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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 severly 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-etiologic. 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,

274).

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 Cryptopsporidium 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 parasitophorus 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 parasitophorus 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 CO_2 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 20^0C but was greatest at 37^0C. 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% CO_2:95% 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

contents.

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 Cryptopsporidium

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

(4).

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 E. coli and/or rotavirus by 4 days after birth (334).

Newborn lambs experimentally infected with 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 Cryptosporidium, euthanatized at specified time intervals and examined for the presence of 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).

Equine infections with Cryptosporidium

Clinical signs of 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-lored 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

(202). 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 artifically 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 prophylatic 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 artifically 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 susceptability 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 possiblity 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 monkies 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 persistant 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 consistant 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 susceptability between colostrum fed and colstrum 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, 5×10^6 versus 30×10^6 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 prevalant 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), malnutrion 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 malnutrion 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 carnii (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 electon 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 refect 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 susceptability 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 susceptable hosts.

The evidence that Cryptosporidium may have a zoonotic potential first surfaced when cryptosporidial infections were estabilished 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 trasmission 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 susceptable 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 polymorpholnuclear 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 electonegatively 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). Fermintation 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 persistant 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 enterpathogen 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 transplatation 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% Jodonal 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 9^0C to 55^0C over a period of 15 to 20 minutes or holding inocula at 45^0C 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 inconsistant results due to variation in host specificity and differences in pathogencity 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 exposured but in which clinical signs were not exhibited (58). Indirect immunofluoresence 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.

Pathognomic 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 consistant 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 carbolfuchsin (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.

Materials and Methods

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 questionaire 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 questionaire (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 fermintation 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 fermintation. 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 questionaires were also compiled and presented in tabular form.

Results

Three hundred eight owners were contacted by letter asking if they would be willing in 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 recived 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 questionaire 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%). Pilus 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 questionaire requesting information on the operation and management of their particular herd. Forty-two of the questionaires were received from uninfected herds and 17 were from infected herds. Information compiled from the questionaire 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

the milk.

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 questionaire 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 questionaire 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 susceptability 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 susceptability 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 suprising.

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 susceptability 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 pathogencity 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

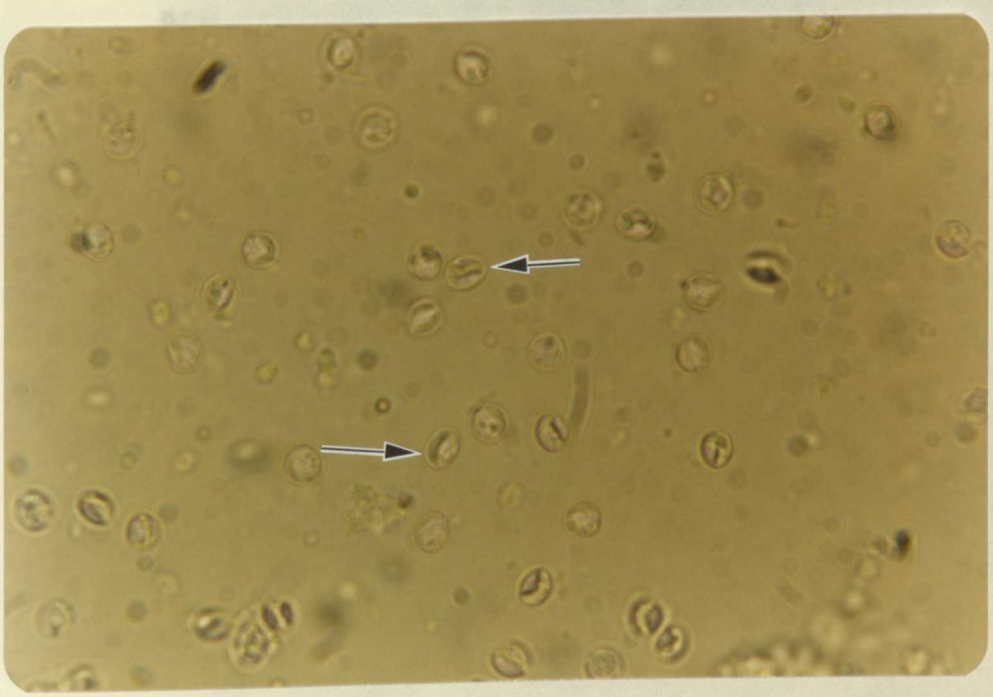
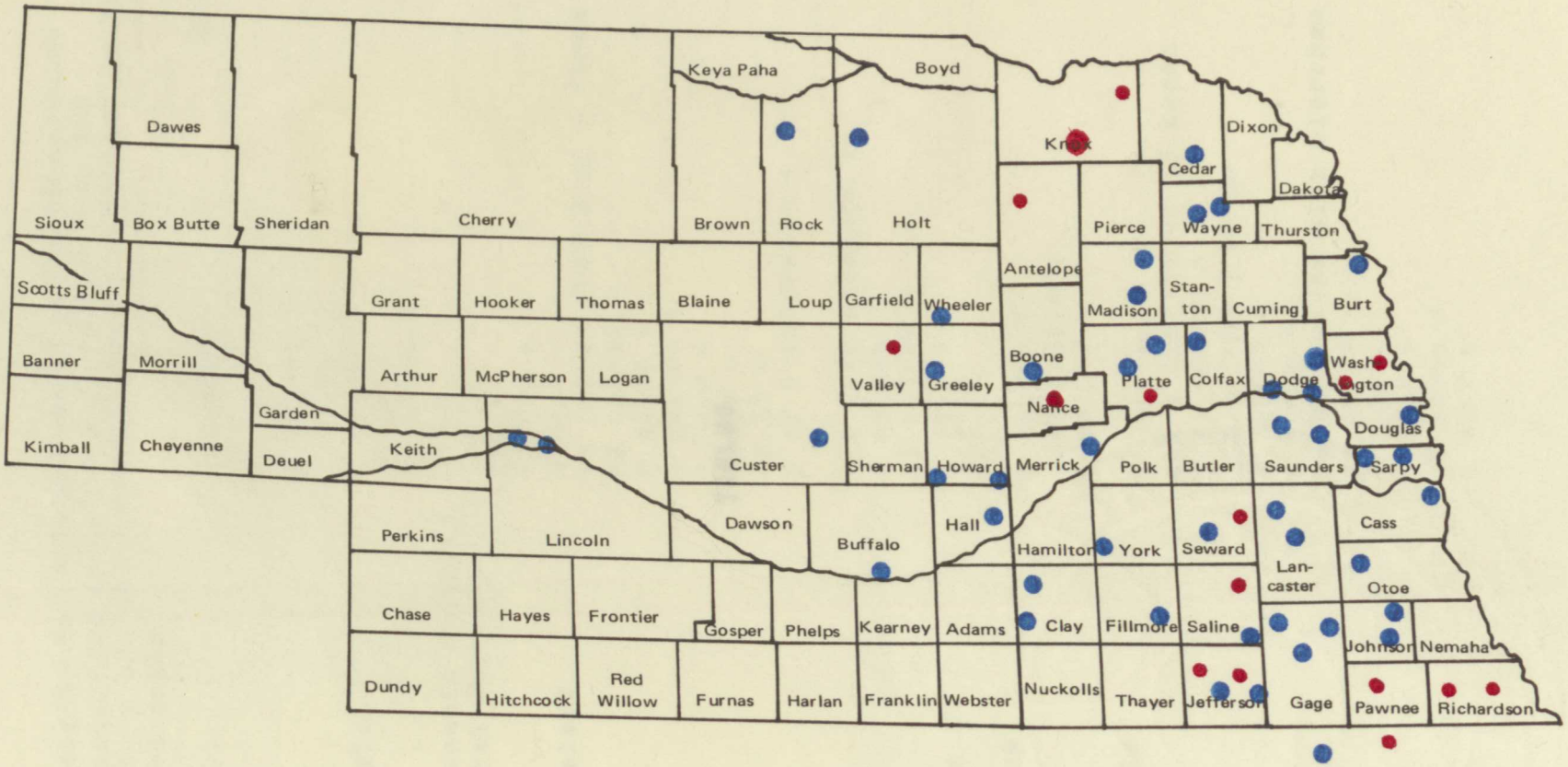


FIGURE 1

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.


Dawes
Sioux
Box Butte
Sheridan
Cherry
Keya Paha
Boyd
Brown
Rock
Holt
Knox
Dixon
Cedar
Dakota
Pierce
Wayne
Thurston
Antelope
Scotts Bluff
Grant
Hooker
Thomas
Blaine
Loup
Garfield
Wheeler
Madison
Stan-
ton
Cuming
Burt
Banner
Morrill
Arthur
McPherson
Logan
Valley
Greeley
Boone
Platte
Colfax
Dodge
Wash-
ington
Nance
Douglas
Garden
Kimball
Cheyenne
Deuel
Keith
Custer
Sherman
Howard
Merrick
Polk
Butler
Saunders
Sarpy
Perkins
Lincoln
Dawson
Buffalo
Hall
Hamilton
York
Seward
Lan-
caster
Cass
Otoe
Chase
Hayes
Frontier
Gosper
Phelps
Kearney
Adams
Clay
Fillmore
Saline
Johnson
Nemaha
Dundy
Hitchcock
Red
Willow
Furnas
Harlan
Franklin
Webster
Nuckolls
Thayer
Jefferson
Gage
Pawnee
Richardson

TABLES

Table 1 - Geographic distribution of reported cases of *Cryptosporidium* infections in cattle from the literature and the incidence of the infection in those reported cases

Country	Incidence	Reference
Europe		
Belgium	2 calves +	21
Bulgaria	2 farms +	160
Czechoslavakia	2 calves +	238
Denmark	16.3% +	132
France	2 calves +	21
German Democratic Republic	51% +	157
German Federal Republic	44% +	96
Hungary	27% +	226
Netherlands	19-85% +	175
Northern Ireland	1 calf +	248
Norway	12 calves from 6 of 9 farms	169
Poland	48 calves +	346
Romania	8 herds +	83
Scotland	4 calves +	291
Sweden	1 herd +	343
Switzerland	14.5% +	298
USSR	87.5% +	245
Australia	5 of 9 outbreaks +	151
Asia		
Bangladesh	14% with diarrhea +	266
Isreal	3 calves +	232
Turkey	56 calves +	55
South Africa	1 calf +	143
Canada	26% +	281
South America		
Argentina	2 calves +	197
Cuba	6/13 calves +	113
Mexico	2 calves +	114
United States		
Connecticut	1 calf +	212
Idaho	64% + from 41 of 73 herds	15
Iowa	8/23 calves +	257
Maryland	26% + from 9 farms	174
Minnesota	14 herds +	36
Nebraska	4 herds +	36
North Dakota	22/257 calves with diarrhea	34
Ohio	20 herds +	121
Oklahoma	1 calf +	237
Oregon	1 calf +	283
South Dakota	16 herds +	36
Tennessee	1 calf +	261

Table 2A - Drugs used to treat human Cryptosporidium infections.

