of the fecal samples were collected when the calves were 5 and 12 days old as requested in the instructions sent to the owners the remaining 10% of the samples were collected at ages other than 5 or 12 ranging from 2 to 22 days of age.

Eighteen of the 71 herds (25%) of the herds had at least 1 calf shedding Cryptosporidium oocysts in the feces. Fifty-two of the 334 calves (15%) and 55 of the 620 samples (8%) were positive for Cryptosporidium. Three of the infected calves shed oocysts at both time intervals at which fecal samples were collected.

Table 3 represents the breakdown of results by age, breed and sex. Oocysts were demonstrated in the feces much more frequently at the 12 day sampling time than at the 5 day sample time. Positive samples were also detected in one fecal sample each at 7 and 13 days of age. Ninety-one percent of the samples were collected from Holstein calves with the remaining 9% representing various other breeds and crosses of dairy cattle. Forty-six of the 52 positive calves were Holstein, 5 were Brown
Swiss and 1 calf was a Holstein-Simmental cross. Sixty-five percent of the calves sampled were from heifers of which 16% were infected.

A 2 X 2 contingency table was constructed as shown in Table 4 to determine if scouring was independent of infection with Cryptosporidium. Scouring in infected herds was determined by the second questionnaire sent to owners of infected herds. Scouring in uninfected herds was determined by the appearance of the feces upon receipt at the laboratory and the previous history of scouring in the herd. Calculation of a chi square with 1 degree of freedom at an alpha level of 5% indicated scouring to be associated with infection with Cryptosporidium. The average herd size from which no positive samples were obtained was 79 animals. Herds with at least one calf detected as shedding Cryptosporidium oocysts had an average size of 105 animals.

Cryptosporidium can occur in association with a variety of enteropathogens such as rotavirus, coronavirus, Clostridium perfringens, Salmonella spp., and Escherichia coli, however, the organism occurred most frequently (41%) as the only detected potential pathogen as detected in Table 5. Seventy-five percent of the calves infected with Cryptosporidium alone exhibited signs of scouring as reported by the owners with 19 of 20 calves surviving the bout and 1 calf dying. Cryptosporidium occurs most frequently in association with E. coli (20%) followed by C. perfringens plus Salmonella spp. (10%); rotavirus, C. perfringens plus E. coli, C. perfringens plus coronavirus, Salmonella spp.
plus *C. perfringens* plus rotavirus (all 4%); and coronavirus or
*C. perfringens* plus rotavirus (2%). Pilius typing for all
isolates of *E. coli* were negative for K88, K99 and 987P pilus
types. Seventy-one percent of all animals infected with
*Cryptosporidium* either alone or in association with other
microorganisms showed signs of scouring with 47 or 49 animals
surviving the bout and 2 calves dying.

The distribution of the herd samples as well the
distribution of all positive herds is shown in Figure 2. The
majority of dairy herds are concentrated in the northeast and
southeast parts of the state with a few scattered throughout the
western part of the state.

Fecal samples were collected from calves starting in March
of 1985 and continued through April of 1986. Over half of the
samples were received in the spring months of April and May and
the fall month of September. These three months also represent
the periods when the majority of positive samples were received.

Fifty-nine of the 71 participating herd owners returned the
questionnaire requesting information on the operation and
management of their particular herd. Forty-two of the
questionnaires were received from uninfected herds and 17 were
from infected herds. Information compiled from the questionnaire
is summarized in Tables 7 and 8. The most common feeding program
in infected and uninfected herds was colostrum followed by whole
milk as shown in Table 7. Feeding programs by all herd owners
except 1 involved colostrum followed by various combinations of
whole milk, milk replacer, fermented colostrum or antibiotics in
As shown in Table 8 the majority of herd owners do not use antibiotics or vitamins in the milk. All except 1 owner indicated the animals being sampled were being treated for other diseases.

Seventy-five percent of all owners indicated having problems with scouring in previous years—some indicating only mild infrequent problems but 15 of the 42 owners from uninfected herds and 13 of 17 owners from infected herds indicated experiencing death loss. Usually a diagnosis of the problem was not made but in those cases in which a diagnosis was made *E. coli* was most frequently reported followed by coccidia, reovirus, *Salmonella* spp. and coronavirus.