Weinstein(350)	Sloper(289)	Stemmermann(299)	CDC(67)
Sulfisoxazole	Mepacrine	Metronidazole	Trimethoprim/
Pyrimethamine	Colistin	Sulfamethoxazole	sulfamethoxazole
Metronidazole	Oxytetracycline	Trimethoprim	Furazolidone
Chloroquine	Metronidazole	Pyrimethamine	Metronidazole
Primaquine	Piperazine	Sulfadiazine	Pyrimethamine/
Loperamide	Thiobendazole	Levamisol	sulfa
Pentemidine	Erythromycin	Amphotericin B	Tetracycline
Sulfathalidine	Penicillin	Cholestyramine	Quinacrine
	Ampicillin		Diloxanide
	Septrin		furoate
	Gentamicin		Ketoconazole
	Cloxacillin		Petamidine
	Carbenicillin		Bovine transfer
			factor
			Amphotericine
			Iodoquinol
			Paromomycin
			Spiramycin
			Clindamycin
			Gamma-Globulin
			Chloroquine
			Primaquine
			Amprolium
			Salinomycin

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
- Sulfaquinoxaline
- Furaltadone
- Enterolyte-N
- Sulfamethazine
- Trinamide
- Amprol
- Phenamidine
- Zoaquin
- 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.

Age of calf (days)	Total	Positive	Negative
2	2	0	2
5	304	4	300
6	13	0	13
7	7	1	6
9	1	0	1
10	6	0	6
11	4	0	4
12	259	49	210
13	12	1	11
14	7	0	7
15	1	0	1
18	1	0	1
19	1	0	1
22	2	0	2
Total # samples	620	55	565
Breed of calf			
Unidentified Breed	1	0	1
Brown Swiss	8	5	3
Short Horn	1	0	1
Ayshire	3	0	3
Angus-Holstein	6	0	6
Guernsey	4	0	4
Angus	1	0	1
Holstein-Long-horn-Shorthorn	4	0	4
Holstein-Simmental	2	1	1
Holstein	305	46	259
Total # calves	334	52	282
Sex of calf			
Male	116	17	99
Female	218	35	183
Total # calves	334	52	282

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

Infected?	Yes	No	Total
Scouring?			
Yes	36	29	65
No	16	253	269
Total	52	282	334

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.

Organisms isolated	Total	Scouring?		Outcome?	
		Yes	No	Survived	Died
C	20	15	5	19	1
C + CP	4	1	3	4	0
C + EC	10	6	4	9	1
C + RV	2	1	1	2	0
C + CV	1	1	0	1	0
C + CP + EC	2	1	1	2	0
C + CP + CV	2	2	0	2	0
C + CP + S	5	5	0	5	0
C + CP + RV	1	1	0	1	0
C + CP + S + RV	2	2	0	2	0

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.

Month and year	Total # samples received that month	Number infected	Percentage	Number uninfected
March 1985	51	3	5	48
April 1985	107	13	12	94
May 1985	152	13	8	139
June 1985	77	4	5	73
July 1985	15	1	6	14
August 1985	35	1	3	34
September 1985	104	14	13	90
October 1985	53	3	6	50
November 1985	13	0	0	13
December 1985	6	3	50	3
January 1986	2	0	0	2
February 1986	1	0	0	1
April 1986	4	0	0	4
Total	620	55		565

Table 7 - Tabulation of the different feeding programs used by the herd owners as reported on the questionaire sent to all owners.

Feed Program	Total	Infected	Uninfected
Colostrum - Whole milk	31	9	22
Colostrum - Whole milk - Milk replacer	12	6	6
Colostrum - Milk replacer	11	1	10
Whole milk only	1	0	1
Colostrum only	1	0	1
Colostrum - Antibiotic milk	1	1	0
Colostrum - Whole milk/Fermented colostrum - Milk replacer	1	0	1
Colostrum - Fermented colostrum	1	0	1
Total	59	17	42

Table 8 - Summary of data from questionaire 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.

	Uninfected		Infected	
	Yes	No	Yes	No
Antibiotics or Vitamins placed in milk?	4	37	1	16
Treatment for any diseases?	1	40	0	17
History of scouring?	30	12	14	3
Death loss	15	27	13	4
Diagnosis?	10	8	3	8

APPENDIX

The following two pages contain the two questionaires used in this study. Questionaire #1 was sent to all owners and asks the owner to relate information on the management practices and operation of the herd. Questionaire #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.

QUESTIONAIRE #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 spearately 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 questionaire 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

1. Abdulla, S.K. (1978). D-xylose study of absorptive function of the small intestine in children with dysentery. Pediatrics 6:39.

2. Acres, S.D.; Babiuk, L.A. (1978). Studies on rotavirus antibody in bovine serum and lacteal secretions using radio-immunoassay. Journal of the American Veterinary Medical Association 173:555-559.

3. Addy P.A.-K.; Aikins-Bekoe, P. (1986). Cryptosporidiosis in diarrhoel children in Kumasi, Ghana. Lancet i:735.

4. Ahourai, P.; Ezzi, A.; Gholami, M.R.; Vandyoosefi,J.; Kargar, R.; Maalhagh, N. (1985). Cryptopsoridium spp. in new born lambs in Iran. Tropical Animal Health and Production 17:6-8.

5. Alpert, G. (1986). Outbreak of cryptosporidiosis in a day-care center. Pediatrics 77:152-157.

6. Alpert, G.; Bell, L.M.; Kirkpatrick, C.E.; Budnick, L.D.; Campos, J.M.; Friedman, H.M.; Plotkin, S.A. (1984). Cryptosporidiosis in a day-care center. [Correspondence]. New England Journal of Medicine 311:860-861.

7. Anderson, B.C. (1981). Patterns of shedding of cryptosporidial oocysts in Idaho calves. Journal of the American Veterinary Medical Association 178:982-984.

8. Anderson, B.C. (1982). Cryptosporidiosis in Idaho lambs--natural and experimental infections. Journal of the American Veterinary Medical Association 181:151-153.

9. Anderson, B.C. (1982). Cryptosporidiosis in calves: Epidemiologic questions, diagnosis and management. Proceedings of the 11th Annual Meting of the American Association of Bovine Practioners pp. 92-94.

10. Anderson, B.C. (1984). Location of cryptsporidia: Review of the literature and experimental infections in calves. American Journal of Veterinary Research 45:1474-1477.

11. Anderson, B.C. (1985). Moist heat inactivation of Cryptosporidium sp. American Journal of Public Health 75:1433-1434.

12. Anderson, B.C. (1986). Abomasal cryptosporidiosis in cattle: A "new" parasitism. Proceedings of the 7th Annual Western Conference for Food Animal Veterinary Medicine p. 22.

13. Anderson, B.C.; Bulgin, M.S. (1981). Enteritis caused by

Cryptosporidium in calves. Veterinary Medicine and Small Animal Clinican 76:865-868.

14. Anderson, B.C.; Donndelinger, T.; Wilkins, R.M.; Smith, J. (1982). Cryptosporidiosis in a veterinary student. Journal of the American Veterinary Medical Association 180:408-409.

15. Anderson, B.C.; Hall, R.F. (1982). Cryptosporidial infection in Idaho dairy calves. Journal of the American Veterinary Medical Association 181:484-485.

16. Anderson D.R.; Dusynski, D.W.; Marquardt W.C. (1968). 3 new coccidia (Protozoa: Telosporea) from kingsnakes, Lampropeltis spp., in Illinois, with a redescription of Eimeria zamenis Phisalix 1921. Journal of Parasitology 54:577-581.

17. Angus, K.W. (1983). Cryptosporidiosis in man, domestic animals and birds: A review. Journal of the Royal Society of Medicine 76:62-70.

18. Angus, K.W.; Appleyard, W.T.; Menzies J.D.; Campbell, I.; Sherwood, D. (1982). An outbreak of diarrhoea associated with cryptopsoridiosis in naturally reared lambs. Veterinary Record 110:129-130.

19. Angus, K.W.; Sherwood, D.; Hutchison, G.; Campbell, I. (1982). Evaluation of the effect of two aldehyde-based disinfectants on the infectivity of faecal cryptosporidia for mice. Research in Veterinary Science 33:379-381.

20. Angus, K.W.; Tzipori, S.; Gray, E.W. (1982). Intestinal lesions in specific-pathogen-free lambs associated with a Cryptosporidium from calves with diarrhoea. Veterinary Pathology 19:67-78.

21. Antoine, H.; Pivont, P.; Gregoire, R.; Bughin, J. (1981). Cryptosporidiose intestinale chez deux veaux nouveau-nes. Point Veterinaire 12:31-32.

22. Arnaud-Battandier, F.; Naceri, M.; Fisher A.; Ricour, C. (1982). Cryptosporidiose intestinale: une cause nouvelle de diarrhee chez l'homme. [Correspondence]. Gastroenterologie Clinique et Biologique 6:1045-1046.

23. Arrowood, M.J.; Mead J.R.; Sterling C.R. (1986). Production of monoclonal antibodies recognizing Cryptosporidium oocysts and sporozoite antigens. Proceedings of the 7th western Conference for Food Animal Veterinary Medicine p. 50.

24. Augustin-Bichl, G.; Boch J.; Henkel, G. (1984). Kryptosporidien-infektionen bei hund und katze. Berliner und Munchener Tierarzliche Wochenschrift 97:179-181.

25. Australia, Queensland Department of Primary Industries. (1982). Annual report 1981-1982. Brisbane, Australia v + 160 pp.

26. Babb, R.R.; Differding J.T.; Trollope, M.l. (1982). Cryptosporidia enteritis in a healthy professional athlete. American Journal of Gastroenterology 77:833-834.

27. Barker, I.K.; Carbonell P.L. (1974). Cryptosporidium agni from lambs, and Cryptosporidium bovis from a calf with observations on the oocyst. Zeitschrift fur Parasitenkunde 44:289-298.

28. Barriga, O.O. (1984). Tratamiento de la cryptosporidiosis [Correspondence]. Parasitologia al Dia 8:62.

29. Baxby, D.; Blundell, N.; Hart, C.A. (1984). The development and performance of a simple, sensitive methos for detection of Cryptosporidium oocysts in faeces. Journal of Hygiene 93: 317-323.

30. Baxby, D.; Getty, B.; Blundell, N.; Ratcliffe, S. (1984). Recognition of whole Cryptosporidium oocysts in feces by negative staining and electron microscopy. Journal of Clinical Microbiology 19:566-567.

31. Baxby, D.; Hart C.A.; Blundell, N. (1985). Shedding of oocysts by immunocompetent individuals with Cryptosporidium. Journal of Hygiene 95:703-709.

32. Baxby, D.; Hart, C.A.; Taylor, C. (1983). Human cryptopsoridiosis; A possible case of hospital cross infection. British Medical Journal 287:1760-1761.

33. Bearup, A.J. (1954). The coccidia of carnivores in Sidney. Australian Veterinary Journal 30:185-186.

34. Berg, I.E. (1984). Cryptosporidiosis. North Dakota Farm Research 41:22-23.

35. Berg, I.E.; Peterson, A.C.; Freeman. T.P. (1978). Ovine cryptosporidiosis. Journal of the American Veterinary Medical Association 173:1586-1587.

36. Bergeland, M.E.; Johnson, D.D.; Shave, H. (1980). Bovine cryptosporidiosis in the north central United States. In Proceedings of the 22nd Annual Meeting of the American Association of Veterinary Laboratory Diagnosticians pp. 131-138.

37. Bennett, M.; Baxby, D.; Blundell, N.; Gaskell, C.J.; Hart, C.A.; Kelly, D.F. (1985) Cryptosporidiosis in the domestic cat. Veterinary Record 116:73-74.

38. Berkowitz, C.D.; Seidel, J.S. (1985) Spontaneous resolution of cryptosporidiosis in a child with acquired immunodeficiency syndrome. American Journal of Diseases of Children 139:967.

39. Bird, R.G. (1981). Protozoa and viruses. Human cryptosporidiosis and concomitant viral enteritis. In Parasitological topics -- a presentation volume to P.C.C. Garnham, F.R.S. on the occasion of his 80th birthday, 1981. pp. 39-47.

40. Bird, R.G.; Smith, M.D. (1980). Cryptosporidiosis in man: Parasite life cycle and fine structural pathology. Journal of Pathology 132:217-233.

41. Blacklow D.B.; Dolan R.; Fedson, D.S. (1972). Acute infectious non-bacterial gastroenteritis: Etiology and pathogenesis. Annals of Internal Medicine 76: 993-1008.

42. Blagburn, B.L.; Current W.L. (1983). Accidental infection of a researcher with human cryptosporidiosis. Journal of Infectious Diseases 148:772-773.

43. Blaser, M.J.; Cohn D.L. (1986). Opportunistic infections in patients with AIDS: Clues to the epidemiology of AIDS and the relative virulence of pathology. Reviews of Infectious Diseases 8:21-29.

44. Boch, J.; Heine, J. (1984). Cryptosporidial infections of domestic animals. Pro Veterinario 3:10-11.

45. Bogaerts, J.; Lepage, P.; Rouvroy, D.; Vandepitte, J. (1984). Cryptosporidium spp., a frequent cause of diarrhea in Central Africa. Journal of Clinical Microbiology 20:874-876.

46. Bond, J.H.; Levitt, M.D. (1976). Fate of soluble carbohydrates in the colon of rats and man. Journal of Clinical Investigation 57:1158-1164.

47. Bond, J.H.; Levitt M.D. (1976). Quantitative measurement of lactose malabsorption. Gastroenterology 70:1058-1062.

48. Booth, C.C. (1980). Clinicopathological conference: Immunodeficiency and cryptosporidiosis. British Medical Journal 281:1123-1127.

49. Box, E.D.; Marchiondo, A.A.; Duszynski, D.W.; Davis, C.P. (1980). Ultrastructure of Sarcocystis sporocysts from passerine birds and opossums: Comments on classification of the genus Isospora. Journal of Parasitology 66:68-74.

50. Brady, E.M.; Margolis, M.L.; Korzeniowski, O.M. (1984). Pulmonary cryptosporidiosis in acquired immune deficiency

syndrome. Journal of the American Medical Association 252:89-90.

51. Brandenborg, L.L.; Goldberg S.B.; Breidenback, W.C. (1970). Human coccidiosis -- a possible cause of malabsorption. The life-cycle in small bowel biopsies as a diagnostic feature. New England Journal of Medicine 283:1306-1313.

52. Britain, Rowett Research Institute (1980). Annual report of studies in animal nutrition and allied sciences. 36:39-40, 47-49.

53. Bronsdon, M.A. (1984). Rapid dimethyl sulfoxide-modified acid-fast stain of Cryptosporidium oocysts in stool specimens. Journal of Clinical Microbiology 19:952-953.

54. Brownstein, D.G.; Strandberg B.D.; Montali R.J.; Bush, M.; Fortner, J. (1977). Cryptosporidium in snakes with hypertrophic gastritis. Veterinary Pathology 14:606-617.

55. Burgu, A. (1984). Turkiye'de buzagilarda Cryptosporidium'larin bulunuse ile ilgili ilk calismalar. Veteriner Fakultesi Dergisi Ankara Universitesi 31:573-585.

56. Bywater, R.J.; Logan E.F. (1974). Sites and characteristics of intestinal water and electrolyte loss in Escherichia coli induced diarrhea in calves. Journal of Comparative Pathology 84:599-610.

57. Campbell, I.; Tzipori, S.; Hutchison, G.; Angus, K.W. (1982). Effect of disinfectants on survival of Cryptosporidium oocysts. Veterinary Record 111:414-415.

58. Campbell, P.N.; Current, W.L. (1983). Demonstration of serum antibodies to Cryptosporidium sp. in normal and immunodeficient humans with confirmed infections. Journal of Clinical Microbiology 18:165-169.

59. Carlson, B.L.; Nielsen S.W. (1982). Cryptosporidiosis in a raccoon. Journal of the American Veterinary Medical Association 181:1405-1406

60. Carter, G.R. (1984). Diagnostic Procedures in Veterinary Bacteriology and Mycology. Springfield, IL.; Charles C. Thomas.

61. Casemore, D.P.; Armstrong M.; Jackson B. (1984). Screening for Cryptosporidium in stools. [Correspondence]. Lancet i:734-735.

62. Casemore, D.P.; Armstrong, M.; Sands, R.L. (1985). Laboratory diagnosis of cryptosporidiosis. Journal of Clinical Pathology 38:1337-1341.

63. Casemore, D.P.; Jackson, B. (1983). Sporadic cryptosporidiosis in children. [Correspondence]. Lancet ii:679.

64. Casemore, D.P.; Jackson, F.B. (1984). Hypothesis: Cryptosporidiosis in human beings is not primarily a zoonosis. Journal of Infection 9:153-156.

65. Casemore, D.P.; Jessop, E.G.; Douce, D.; Jackson, F.B. (1986). Cryptosporidium plus Campylobacter: An outbreak in a semi-rural population. Journal of Hygiene 96:95-105.

66. Casemore, D.P.; Sands R.L.; Curry A. (1985). Cryptosporidium sp., a "new" human pathogen. Journal of Clinical Pathology 38:1321-1336.

67. Centers for Disease Control. (1982). Cryptosporidiosis: Assesment of chemotheraphy of males with acquired immune deficiency syndrome. Morbidity and Mortality Weekly Report 31:589-592.

68. Centers for Disease Control. (1984). Cryptosporidiosis among children attending day-care centers in GA, PE, MI, CA, N Mex. Morbidity and Mortality Weekly Report 33:599-601.

69. Centers for Disease Control. (1984). Update: Treatment of cryptosporidiosis in patients with AIDS. Morbidity and Mortality Weekly Report 33:117-119.

70. Christie, E.; Pappas, P.W.; Dubey, J.P. (1978). Ultrastructure of excystment of Toxoplasma gondii oocysts. Journal of Parasitology 25:438-443.

71. Cockrell, B.Y.; Valerio, M.G.; Garner, F.M. (1974). Cryptosporidium in the intestines of rhesus monkeys (Macaca mulatta). Laboratory Animal Science 24:881-886.