The type of housing in which the calves were kept varied among owners. The majority of owners from *Cryptosporidium*-infected herds indicated their calves were housed separately in hutches on well-drained ground until the animals were 3 to 4 weeks of age at which time they were placed in groups. Two of these owners indicated they placed their calves in groups from birth. Owners from uninfected herds indicated keeping their calves separately in similar units on well-drained ground or slatted floors. Four of these owners indicated housing their calves in groups.

Owners from *Cryptosporidium*-infected herds indicated having a variety of cleaning schedules for housing units in which calves were kept. Several owners responded by indicating pens were cleaned twice a day, others once a year and still others
indicated cleaning the cages after every calf. The majority of these owners cleaned hutches after every calf. Owners from uninfected herds indicated a similar cleaning schedule.

Fourteen of the eighteen owners from Cryptosporidium-infected herds returned the questionnaire asking about information in an attempt to characterize the infection with Cryptosporidium. All owners except one indicated they used various commercial treatments such as scour pills and boluses to treat the infection. The cause of scouring was never diagnosed in any cases where scouring did indeed occur. Eleven owners indicated their calves survived the bout of scouring. The remaining two owners had scouring calves die during or shortly after the time interval in which the fecal samples were collected. Twelve of the owners indicated scouring problems continued in their herds in calves born subsequent to the calves from which fecal samples were collected for this study.
Discussion

Cryptosporidium has emerged as a potential pathogen of cattle only within the past decade having first been reported in 1971. The organism most likely caused infections in cattle before that time but simply went unnoticed because of its small size and the specialized techniques necessary to diagnose the infection.

With the advent of the Acquired Immune Deficiency Syndrome in the early 1980s and the finding that cryptosporidial infections produced life-threatening diarrhea in immunocompromised individuals, interest in this organism intensified and diagnostic procedures for identification of the organism improved. Attention also became focused on this organism when it was found that Cryptosporidium-infected calves could be the source of human acquired infections.

When Cryptosporidium was first described in humans in the late 1970's it was considered to be a rare opportunistic infection occurring only in young children or patients whose immune systems had been suppressed as a result of immunosuppressive drugs or other abnormalities. The finding that Cryptosporidium infections were associated with AIDS patients produced large amounts of literature describing the clinical course of infection in these patients as well as the pathologic effects of infection and its relation to the immune status of the patient. With the improved diagnostic procedures brought about by a necessity for identification of the infection routine screening of fecal samples for detection of Cryptosporidium
became incorporated into diagnostic procedures for identification of enteropathogens in the general population. This led to the survey work in which the organism was found to occur in immunocompetent individuals in the general population. This work in turn lead to speculation on the epidemiology of the infection and expanded its proposed mode of transmission from direct animal-to-man contact to person-to-person contact.

Survey and experimental work on bovine cryptosporidiosis has also intensified within the last decade. Many veterinary diagnostic centers routinely exam fecal samples in young calves for the presence of *Cryptosporidium* and diagnosticians consider the organism in the differential diagnosis of the neonatal scouring syndrome.

As shown in Table 1, *Cryptosporidium* infections have been reported in the literature to occur in 13 states. The organism is most likely diagnosed in veterinary clinics throughout the country but simply does not get reported in the literature. Bergeland (36) reported *Cryptosporidium* in 4 herds in Nebraska. Subsequent to that there have been no reports of *Cryptosporidium* infections in Nebraska calves. The present study represents the first systematic survey of the infection in dairy calves in Nebraska.

The present study is modeled closely after similar survey studies conducted by Anderson and Hall in Idaho (15) and Leek and Fayer in Maryland (174). Both of these studies reported higher prevalence rates for both the number of infected herds and the total number of positive samples with 36 of 136 calves from 9 of
12 farms positive in Maryland and 110 of 284 calves from 41 of 73 herds positive in Idaho. This compares with 52 of 334 calves from 18 of 71 herds positive in this study. Surveys conducted in other countries have shown a prevalence of 26% in Canada (281), 37.6% in the German Federal Republic (97), 27% in Hungary (222), 28% in Switzerland (222), and 40% in Czechoslovakia (239).

Factors such as management, stress brought on by environment and raising and other unknown factors make comparisons between these different rates of incidence difficult for the various studies. However, the difference in the infection rates is probably not significant nor is it important. The important factor to extract from this study and the other studies mentioned is that Cryptosporidium has been reported from these different areas, infection appears to be widespread and a correlation appears to exist between infection and diarrhea.

There does not appear to be any differential susceptibility to infection due to factors such as sex and breed, however, the data collected in this study would not show definitive trends in either of these factors since the majority of samples were from holstein females.

The purpose of analyzing samples from the calves when they were 5 and 12 days of age was to make some tentative statements about the time after birth at which the calves became infected. Work by Anderson (7) demonstrated that the optimum time for detection of oocysts during the first week of life was 5 days of age and the second week of life at day 12. The purpose of this study was simply to determine the incidence of Cryptosporidium in
the state of Nebraska, therefore, these two days were chosen as collection times in an effort to increase chances of detection of oocysts in feces of infected calves. Further studies could be conducted in which daily sampling could be performed in order to make a more precise determination of shedding patterns of the oocysts in relation to the age of the calf.

In collecting information from the questionnaire on feeding programs, the use of antibiotics in milk and housing and cleaning schedules, it was hoped that patterns would arise that would allow statements to be made about increased susceptibility based on any of these factors. All owners except 1 fed their calves colostrum followed by various combinations of whole milk or milk replacer. Therefore, statements about susceptibility based on feeding or non-feeding of colostrum cannot be made. The majority of owners did not use antibiotics in the milk. Antibiotics have not been shown to have a therapeutic or preventive effect on the clinical signs produced by the organism, therefore, differences in susceptibility would not have been noted based on the presence or absence of these compounds in the milk. A history of at least mild scouring in calves in previous years seemed to be common in all participating herds. Considering the variety of factors which can lead to scouring in young calves such as infection with various enteropathogens, stress brought on by the environment or handling and diet, these kinds of data should not be surprising.

As with any potential pathogen a seasonal variation would be expected to exist with Cryptosporidium in which infection rates would increase during months in which climatic conditions would
place an additional stress on the animals. This trend could be noted in these data as more positive samples were obtained in the spring and the fall. However, these results could have occurred due to uneven distribution of times in which the samples were received since the majority of the samples were received during those three months. Looking at the percentage of total samples received during any particular month which were positive, 50% of the samples received in December were positive. However, this is most likely due to low numbers since only 6 samples were received during that month. This study gives a hint of seasonal variation in Cryptosporidium infection but a study conducted in which an equal number of calves would be examined monthly for a year to determine the presence or absence of Cryptosporidium would provide a stronger basis for the conclusion that seasonal variation exists in cryptosporidial infections.

Anderson (15) noted in data collected from dairy calves in Idaho that the average size of positive herds was larger than that of negative herds however the difference was not statistically significant. In the present study the average herd size for infected herds was larger than the average herd size for uninfected herds. This difference was shown to be statistically significant. Intensive rearing in which large numbers of animals are confined to a smaller area could be a contributing factor to this difference in infection rates.

Due to the wide variation in housing and cleaning schedules reported by the owners it was not possible to correlate any particular management practice with increased susceptibility to
infection. It would be expected that calves housed in groups on poorly drained ground in which the areas were not cleaned often would increase susceptibility to infection by Cryptosporidium as well as other enteropathogenic organisms. However, these types of practices were not reported by owners from infected herds. In fact one owner whose calves were infected with Cryptosporidium reported cleaning hutches twice per day. The majority of owners from Cryptosporidium-infected and negative herds reported housing calves separately in hutches and cleaning after each calf was moved out of the hutch. It is doubtful that a dairy herd owner would report unhygienic conditions. Therefore on-site visits to all infected herds would have allowed for observations on how extrinsic factors such as housing and the extent of cleaning affect susceptibility to cryptosporidial infections. Time and distance factors precluded such on-site observations.