72. Collier, J.C.; Miller, R.A.; Meyers, J.D. (1984). Cryptosporidiosis after marrow transplantation: person-to-person transmission and treatment with spiramycin. Annales of Internal Medicine 101:205-206.

73. Contrepois, M.; Gouet, P.; Naciri, M. (1984). Cryptosporidiose experimental chez des chevreaux et des agneaux axeniques. In Les maladies de la chevre. Colloque International, Niort France, 9-11 ocgobre 1984. pp. 453-463.

74 Contrepois, M.; Vallet, A. (1984). Cryptosporidiose et diarrhee neonatale en elevage bovin. Point Veterinaire 16:47-53.

75. Cooper, D.A.; Wodak, A.; Marriot, D.J.E.; Harkness, J.L.; Ralston, M.; Hill, A.; Penny, R. (1984). Cryptosporidiosis

in the acquired immune deficiency syndrome. Pathology 16:455-457.

76. Cross, J.H.; Alcantara, A.; Alquiza, L.; Zaraspe, G.; Ranoa, C. (1985). Cryptosporidiosis in Philippine children. Southeast Asian Journal of Tropical Medicine 16:257-260.

77. Current, W.L. (1984). Cryptosporidium and cryptosporidiosis. In UCLA Symposium on Molecular and Cellular Biology Gottlieb, M.S.; Groopman, J.E. ed. 16:355-373.

78. Current, W.L. (1985). Cryptosporidiosis. Journal of the American Veterinary Medical Association 187:1309-1404.

79. Current, W.L.; Haynes, T.B. (1984). Complete development of Cryptosporidium in cell culture. Science, USA 224:603-605.

80. Current, W.L.; Long, P.L. (1983). Development of human and calf Cryptosporidium in chicken embryos. Journal of Infectious Diseases 148:1108-1113.

81. Current, W.L.; Reese, N.C. (1986). Comparison of endogenous development of 3 isolates of Cryptosporidium in suckling mice. Journal of Protozoology 33:98-108.

82. Current, W.L.; Reese, N.C.; Ernst, J.V.; Bailey, W.S.; Heyman, M.B.; Weinstein, W.M. (1983). Human cryptosporidiosis in immunocompetent and immunodeficient persons. New England Journal of Medicine 308:1252-1257.

83. Dan, S.; Cristescu, P.; Neagoe, V. (1983). Identificarea genului Cryptosporidium in Romania. Revista de Cresterea Animalelor 33:42-44.

84. D'Antonio, R.G.; Winn, R.E.; Taylor, J.P.; Gustafson, T.L.; Current. W.L.; Rhodes, M.M.; Gary, G.W.; Zarjac, R.A. (1985). A waterborne outbreak of cryptosporidiosis in normal hosts. Annals of Internal Medicine 103:886-888.

85. De Mol, P.; Mukashema, S.; Bogaerts, J.; Hemelhof, W.; Butzler, J.P. (1984). Cryptosporidium related to measles diarrhoea in Rwanda. [Correspondence]. Lancet ii:42-43.

86. Dhillon, A.S.; Thacker, H.L.; Dietzel, A.V.; Winterfield, R.W. (1981). Respiratory cryptosporidiosis in broiler chickens. Avian Diseases 25:747-751.

87. Doster, A.R.; Mahaffey, E.A.; McClearen, J.R. (1979). Cryptosporidia in the cloacal coprodeum of red-lored parrots (Amazona autumnalis). Avian Diseases 23:654-661.

88. Dryjanski, J.; Gold. J.W.M.; Ritchie, M.T.; Kurtz,R.C.; Linn, S.L.; Armstrong, D. (1986). Cryptosporidiosis: Case

report in a health team worker. American Journal of Medicine 80:751-752.

89. Dubey, J.P. (1963). Observations on the coccidian oocysts from the Indian jungle cat (Felis chaus). Indian Journal of Microbiology 3:103-105.

90. Ducatelle, R.; Maenhout, D.; Charlier, G.; Miry, C.; Coussement, W.; Hoorens, J. (1983). Cryptosporidiosis in goats and in mouflon sheep. Vlaams Diergeneeskundig Tijdschrift 52:7-17.

91. Duszynski, D. (1969). Two new coccidia (Protozoa: Eimeriidae) from Costa Rican lizards with a review of the Eimeria from lizards. Journal of Protozoology 16:581-585.

92. Fayer, R.; Ernst, J.V.; Miller, R.G.; Leek, R.G. (1985). Factors contributing to clinical illness in calves experimentally infected with a bovine isolate of Cryptosporidium. Proceedings of the Helminthological Society of Washington 52:64-70.

93. Fayer, R.; Leek, R.G. (1973). Excystation of Sarcocystis fusiformis sporocysts from dogs. Proceedings of the Helmintological Society of Washington 40:294-296.

94. Fayer, R.; Leek, R.G. (1984). The effects of reducing conditions, medium, pH, temperature, and time on in vitro excystation of Cryptosporidium. Journal of Protozoology 31:567-569.

95. Fenwick, B.W. (1983). Cryptosporidiosis in a neonatal gazella. Journal of the American Veterinary Medical Association 183:1331-1332.

96. Fiedler, H.-H. (1985). Zur verbreitung von Kryptosporidien unter norddeutschen Rinderbestanden. Tierarztliche Umschau 40:526-528.

97. Fiedler, H.-H.; Bahr, K.H.; Hirchert, R. (1982). Beitrag zur Kryptosporidieninvasion bei Kalbern. Tierarztliche Umschau 37:497-500.

98. Fischer, O. (1982). Kryptosporidioza telat v obdobi mlecne vyzivy. Veterinarni Medicina 27:465-471.

99. Fletcher, A.; Sims, T.A.; Talbot, I.C. (1982). Cryptsoporidial enteritis without general or selective immune deficiency. British Medical Journal 285:22-23.

100. Fletcher, O.J.; Munnell, J.F.; Page, R.K. (1975). Cryptosporidiosis of the bursa of Fabricius of chickens. Avian Diseases 19:630-639.

101. Flewett, T.H. (1978). Electron microscopy in the diagnosis of infectious diarrhea. Journal of the Veterinary Medical Association 173:538-543.

102. Forgacs, P.; Tarshis A.; Ma, P.; Federman, M.; Mele, L.; Silverman, M.L.; Shea, J.A. (1983). Intestinal and bronchial cryptosporidiosis in an immunodeficient homosexual man. Annals of Internal medicine 99:793-794.

103. Frenkel, J.K. (1977). Besnoitia wallacei of cats and rodents: with a reclassification of other cyst-forming isosporid coccidia. Journal of Parasitology 63:611-628.

104. Freter R.; De.S.P.; Mondal, A.; Shrirastava, D.L.; Sunderman, F.W. (1965). Coproantibody and serum antibody in cholera patients. Journal of Infectious Diseases 115:83-87.

105. Fukushima, K.; Helman, R.G. (1984). Cryptosporidiosis in a pup with distemper. Veterinary Pathology 21:247-248.

106. Gajadhar, A.A.; Caron, J.P.; Allen, J.R. Cryptosporidiosis in two foals. Canadian Veterinary Journal 26:132-134.

107. Garcia, L.S.; Bruckner, D.A.; Brewer, T.C.; Shimizu, R.Y. (1983). Techniques for the recovery and identification of Cryptosporidium oocysts from stool specimens. Journal of Clinical Microbiology 18:185-190.

108. Gardiner, C.H.; Imes, G.D. Jr. (1984). Cryptosporidium sp. in the kidney of a black-throated finch. Journal of the American Veterinary Medical Association 185:1401-1402.

109. Gibson, J.A.; Hill, M.W.M.; Huber, M.J. (1983). Cryptosporidiosis in Arabian foals with severe combined immunodeficiency. Australia Veterinary Journal 60:378-379.

110. Glisson, J.R.; Brown, T.P.; Brugh, M.; Page, R.K.; Kleven, S.H.; David, R.B. (1984). Sinusitis in turkeys associated with respiratory cryptosporidiosis. Avian Diseases 28:783-790.

111. Goebel, E.; Braendler, U. (1982). Ultrastructure of microgametogenesis, microgametes and gametogamy of Cryptopsoridium sp. in the small intestine of mice. Protistologica 18:331-344.

112. Goebel, E.; Bretschneider, M. (1985). Mikromorphologische untersuchungen zur wirksamkeit von lasalocid auf die entwicklungsstadien von Cryptosporidium. In Bericht des 16. Kongresses der Deuteschen Veterinarmedizinischen Gesellschaft. Bad Nauheim, 17-20, April 1985 pp. 278-282.

113. Gomez, E.; Alonso, M.; Blandino, T.; Frias, M.T.; Merino,

N. (1982). Cryptosporidium spp. en terneros de und recria de la provincia Habana. Revista de Salud Animal 4:65-67.

114. Gonzalez Morteo, C.; Gomez Estrella, S.; Aluja, A.S. De. (1983). Criptosporidiosis en bovinos lactantes (histopatologia, microscopia electronica de transmision y de barrido). Veterinaria Mexico 14:12-22.

115. Gottlieb, M.S.; Groopman, J.E.; Weinstein, W.M.; Fahey, J.L.; Detels, R. (1983). The Acquired Immune Deficinecy Syndrome. Annals of Internal Medicine 99:208-220.

116. Gouet, P.; Contrepois, M.; Dubourguier, H.C.; Rious Y.; Scherrer R. (1978). Experimental production of diarrhoea in colostrum deprived axenic and gnotoxenic calves with exnteropathogenic Escherichia coli, rotavirus, coronavirus and in a combined infection of rotavirus and E. coli. Annales de Recherches Veterinaires 9:433-440

117. Guerrant, R.L. (1985). Microbial toxins and diarrhoel diseases: introduction and overview. In Microbial toxins and diarrhoeal disease. (Ciba Foundation Symposium 112 -- held 10-12 July 1984. London) [Edited by: Evered, D.; Whelan, J.]. pp. 1-13.

118. Gunther, H. (1983). Kryptosporidien beim kalb--bedeutung, nachweis, bekampfung. Monatschefte fur Veterinarmedizin 38:653-655.

119. Gunther, H. (1984). Bekampfung der bovinen kryptosporidiose. Monatshefte fur Veterinarmedizin 39:730-733.

120. Hampton, J.C. Rosario, B. (1966). The attachment of protozoan parasites to intestinal epithelial cells of the mouse. Journal of Parasitology 52:939-949.

121. Hancock , D.D. (1982). Epidemiology of Cryptosporidium infection in neonatal heifers in 20 Ohio dairy farms. Abstract presented at the 63rd Annual Meeting, Conference of Research Workers in Animal Diseases, 1982.

122. Harari, M.D. West, B.; Dwyer, B. (1986). Cryptosporidium as cause of laryngotracheitis in an infant. Lancet 1:1207.

123. Hart, C.A.; Baxby, D.; Blundell, N. (1984). Gastro-enteritis due to Cryptosporidium: A prospective survey in a children's hospital. Journal of Infection 9:264-270.

124. Hee Oh, Sung; Jaffe, N.; Fainstein, V.; Pickering, L.K. (1984). Cryptosporidiosis and anticancer theraphy. Journal of Pediatrics 104:963-964.

125. Heine, J. (1980). Crytosporidien-infektionen beim kalb. In

Symposium Parasitosen der Wiederkauer am 14. und 15. November 1980 in Rothenburg ob der tauber. Referate. pp. 86-88.

126. Heine, J. (1982). Eine einfache nachweismethode fur kryptopsoridien im kot. Zentralblatt fur Veterinarmedizin. B 29:324-327.

127. Heine J.; Boch, J. (1981). Kryptosporidien-infektionen beim kalb. Nachweis, vorkommen und experimentelle ubertragung. Berliner und Munchener Tierarztliche Wochenschrift 94:289-292.

128. Heine, J.; Moon, H.W.; Woodmansee, D.B. (1984). Persistent Cryptosporidium infection in congentially athymic (nude) mice. Infection and Immunity 43:856-859.

129. Heine, J.; Moon, H.W.; Woodmansee, D.B.; Pohlenz, J.F.L. (1984/1985). Experimental tracheal and conjunctival infections with Cryptosporidium sp. in pigs. 17:17-25.

130. Heine, J.; Pohlenz, J.F.L.; Moon, H.W.; Woode, G.N. (1984). Enteric lesions and diarrhea in gnotobiotic calves monoinfected with Cryptosporidium species. Journal of Infectious Diseases 150:768-775.

131. Henkel, G. (1983). Kryptosporidiose des Menschen. Munchener Medizinsche Wochenschrift 125:1099-1102.

132. Henriksen, S.A.; Krogh, H.V. (1980). A note on bovine infections with cryptosporidia in Denmark. Nordisk Veterinaer Medicin 32:501.

133. Henriksen, S.A.; Pohlenz, J.F.L. (1981). Staining of cryptosporidia by a modified Ziehl-Neelsen technique. Acta Veterinaria Scandinavica 22:594-596.

134. Henry, M.C.; De Clercq, D.; Lokombe, B.; Kayembe, K.; Kapita, B.; Mamba, K.; Mbemdi, N.; Mazebo, P. (1986). Parasitological observations of chronic diarrhea in suspected AIDS adult patients in Kinshasa (Zaire). Transactions of the Royal Society of Tropical Medicine and Hygiene 80:309-310.

135. Hibbert, L.E.; Hammond, D.M. (1968). Effects of temperature on in vitro excystation of various Eimeria species. Experimental Parasitology 23:161-170.

136. Hoerr, F.J.; Ranck, F.M.; Hastings, T.F. (1983). Respiratory cryptosporidiosis in turkeys. Journal of the American Veterinary Medical Association 173:1591-1593.

137. Hojlyng, N.; Molbak, K.; Jepen, S. (1985). Cryptosporidiosis

in human beings is not primarily a zoonois. Journal of Infection 11:270-271.

138. Hojlyng, N.; Molbak, K.; Jepsen, S.; Hansson, A.P. (1984). Cryptosporidiosis in Liberian children. Lancet i:734.

139. Holten-Andersen, W.; Gerstoft, J.; Henriksen, S.A.; Pedersen, N.S. (1984). Prevalence of Cryptosporidium among patients with acute enteric infection. Journal of Infection 9:277-282.

140. Hoover, D.M.; Hoerr, F.J.; Carlton, W.W.; Hinsman, E.J.; Ferguson, H.W. (1981). Enteric cryptosporidiosis in a naso tang, Naso lituratus Bloch and Schneider. Journal of Fish Diseases 4:425-428.

141. Horen, W.P. (1983). Detection of Cryptosporidium in human fecal specimens. Journal of Parasitology 69:622-624.

142. House, J.A. (1978). Economic impact of rotavirus and other neonatal disease agents of animals. Journal of the American Veterinary Medical Association 173:573-576.

143. Howerth, E.W. (1981). Bovine cryptosporidiosis. Journal of the South African Veterinary Association 52:251-253.

144. Hunt, D.A.; Shannon, R.; Palmer, S.R.; Jephcott, A.E. (1984). Cryptopsporidiosis in an urban community. British Medical Journal 289:814-816.

145. Ikeda, M. (1960). Factors necessary for Eimeria tenella infection in the chicken. VI. Excystation of oocysts in vitro. Japanese Journal of Parasitology 28:285-307.

146. Inman, L.R.; Takeuchi, A. (1979). Spontaneous cryptosporidiosis in an adult female rabbit. Veterinary Pathology 16:89-95.

147. Iseki, M. (1979). Cryptosporidium felis sp.n. (Protozoa: Eimeriorina) from the domestic cat. Japanese Journal of Parasitology 28:285-307.

148. Issacs, D. (1985). Cryptosporidium and diarrhoea. Archives of Disease in Childhood 60:608-609.

149. Isaacs, D.; Hunt, G.H.; Phillips, A.D.; Price, E.H.; Raafat, F.; Walker-Smith, J.A. (1985). Cryptosporidiosis in immunocompetent children. Journal of Clinical Pathology 38: 76-81.

150. Itakura, C.; Goryo, M.; Umemura, T. (1984). Cryptosporidial infection in chickens. Avian Pathology 13:487-499.

151. Jerrett, I.V.; Snodgrass, D.R. (1981). Cryptosporidia associated with outbreaks of neonatal calf diarrhoea. Australian Veterinary Journal 57:434-435.

152. Jervis, H.B.; Merrill, T.G.; Sprinz, H. (1966). Coccidiosis in the guinea pig small intestine due to a Cryptosporidium. American Journal of Veterinary Research 27:408-414.

153. Jokipii, A.M.M.; Hemila, M.; Jokipii, L. (1985). Prospective study of acquistion of Cryptosporidium, Giardia lamblia, and gastrointestinal illness. Lancet ii:487-489.

154. Jokipii, L.; Pohjolo, S.; Jokippi, A.M.M. (1983). Cryptosporidium: A frequent finding in patients with gastrointestinal symptoms. Lancet ii:358-361.

155. Jokipii, L.; Pohjola, S.; Jokipii, A.M.M. (1985). Cryptosporidiosis associated with traveling and giardiasis. Gastroenterology 89:838-842.

156. Jonas, C.; Deprez, C.; De Maubeuge, J.; Taelman, H.; Panzer, J.M.; Deltenre, M. (1983). Cryptosporidium in patient with acquired innumodeficiency syndrome. [Correspondence]. Lancet ii:964.

157. Jungmann, R.; Hiepe, T. (1983). Vorkommen und intravitaldiagnostik der kryptopsoridiose bei neugeboronen kalbern (kurzmitteilung). Monatshefte fur Veterinarmedizin 38:299-300.

158. Kansouzidou-Kanakoudis, A.; Danielides, B.; Arvanitidou-Vayonas, T. (1984). [Cryptosporidial enteritis.] Acta Microbiologica Hellenica 29:369-378.

159. Kennedy, G.A.; Kreitner, G.L.; Strafuss, A.C. (1977). Cryptosporidiosis in three pigs. Journal of the American Veterinary Medical Association 170:348-350.

160. Khalacheve, M.; Belchev, D. (1984). [Cryptosporidium oocysts in calves]. Veterinarna Sbirka 82:30-31.

161. Kiupel, H.; Bergmann, V. (1982). Nachweis von kryptosporidien bei kalbern mit diarroe. Monatshefte fur Veterinar-Medizin 37:392-393.

162. Kobiler, D.; Mirelman, D. (1980). Lectin activity in Entamoeba histolytica trophozoites. Infection and Immunity 29:221-225.

163. Koch, K.L.; Phillips, D.J.; Aber, R.C.; Current, W.L. (1985). Cryptosporidiosis in hospital personnel: Evidence for person-to-person transmission. Annals of Internal Medicine 102:593-596.

164. Koch, K.L.; Shankey, T.V.; Weinstein, G.S.; Dye, R.E.; Abt, A.B.; Current, W.L.; Eyster, M.E. (1983). Cryptosporidiosis in a patient with hemophilia, common variable hypogammaglobulinemia, and the acquired immunodeficiency syndrome. Annals of Internal Medicine 99:337-340.