It appears that infection with Cryptosporidium can lead to clinical signs of loose, watery stools. However, other factors which could result in scouring were not investigated as possible causes such as infection with bovine virus diarrhea virus, mucosal disease, Johne's disease, digestive upset, nutritional problems, overeating, toxic mastitis or infection with Ostertagia. Results of this study appear to agree with the clinical picture postulated by Tzipori (316) in which field outbreaks of cryptosporidial infections are characterized by high morbidity and low mortality.

It was demonstrated in this study that Cryptosporidium does occur in the state of Nebraska in dairy calves. Further studies
should be conducted in the future to determine the pathogenicity of the Nebraska strain and also the effect of different management practices on the incidence of Cryptosporidium.
Conclusion

A total of 620 fecal samples from 334 dairy calves from 71 herds in Nebraska were examined for cryptosporidial oocysts using the Sheather's sugar flotation technique. Fifty-five of the 620 fecal samples from 52 of the 334 calves were positive for Cryptosporidium. The positive calves were from 18 of 71 herds. Forty-nine positive fecal samples were examined for the following enteropathogens: Enterotoxigenic Escherichia coli, Clostridium perfringens, rotavirus, coronavirus, and Salmonella spp. Twenty of the calves were infected with Cryptosporidium alone, 15 of which scoured and 1 of which eventually died. One or more of the aforementioned enteropathogens were observed in the remaining 29 samples. Results of this study suggest an association between infection with Cryptosporidium and scouring.
FIGURES
Figure 1 - Appearance of *Cryptosporidium* oocysts from a fecal sample using the Sheather's sugar flotation technique. Oocysts are under oil immersion at a magnification of 100X.
FIGURE 1
Figure 2 - Distribution of the participating herds. Herds in which at least one calf was shedding oocysts in the feces are represented by a red dot. Negative herds are represented by a blue dot.
TABLES
<table>
<thead>
<tr>
<th>Country</th>
<th>Incidence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Europe</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Belgium</td>
<td>2 calves +</td>
<td>21</td>
</tr>
<tr>
<td>Bulgaria</td>
<td>2 farms +</td>
<td>160</td>
</tr>
<tr>
<td>Czechoslovakia</td>
<td>2 calves +</td>
<td>238</td>
</tr>
<tr>
<td>Denmark</td>
<td>16.3% +</td>
<td>132</td>
</tr>
<tr>
<td>France</td>
<td>2 calves +</td>
<td>21</td>
</tr>
<tr>
<td>German Democratic Republic</td>
<td>51% +</td>
<td>157</td>
</tr>
<tr>
<td>German Federal Republic</td>
<td>44% +</td>
<td>96</td>
</tr>
<tr>
<td>Hungary</td>
<td>27% +</td>
<td>226</td>
</tr>
<tr>
<td>Netherlands</td>
<td>19-85% +</td>
<td>175</td>
</tr>
<tr>
<td>Northern Ireland</td>
<td>1 calf +</td>
<td>248</td>
</tr>
<tr>
<td>Norway</td>
<td>12 calves from 6 of 9 farms</td>
<td>169</td>
</tr>
<tr>
<td>Poland</td>
<td>48 calves +</td>
<td>346</td>
</tr>
<tr>
<td>Poland</td>
<td>8 herds +</td>
<td>83</td>
</tr>
<tr>
<td>Romania</td>
<td>4 calves +</td>
<td>291</td>
</tr>
<tr>
<td>Scotland</td>
<td>1 herd +</td>
<td>313</td>
</tr>
<tr>
<td>Sweden</td>
<td>14.5% +</td>
<td>298</td>
</tr>
<tr>
<td>Switzerland</td>
<td>87.5% +</td>
<td>245</td>
</tr>
<tr>
<td>USSR</td>
<td>5 of 9 outbreaks +</td>
<td>151</td>
</tr>
<tr>
<td><strong>Australia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bangladesh</td>
<td>14% with diarrhea +</td>
<td>266</td>
</tr>
<tr>
<td>Israel</td>
<td>3 calves +</td>
<td>232</td>
</tr>
<tr>
<td>Turkey</td>
<td>56 calves +</td>
<td>55</td>
</tr>
<tr>
<td>South Africa</td>
<td>1 calf +</td>
<td>143</td>
</tr>
<tr>
<td><strong>Canada</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Argentina</td>
<td>2 calves +</td>
<td>197</td>
</tr>
<tr>
<td>Cuba</td>
<td>6/13 calves +</td>
<td>113</td>
</tr>
<tr>
<td><strong>United States</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Connecticut</td>
<td>1 calf +</td>
<td>212</td>
</tr>
<tr>
<td>Idaho</td>
<td>64% + from 41 of 73 herds</td>
<td>15</td>
</tr>
<tr>
<td>Iowa</td>
<td>8/23 calves +</td>
<td>257</td>
</tr>
<tr>
<td>Maryland</td>
<td>26% + from 9 farms</td>
<td>174</td>
</tr>
<tr>
<td>Minnesota</td>
<td>14 herds +</td>
<td>36</td>
</tr>
<tr>
<td>Nebraska</td>
<td>4 herds +</td>
<td>36</td>
</tr>
<tr>
<td>North Dakota</td>
<td>22/257 calves with diarrhea</td>
<td>34</td>
</tr>
<tr>
<td>Ohio</td>
<td>20 herds +</td>
<td>121</td>
</tr>
<tr>
<td>Oklahoma</td>
<td>1 calf +</td>
<td>237</td>
</tr>
<tr>
<td>Oregon</td>
<td>1 calf +</td>
<td>283</td>
</tr>
<tr>
<td>South Dakota</td>
<td>16 herds +</td>
<td>36</td>
</tr>
<tr>
<td>Tennessee</td>
<td>1 calf +</td>
<td>261</td>
</tr>
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</table>
### Table 2A - Drugs used to treat human Cryptosporidium infections.