165. Kocoshis, S.A.; Cibull, M.L.; Davis T.E.; Hinton, J.T.; Seip, M.; Banwell, J.G. (1984). Case Report: Intestinal and Pulmonary cryptosporidiosis in an infant with severe combined immune deficiency. Journal of Pediatric Gastroenterology and Nutrition 3:149-157.

166. Korsholm, H.; Henriksen, S.A. (1984). Infection with Cryptosporidium in a roe deer (Capreolus capreolus L.). Nordisk Veterinaermedicin 36:266.

167. Kovatch, R.M.; White, J.D. (1972). Cryptosporidiosis in two juvenile rhesus monkies. Veterinary Pathology 9:426-440.

168. Krogh, H.V.; Henriksen, S.A. (1985). Bovine cryptosporidiosis in Denmark. II. Cryptosporidia associated with neonatal calf diarrhea. Nordisk Veterinaermedicin 37:42-47.

169. Landsverk, T.; Tharaldsen J. (1984). Kryptosporidiose--arsak til diare hos kalv i Norge. Norsk Veterinaertidsskrift 96:451-455.

170. Langone, J. (1985). AIDS. Discover December 1985:28-53.

171. Lasser, K.H.; Lewin, K.J.; Ryning, F.W. (1979). Cryptosporidial enteritis in a patient with congenital hypogammaglobulinemia. Human Pathology 10:234-240.

172. Leece, J.G.; Balsbaugh, R.K.; Clare, D.A.; King, M.M. (1982). Rotavirus and hemolytic enteropathogenic Escherichia coli in weanling diarrhea of pigs. Journal of Clinical Microbiology 16:715-718.

173. Le Charpentier, Y.; Galian, A.; Messing, B.; Andreani, T.; Modigliani, R.; Lavergne, A.; Hoang, C.; Puel, F.; Houin, R. (1982). Diagnostic ultrastructural d'une infection intestinale humaine a Cryptosporidium sp. Annales de Pathologie 2:336-338.

174. Leek, R.G.; Fayer, R. (1984). Prevalence of Cryptosporidium infections, and their relation to diarrhea in calves on 12 dairy farms in Maryland. Proceedings of the Helminthological Society of Washington 51:360-361.

175. Leeuw, P.W.De; Moerman, A.; Pol, J.M.A.; Talmon, F.P.; Zijderveld, F.G.Van. (1984). Epidemiological observations on cryptosporidiosis in dairy herds in the Netherlands.

Proceedings of the 13th World Congress on Diseases of Cattle, Durban, South Africa 1:104-109.

176. Lefkowitch, J.H.; Krumholz, S.; Feng-Chen, K.C.; Griffin, P.; Despommier, D.; Brasitus, T.A. (1984). Cryptosporidiosis of the human small intestine: A light and electron microscopic study. Human Pathology 15:746-752.

177. Leger L. (1911). Caryospora simplex, coccidie monosporee et la classification des coccidies. Archiv fur Protistenkunde 22:71-88.

178. Lennette, E.H.; Balows, A.; Hausler, W.S.Jr.; Truant, J.R.; (1980). Manual of Clinical Microbiology Washington, D.C., American Society of Microbiology.

179. Leoni, A. (1984). La criptosporidiosi degli ovini. Atti della Societa Italiana delle Scienze Veterinarie 38:561-565.

180. Lerner, C.W.; Tapper, M.L. (1984). Opportunistic infections complicating the acquired immune deficiency syndrome: Clinical features of 25 cases. Medicine 63:155-164.

181. Levine, N.D. (1977). Nomenclature of Sarcocystis in the ox and sheep and of fecal coccidia of the dog and cat. Journal of Parasitology 63:36-51.

182. Levine, N.D. (1984). Taxonomy and review of the coccidian genus Cryptopsporidium (Protozoa, Apicomplexa). Journal of Parasitology 31:94-98.

183. Levine, N.D. (1985). Phylum II. Apicomplexa Levine, 1970. In An Illustrated Guide to the Protozoa, Lee, J.J.; Hutner, S.H.; Bovee, E.C.; editors. Lawrence, KS.; Society of Protozoologists pp. 322-357.

184. Levine, N.D.; Tadros, W. (1980). Named species and hosts of Sarcocystis (Protozoa: Apicomplex: Sarcocystidae) Systemic Parasitology 2:41-59.

185. Lewis, I.J.; Hart, C.A.; Baxby, D. (1985). Diarrhoea due to Cryptosporidium in acute lymphoblastic leukaemia. Archives of Diseases in Childhood 60:60-62.

186. Lin, J.A.; Shieh, H.K.; Wang, J.S. (1984). Studies on the avian cryptopsoridiosis: Pathological and electron microscopic observation. Taiwan Journal of Veterinary Medicine and Animal Husbandry 44:43-52.

187. Lindenbaum, J. (1965). Malabsorption during and after recovery from acute intestinal infection. British Medical Journal ii:326-329.

188. Lindsay, D.S.; Blagburn, B.L.; Sundermann, C.A.; Hoerr, F.J.; Ernst, J.A. (1986). Experimental Cryptosporidium infections in chickens: Oocyst structure and tissue specificity. American Journal of Veterinary Research 47:876-879.

189. Links, I.J. (1982). Cryptosporidial infection of piglets. Australian Veterinary Journal 58:60-62.

190. Lipp, D. (1985). Cryptosporidiosis in general practice. British Medical Journal 290:1788.

191. Lodinova, R.; Jouja, V.; Wagner, V. (1973). Serum immunoglobulins and coproantibody formation in infants after artifical intestinal colonization with Escherichia coli 083 and oral lysozyme administration. Pediatric Research 7:659-669.

192. Lopez-Brea, M.; Garcia-Picazo, L.; Del Rey, M.; Jimenez, M.L. (1985). Cryptosporidium in stool specimens in Madrid. [Correspondence]. Transactions of the Royal Society of Tropical Medicine and Hygiene 79:422-423.

193. Lumb, R.; Erlich, J.; Davidson, G.P. (1985). Cryptosporidia detection. Medical Journal of Australia 142:329-330.

194. Ma, P.; Kaufman, D.L.; Helmick, C.G.; D'Souza, A.J.; Navin, T.R. (1985). Cryptosporidiosis in tourists returning from the Caribbean. New England Journal of Medicine 312:647.

195. Ma, P.; Soave, R. (1983). Three-step examination for cryptosporidiosis in 10 homosexual men with protracted watery diarrhea. Journal of Infectious Diseases 147:824-828.

196. Ma.P.; Villanueva, T.G.; Kaufaman, D.; Gillooley, J.F. (1984). Respiratory cryptosporidiosis in the acquired immune deficiency syndrome. Use of modified cold Kinyoun and Hemacolor stains for rapid diagnosis. Journal of the American Medical Association 252:1298-1301.

197. Magnasco, E.J.; Odeon, A.C. (1982). Primera observacion de cyrptosporidios en terneros enfermos de diarrea neonatal en la Republica Argentina. Gaceta Veterinaria 44:670-673.

198. Magnasco, E.J.; Odeon, A.C. (1984). Cryptosporidios en la diarrea neonatal de los terneros. Veterinaria, Argentina 1:740-748.

199. Malebranche, R.; et al. (1983). Acquired Immune Deficiency Syndrome with severe gastrointestinal manifestations in Haiti. Lancet ii:873-878.

200. Maniuel, C.; Filipovich, A.; Snover, D.C. (1985).

Cryptopsoridiosis as a cause of diarrhea following bone marrow transplantation. Diseases of the Colon and Rectum 28:741-747.

201. Marcial, M.A.; Madara, J.L. (1986). Cryptosporidium: Cellular localization, structural analysis of absorptive cell-parasite membrane-membrane interactions in guinea pigs and suggestion of protozoan transport of M cells. Gastroenterology 90:583-594.

202. Mason, R.W.; Hartley, W.J. (1980). Respiratory cryptosporidiosis in a peacock chick. Avian Diseases 24:771-776.

203. Mason, R.W.; Hartley, W.J.; Tilt, L. (1981). Intestinal cryptosporidiosis in a kid goat. Australian Veterinary Journal 57:386-388.

204. Mata, L.; Bolanos, H.; Pizarro, D.; Vives, M. (1984). Criptosporidiosis en ninos de Costa Rica: estudio transveral y longitudinal. Revista de Biologia Tropical 32:129-135.

205. Mata, L.; Bolanos, H.; Pizarro, D.; Vives, M. (1984). Cryptosporidiosis in children from some highland Costa Rican rural and urban areas. American Journal of Tropical Medicine and Hygiene 33:24-29.

206. Mathan, M.; Venkastesan, S.; George, R.; Mathew, M.; Mathan, V.I. (1985). Cryptosporidium and diarrhoea in southern Indian children. Lancet ii:1172-1175.

207. Matovelo, J.A.; Landsverk, T.; Amaya Posada, G. (1984). Cryptosporidiosis in Tanzanian goat kids: Scanning and transmission electron microscopic observations. Acta Veterinaria Scandinavica 25:322-326.

208. McColl, D.; Mooney, T.J. (1984). Cryptosporidia in Tasmania. [Correspondence]. Medical Journal of Australia 141:900-901.

209. McKenzie, R.A.; Green, P.E.; Hartley, W.J.; Pollitt, C.C. (1978). Cryptosporidium in a red-bellied black snake (Pseudechis porphyriacus). [Correspondence]. Australian Veterinary Journal 54:365-366.

210. McNulty, M.S. (1985). Enteric infections in young animals. In Infectious Diarrhoea in the Young, Tzipori, S.; ed. Elsevier Science Publishers, Amsterdam (Biomedical Division) pp. 231-239.

211. Meisel, J.L.; Perera, D.R.; Meligro, C.; Rubin, C.E. (1976). Overwhelming watery diarrhea associated with a Cryptosporidium in an immunosuppressed patient. Gastroenterology 70:1156-1160.

212. Meuten, D.J.; Van Kruiningen, H.J.; Lein, D.H. (1974). Cryptosporidiosis in a calf. Journal of the American Veterinary Medical Association 165:914-917.

213. Miller, R.A.; Holmberg, R.E.Jr.; Clausen, C.R. (1983). Life-threatening diarrhea caused by Cryptosporidium in a child undergoing theraphy for acute lymphocytic leukemia. Journal of Pediatrics 103:256-259.

214. Miller, R.A.; Wasserheit, J.N.; Kirihara, J.; Coyle, M.B. (1984). Detection of Cryptosporidium oocysts in sputum during screening for myobacteria. Journal of Clinical Microbiology 20:1192-1193.

215. Modigliani, R.; Bories, C.; Le Charpentier, Y.; Salmeron, M.; Messing, B.; Galian, A.; Rambaud, J.C.; Lavergne, A.; Desportes, I.; Cochand-Prioleet, B. (1985). Diarrhoea and malabsorption in acquired immune deficinecy syndrome: A study of four cases with special emphasis on opportunistic protozoan infestations. Gut 26:179-187.

216. Montessori, G.A.; Bischoff, L. (1985). Cryptosporidiosis: a cause of summer diarrhea in children. Canadian Medical Association Journal 132:1285.

217. Moon, H.W.; Bemrick, W.J. (1981). Faecal transmission of calf cryptosporidia between calves and pigs. Veterinary Pathology 18:248-255.

218. Moon, H.W.; Schwartz, A.; Welch, M.J.; McCann, P.P.; Runnels, P.L. (1982). Experimental fecal transmission of human cryptosporidia to pigs, and attempted treatment with an ornithine decarboxylase inhibitor. Veterinary Pathology 19:700-707.

219. Moon, H.W.; Woode, G.N.; Ahrens, F.A. (1982). Attempted chemoprophylaxis of cryptosporidiosis in calves. Veterinary Record 110: 181.

220. Morin, M.S.; Lariviere, S.; Lallier, R. (1976). Pathological and microbiological observations made on spontaneous cases of acute neonatal calf diarrhea. Canadian Journal of Comparative Medicine 40:228-240.

221. Naciri, M.; Yvore, P.; Levieux, D. (1984). Cryptosporidiose experimentale du chevreau. Influence de la prise du colostrum. Essais de traitments. In Les maladies de la chevre. Colloque International, Niort, France, 9-11 october 1984 pp. 465-471.

222. Nagy, B.; Antal, A.; Lakner, J. (1980). Significance of intestinal cryptosporidiosis in calf diarrhea. In Proceedings of the 2nd International Symposium of Veterinary

Laboratory Diagnosticians, 24-26 June 1980, Lucerne, Switzerland. 3:431-434.

223. Nagy, B.; Antal, A.; Ratz, F. (1979). A borjak cryptosporidiosisanak magyarorszagi elofordulase. Magyar Allatorvosok Lapja 34:585-588.

224. Nagy, B.; Bozso, M.; Palfi, V.; Nagy, G.; Sahiby, M.A. (1984). Studies on cryptosporidial infection of goat kids. In Les maladies de la chevre. Colloque International, Niort, France, 9-11 octobre 1984. pp. 443-451.

225. Nagy, B.; Nagy, G.; Palfi, V.; Bozso, M. (1983). Occurence of cryptosporidia, rotaviruses, coronavirus-like particles and K99+ Escherichia coli in goat kids and lambs. In Proceedings of the Third International Symposium of the World Association of Veterinary Laboratory Diagnosticians. June 13-15, 1983. 2:525-531.

226. Nagy, B,; Pohlenz, J. (1982). Die bovine kryptopsoridiose. Diagnose und therapie. Tierarztliche Praxis 10:163-172.

227. Navin, T.R.; Juranek, D.D. (1984). Cryptosporidiosis: Clinical, epidemiolgic, and parasitologic review. Reviews of Infectious Diseases 6:313-327.

228. Nichols, G.L.; Thom, B.T. (1984). Screening for Cryptosporidium in stools. [Correspondence]. Lancet 1:734-735.

229. Nicolas, J.A.; Dubost, G.; Gayaud, C.; Noel, F. (1984). Importance des cryptosporidies dans les diarrhees neonatales du veau. Point Veterinaire 16:72-73.

230. Nime, F.A.; Burek, J.D.; Page, D.L.; Holscher, M.A.; Yardley, J.H. (1976). Acute enterocolitis in human being infected with the protozoan Cryptosporidium. Gastroenterology 70:592-598.

231. Nishikawa, H. (et al). (1984). [Four cases of cryptosporidial parasitization in chickens.] Journal of the Japan Veterinary Medical Association 37:667-669.

232. Nobel, T.A.; Kuttin, E.; Yakobson, B.; Perl, S. (1982). First diagnosis of bovine cryptosporidiosis in Isreal. Refuah Veterinarith 39:10-15.

233. O'Donoghue, P.J. (1985). Cryptosporidium infections in man, animals, birds and fish. Australian Veterinary Journal 62:253-258.

234. Orr, M.B.; Mackintosh, C.G.; Suttie, J.M. (1985). Cryptosporidiosis in deer calves. [Correspondence]. New

Zealand Veterinary Journal 33:151-152.

235. Overstreet, R.M.; Hawkins, W.E.; Fournie, J.W. (1984). The coccidian genus Calyptospora n.g. and family Calyptosporidae n. fam. (Apicomplexa), with members infecting primarily fishes. Journal of Protozoology 31:332-339.

236. Owen, R.L. (1984). Enteric disease in acquired immunodeficinecy syndrome (AIDS). In Current perspectives in parasitic disease. Proceedings of the Southeast Asian Symposium on parasitology and modern medicine held in Hong Kong, 9-12 December 1983. [Edited by Ko, R.C.]. pp. 133-142.

237. Panciera, R.J.; Thomassen, R.W.; Garner, F.M. (1971). Cryptosporidial infection in a calf. Veterinary Pathology 8:479-484.

238. Pavlasek, I. (1981). First record of Cryptosporidium sp. in calves in Czechoslovakia. Folia Parasitologica 28:187-189.

239. Pavlasek, I. (1982). Vyskyt Cryptosporidium sp. u nucene odporazenych telat a prubeh vylucovani oocyst tohoto prvoka u telat na dvon jarmach Jihoceskeho kraje. Veterinarni Medicina 27:729-740.

240. Pavlasek, I. (1983). Cryptosporidium sp. in Cyprinus carpio Linne, 1758 in Czechoslovakia. Folia Parasitologica 30:248.

241. Pavlasek, I. (1984). First record of developmental stages of Cryptosporidium sp. in various organs of experimentally infected mice and spontaneously infected calves. Folia Parasitologica 31:191-192.

242. Pavlasek, I. (1984). Ucinek dezinfekcnich prostredku na infekceschopnost oocyst Cryptosporidium sp. Ceskoslovenska Epidemiologie, Mikrobiologie, Imunologie 33:97-101.

243. Pavlasek, I. (1985). Prvni nalez spontanni kryptosporidiozni infekce kocky domaci v CSSR. Veterinarstvi 35:125-126.

244. Pavlasek, I. (1983). Vliv jednorazove dezinfekce farmy na prubeh kryptosporidioznich infekci telat. Veterinarni Medicina 28:449-455.

245. Pavlasek, I.; Nikitin, V.F. (1984). [Eimeria in calves on industrial farms] Moscow, USSR 5:44-45.

246. Pavlasek, I.; Nikitin, V.G. (1983). Finding of Cryptosporidium sp. in calves in the USSR. Folia Parasitologica 30:4.

247. Pavlasek, I.; Zikmund, B.; Klima, F. (1983). Vliv ruzneho zpusobu ustajeni telat po narozeni na vyskyt Cryptosporidium

sp. Veterinarni Medicina 28:31-36.

248. Pearson, G.R.; Logan, E.F. (1978). Demonstration of cryptosporidia in the small intestine of a calf by light, transmission electron and scanning electron microscopy. Veterinary Record 103:212-213.

249. Pearson, G.R.; Logan, E.F. (1983). The pathology of neonatal enteritis in calves with observations on E. coli, rotavirus, and Cryptosporidium. Annales de Recherches Veterinaires 14:422-425.

250. Pearson, G.R.; Logan, E.F.; McNulty, M.S. (1982). Distribution of cryptosporidia within the gastrointestinal tract of young calves. Research in Veterinary Science 33:228-231.

251. Perez-Schael, I.; Boher, Y.; Mata, L.; Perez, M.; Tapia, F.J. (1985). Cryptosporidiosis in Venezuelan children with acute diarrhea. American Journal of Tropical Medicine and Hygiene 34:721-722.

252. Pitlik, S.D.; Fainstein, V.; Rios, A.; Guarda, L.; Mansell, P.W.A.; Hersh, E.M. (1983). Cryptosporidial cholecystitis. [Correspondence]. New England Journal of Medicine 308:967.

253. Pivont, P.; Antoine, H.; Gregoire, R.; Bughin, J. (1981). Cryptosporidies chez un veau nouveau-ne. Annales de Medecine Veterinaire 125:557-559.

254. Pohjola, S. (1984). Negative staining method with nigrosin for the detection of cryptosporidial oocysts: A comparative study. Research in Veterinary Science 36:217-219.

255. Pohjola, S. (1984). Survey of cryptopsporidiosis in feces of normal healthy dogs. Norkisk Veterinaermedicin 36:189-190.

256. Pohlenz, J.; Bemrick, W.J.; Moon, H.W.; Cheville, N.F. (1978). Bovine cryptosporidiosis: A transmission and scanning electron microscopic study of some stages in the life cycle and of the host-parasite relationship. Veterinary Pathology 15:417-427.