<table>
<thead>
<tr>
<th>Weinstein(350)</th>
<th>Sloper(289)</th>
<th>Stemmermann(299)</th>
<th>CDC(67)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfisoxazole</td>
<td>Mepacrine</td>
<td>Metronidazole</td>
<td>Trimethoprim/</td>
</tr>
<tr>
<td>Pyrimethamine</td>
<td>Colistin</td>
<td>Sulfamethoxazole</td>
<td>sulfamethoxazo</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>Oxytetracycline</td>
<td>Trimethoprim</td>
<td>le</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>Metronidazole</td>
<td>Pyrimethamine</td>
<td>Metronidazole</td>
</tr>
<tr>
<td>Primaquine</td>
<td>Piperazine</td>
<td>Sulfadiazine</td>
<td>Pyrimethamine/</td>
</tr>
<tr>
<td>Loperamide</td>
<td>Thiobendazole</td>
<td>Levamisol</td>
<td>sulfa</td>
</tr>
<tr>
<td>Pentamidine</td>
<td>Erythromycin</td>
<td>Amphotericin B</td>
<td>Tetracycline</td>
</tr>
<tr>
<td>Sulfathalidine</td>
<td>Penicillin</td>
<td>Cholestyramine</td>
<td>Quinacrine</td>
</tr>
<tr>
<td></td>
<td>Ampicillin</td>
<td></td>
<td>Diloxanide</td>
</tr>
<tr>
<td></td>
<td>Septin</td>
<td></td>
<td>furoate</td>
</tr>
<tr>
<td></td>
<td>Gentamicin</td>
<td></td>
<td>Ketoconazole</td>
</tr>
<tr>
<td></td>
<td>Cloxacillin</td>
<td></td>
<td>Petamidine</td>
</tr>
<tr>
<td></td>
<td>Carbenicillin</td>
<td></td>
<td>Bovine transfer</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>factor</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Amphotericine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Iodoquinol</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Paromomycin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Spiramycin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Clindamycin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Gamma-Globulin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chloroquine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Primaquine</td>
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<td></td>
<td></td>
<td></td>
<td>Amprolium</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Salinomycin</td>
</tr>
</tbody>
</table>

**Veldhuyzen Van Zanten (341)**

- Amprolium
Table 2B - Compounds used experimentally to treat *Cryptosporidium* infections in:

**Calves (Moon, 219)**

- Amprolium
- Sulfadimidine
- Trimethoprim-
  - sulfadiazine
- Dimetridazole
- Metronidazole
- Ipronidazole
- Quinacrine
- Monensin
- Laslocid

**Mice (Tzipori, 327)**

- Ethopabate
- Nicarbazin
- Sulfadinoxaline
- Furalfadone
- Enterolyte-N
- Sulfamethazine
- Trinamide
- Amprol
- Phenamidine
- Zoquin
- Halofuginone
- Salinomycin
- Monensin
- Emtryl
- Apprinocid
- Amprolium
Table 3 - The incidence of cryptosporidial infections separated by number of samples received from calves of that indicated age, breed or sex.

<table>
<thead>
<tr>
<th>Age of calf (days)</th>
<th>Total</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>304</td>
<td>4</td>
<td>300</td>
</tr>
<tr>
<td>6</td>
<td>13</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>11</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>12</td>
<td>259</td>
<td>49</td>
<td>210</td>
</tr>
<tr>
<td>13</td>
<td>12</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>14</td>
<td>7</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>15</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>16</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>19</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>22</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total # samples</td>
<td>620</td>
<td>55</td>
<td>565</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Breed of calf</th>
<th>Total</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unidentified</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Breed Brown Swiss</td>
<td>8</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Breed Short Horn</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Breed Ayshire</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Breed Angus-Holstein</td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Breed Guernsey</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Breed Angus</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Breed Holstein-Longhorn-Shorthorn</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Breed Holstein-Simmental</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Breed Holstein</td>
<td>305</td>
<td>46</td>
<td>259</td>
</tr>
<tr>
<td>Total # calves</td>
<td>334</td>
<td>52</td>
<td>282</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sex of calf</th>
<th>Total</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>116</td>
<td>17</td>
<td>99</td>
</tr>
<tr>
<td>Female</td>
<td>218</td>
<td>35</td>
<td>183</td>
</tr>
<tr>
<td>Total # calves</td>
<td>334</td>
<td>52</td>
<td>282</td>
</tr>
</tbody>
</table>


Table 4 - 2 x 2 Contingency table used to determine if scouring is independent of infection with Cryptosporidium.

<table>
<thead>
<tr>
<th>Infected?</th>
<th>Yes</th>
<th>No</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scouring?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>36</td>
<td>29</td>
<td>65</td>
</tr>
<tr>
<td>No</td>
<td>16</td>
<td>253</td>
<td>269</td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
<td>282</td>
<td>334</td>
</tr>
</tbody>
</table>
Table 5 - Results of tests performed on fecal samples positive for Cryptosporidium to determine the presence of bacterial and viral pathogens. Total column represents the total number of fecal samples for which that particular combination of organisms were isolated. Scouring column represents the number out of that total that were and were not scouring. The outcome column represents how many of the calves from which the fecal samples were collected survived during the collection period and after and how many of the calves died during or shortly after the collection period.

<table>
<thead>
<tr>
<th>Organisms isolated</th>
<th>Total</th>
<th>Scouring?</th>
<th>Outcome?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>C</td>
<td>20</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>C + CP</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>C + EC</td>
<td>10</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>C + RV</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>C + CV</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>C + CP + EC</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>C + CP + CV</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>C + CP + S</td>
<td>5</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>C + CP + RV</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>C + CP + S + RV</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

Key:

C = Cryptosporidium
CP = Clostridium perfringens
EC = Escherichia coli (pilus typing was negative for all isolates)
S = Salmonella
CV = Coronavirus
RV = Rotavirus
Table 6 - Number of fecal samples collected in each of the months March 1985 through April 1986. The percentage column represents the percentage of samples received that month which were positive for Cryptosporidium.

<table>
<thead>
<tr>
<th>Month and year</th>
<th>Total # samples received that month</th>
<th>Number infected</th>
<th>Percentage</th>
<th>Number uninfected</th>
</tr>
</thead>
<tbody>
<tr>
<td>March 1985</td>
<td>51</td>
<td>3</td>
<td>5</td>
<td>48</td>
</tr>
<tr>
<td>April 1985</td>
<td>107</td>
<td>13</td>
<td>12</td>
<td>94</td>
</tr>
<tr>
<td>May 1985</td>
<td>152</td>
<td>13</td>
<td>8</td>
<td>139</td>
</tr>
<tr>
<td>June 1985</td>
<td>77</td>
<td>4</td>
<td>5</td>
<td>73</td>
</tr>
<tr>
<td>July 1985</td>
<td>15</td>
<td>1</td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>August 1985</td>
<td>35</td>
<td>1</td>
<td>3</td>
<td>34</td>
</tr>
<tr>
<td>September 1985</td>
<td>104</td>
<td>14</td>
<td>13</td>
<td>90</td>
</tr>
<tr>
<td>October 1985</td>
<td>53</td>
<td>3</td>
<td>6</td>
<td>50</td>
</tr>
<tr>
<td>November 1985</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>December 1985</td>
<td>6</td>
<td>3</td>
<td>50</td>
<td>3</td>
</tr>
<tr>
<td>January 1986</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>February 1986</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>April 1986</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>620</td>
<td>55</td>
<td></td>
<td>565</td>
</tr>
</tbody>
</table>
**Table 7 - Tabulation of the different feeding programs used by the herd owners as reported on the questionnaire sent to all owners.**

<table>
<thead>
<tr>
<th>Feed Program</th>
<th>Total</th>
<th>Infected</th>
<th>Uninfected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colostrum - Whole milk</td>
<td>31</td>
<td>9</td>
<td>22</td>
</tr>
<tr>
<td>Colostrum - Whole milk - Milk replacer</td>
<td>12</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Colostrum - Milk replacer</td>
<td>11</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Whole milk only</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Colostrum only</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Colostrum - Antibiotic milk</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Colostrum - Whole milk/Fermented colostrum - Milk replacer</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Colostrum - Fermented colostrum</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>59</td>
<td>17</td>
<td>42</td>
</tr>
</tbody>
</table>
Table 8 - Summary of data from questionnaire sent to all herd owners on whether antibiotics were placed in the milk, if animals were being treated for any diseases at the time the samples were collected, if the owners had any previous history of scouring, if any calves were lost to the scouring and if the cause of the scouring was diagnosed.

<table>
<thead>
<tr>
<th></th>
<th>Uninfected Yes</th>
<th>Uninfected No</th>
<th>Infected Yes</th>
<th>Infected No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotics or Vitamins placed in milk?</td>
<td>4</td>
<td>37</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>Treatment for any diseases?</td>
<td>1</td>
<td>40</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>History of scouring?</td>
<td>30</td>
<td>12</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td>Death loss</td>
<td>15</td>
<td>27</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td>Diagnosis?</td>
<td>10</td>
<td>8</td>
<td>3</td>
<td>8</td>
</tr>
</tbody>
</table>
APPENDIX

The following two pages contain the two questionnaires used in this study. Questionnaire #1 was sent to all owners and asks the owner to relate information on the management practices and operation of the herd. Questionnaire #2 was sent to all owners of calves shown to be positive for Cryptosporidium. The questions were asked in an attempt to gain an understanding of the clinical course of infection with Cryptosporidium.
QUESTIONNAIRE #1

Owner Name:

1. Feeding of Newborn calves: (i.e. colostrum followed by whole milk, milk replacer, milk replacer alone?)

2. Are antibiotics or vitamins placed in the milk? Were the calves being treated for any diseases at the time the fecal samples were collected?

3. How are your newborn calves housed? (i.e. is each calf housed separately or in groups, in hutches or pens, on concrete floors or on well-drained ground, etc.?)

4. How often is bedding and manure removed from these housing units?

5. Have you had problems with scouring in young calves in previous years? Did you lose any of these calves to scouring? Was the cause of the scouring ever diagnosed?

6. What types of vaccinations do you give your young calves?

Please send the completed questionnaire to us with one of the fecal samples.
Questionaire #2

Owner name:

1. Did you consider any of these calves to be scouring at the time these fecal samples were taken?

2. If so did you treat them for the problem? What did you use for treatment and did it help?

3. Was the cause of the scouring ever diagnosed?

4. Did the calves survive the scouring? If they did how are they doing now?

5. Have any other calves born since this time scoured?
References


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