257. Pohlenz, J.; Moon, H.W.; Cheville, N.F.; Bemrick, W.J. (1978). Cryptosporidiosis as a probable factor in neonatal diarrhea of calves. Journal of the American Veterinary Medical Association 172:452-457.

258. Pol, J.M.; Schreuder, B.E.C.; Kok, G.J.; de Leeuw P.W. (1982). Cryptosporidium: A new factor in the etiology of neonatal diarrhoea in calves. Tijdschrift voor Diergeneeskunde 107:503-510.

259. Poonacha, K.B.; Pippin, C. (1982). Intestinal cryptosporidiosis in a cat. Veterinary Pathology 19:708-710.

260. Portnoy, D.; Whiteside, M.E.; Buckley, E.; MacLeod. C.L. (1984). Treatment of intestinal cryptosporidiosis with spiramycin. Annals of Internal Medicine 101:202-204.

261. Powell, H.S.; Holsher, M.A.; Heath, J.E.; Beesley, F.F. (1976). Bovine cryptosporidiosis (a case report). Veterinary Medicine and Small Animal Clinician 71:205-207.

262. Proctor, S.J. (1974). Cryptosporidium anserinum sp. n. (Sporozoa) in a domestic goose Anser anser L. from Iowa. Journal of Protozoology 21:664-666.

263. Radostits, O.M. (1985). A veterinary clinician's perspective of diarrhea in neonatal food-producing animals. In Infectious Diarrhea in the Young, Tzipori, S. ed.; Elsevier Science Publishers, Amsterdam (Biomedical Division) pp. 9-18.

264. Radu, S.; Dan S. (1985). Identificarea cryptosporidiilor la puii broiler de curca si de gaina in Romania. Revista de Cresterea Animalelor 6:55-58.

265. Rahman, A.S.M.H.; Sanyal, S.C.; Al-Mahmud, K.A.; Sobhan A. (1985). Cryptosporidium diarrhea in calves and their handlers in Bangladesh. Indian Journal of Medical Research 82: 510-515.

266. Rahman, A.S.M.H.; Sanyal, S.C.; Al-Mahmud, K.A.; Sobhan, A.; Hossain, K.S.; Anderson, B.C. (1984). Cryptosporidiosis in calves and their handlers in Bangladesh. Lancet ii:221.

267. Ramisse, J.; Lepareur, F.; Poudelet, M.; Brebion, M.; Moinet, I. (1984). Mise en evidence de rotavirus et de cyrptosporidies dan les diarrhees des jeunes agneaux. Point Veterinaire 16:73-75.

268. Ranck, F.M.Jr. (1979). Cryptosporidiosis -- a possible coccidial respiratory pathogen in turkeys. In Proceedings of 28th Western Poultry Disease Conference and 13th Poultry Health Symposium, March 19-22, 1979 pp. 49-51.

269. Randell, C.J. (1982). Cryptosporidiosis of the bursa of Fabricius and trachea in broilers. Avian Pathology 11:95-102.

270. Randell, C.J. (1986). Conjunctivitis in pheasants associated with cryptosporidial infection. Veterinary Record 118:211.

271. Ratnam, S.; Paddock, J.; McDonald, E.; Whitty, D.; Jong, M.; Cooper, R. (1985). Occurrence of Cryptosporidium oocysts in

fecal samples submitted for routine microbiological examination. Journal of Clinical Microbiology 22:402-404.

272. Reduker, D.W.; Speer, C.A. (1985). Factors influencing excystation in Cryptosporidium oocysts in cattle. Journal of Parasitology 71:112-115.

273. Reduker, D.W.; Speer, C.A.; Blixt, J.A. (1985). Ultrastructural changes in the oocyst wall during excystation of Cryptosporidium parvum (Apicomplexa: Eucoccidiorida). Canadian Journal of Zoology 63:1892-1896.

274. Reese, N.C.; Current, W.L.; Ernst, J.V.; Bailey, W.S. (1982). Cryptosporidiosis of man and calf: A case report and results of experimental infections in mice and rats. American Journal of Tropical Medicine and Hygiene 31:226-229.

275. Rehg, J.E.; Lawton, G.W.; Pakes, S.P. (1979). Cryptosporidium cuniculus in the rabbit (Oryctolagus cuniculus). Laboratory Animal Science 29:656-660.

276. Reinemeyer, C.R.; Kline, R.C.; Stauffer, G.D. (1984). Absence of Cryptosporidium oocysts in faeces of neonatal foals. Equine Veterinary Journal 16:217-218.

277. Ribeiro, C.D.; Palmer, S.R. (1986). Family outbreak of cryptosporidiosis. British Medical Journal 292:377.

278. Runnels, P.L.; Moon, H.W.; Whipp, S.C.; Mattheras, P.J.; Woode, G.N. (1980). In: Proceedings of the Third International Symposium on Neonatal Diarrhoea., Acres, S.D.; Forman, A.J.; Fast, H.; eds. VIDO, Saskatchewan pp. 343-358.

279. Ryan, M.J.; Sundberg, J.P.; Sauershell, R.J.; Todd, K.S. (1986). Cryptosporidium in a wild cottontail rabbit. Journal of Wildlife Diseases 22:267-275.

280. Ryley, J.F. (1973). Cytochemistry, physiology, and biochemistry. In: The Coccidia, Hammond, D.M.; Long, P.L.; eds. Baltimore, University Park Press pp. 145-181.

281. Sanford, S.E.; Josephson, G.K.A. (1982). Bovine cryptosporidiosis: Clinical and pathological findings in forty-two scouring neonatal calves. Canadian Veterinary Journal 23:343-347.

282. Schmidt, U.; Neinhoff, H. (1982). Kryptosporidiose beim schwein. Deutsche Tierarztliche Wochenschrift 89:437-439.

283. Schmitz, J.A.; Smith, D.H. (1975). Cryptosporidium infection in a calf. Journal of the American Veterinary Medical

Association 167:731-732.

284. Shahid, N.S.; Rahman, A.S.M.H.; Anderson, B.C.; Mata, L.J.; Sanyal, S.C. (1985). Cryptosporidiosis in Bangladesh. British Medical Journal 290:114-115.

285. Sheather, A.L. (1923). Detection of worm eggs in the faeces of animals and some experiences in the treatment of parasitic gastritis in cattle. Journal of Comparative Pathology and Theraphy 36: 71-90.

286. Sisk, D.B.; Gosser, H.S.; Styer, E.L.; Branch, L.O. (1984). Intestinal cryptosporidiosis in two pups. Journal of the American Veterinary Medical Association 184:835-836.

287. Skeels, M.R.; Sokolow, R.; Vance Hubbard, C.; Foster, L.R. (1985). Screening for co-infection with Cryptopsporidium and Giardia in Oregon public health clinic patients. American Journal of Public Health 76:270-273.

288. Slavin, D. (1955). Cryptosporidium meleagridis (sp. nov.) Journal of Comparative Pathology and Theraphy 65:262-266.

289. Sloper, K.S; Dourmashkin. R.R.; Bird, R.B.; Slavin, G.; Webster, A.D.B. (1982). Chronic malabsorption due to cryptosporidiosis in a child with immunoglobulin deficiency. Gut 23:80-82.

290. Snodgrass, D.R.; Angus, K.W.; Gray, E.W. (1984) Experimental cryptosporidiosis in germfree lambs. Journal of Comparative Pathology 94:141-152.

291. Snodgrass, D.R.; Angus, K.W.; Gray, E.W.; Keir, W.A.; Clerihew, L.W. (1980). Cryptosporidia associated with rotavirus and an Escherichia coli in an outbreak of calf scour. Veterinary Record 106:458-460.

292. Snyder, B. (1970) Pitfalls in the gram stain. Laboratory medicine p. 41.

293. Snyder, J.D.; Merson, M.H. (1982). The magnitude of the global problem of acute diarrhoeal disease: a review of active surveillance data. Bulletin of the World Health Organization 60:605-613.

294. Snyder, S.P.; England, J.J.; McChesney, A.E. (1978). Cryptosporidiosis in immunodeficient Arabian foals. Veterinary Pathology 15:12-17.

295. Soave, R.; Ma, P. (1985). Cryptosporidiosis. Archives of Internal Medicine 145:70-72.

296. Soph, M.; Lavicka, M.; Zajicek, D. (1984). Histologicka

diagnostika kryptosporidiozy u telat; overeni vyskytu u telat porazenych MPZ Praha-Pisnice. Veterinarstvi 34:107-108.

297. Soule, C.; Plateau, E.; Perret, C.; Chermette, R.; Feton, M.M. (1983). Observation de cryptosporidies chez le poulain. Note preliminaire. Recueil de Medecine Veterinaire 159:719-720.

298. Spillman, S.K.; Eckert, J.; Merk, W.; Frey, R. (1986). Cryptosporidium in calves in Switzerland. Schweizer Archiv fur Tierkeilkunde 128:111-119.

299. Stemmermann, G.N.; Hayashi, G.; Glober, G.A.; Oishi, N.; Frankel, R.I. (1980). Cryptosporidiosis. Report of a fatal case complicated by disseminated toxoplasmosis. American Journal of Medicine 69:637-642.

300. Sterling, C.R.; Seegar, K.; Sinclair, N.A. (1986). Cryptosporidium as a causative agent of traveler's diarrhea. Journal of Infectious Diseases 153:380-384.

301. Sundberg, J.P.; Hill, D.; Ryan, M.J. (1982). Cryptosporidiosis in a gray squirrel. Journal of the American Veterinary Medical Association 181:1420-1422.

302. Sutherland, R.J. (1982). Gastrointestinal disturbances in goat kids. [Laboratory Reports]. Surveillance 9:25-27.

303. Szabo, J.R.; Moore, K. (1984). Cryptosporidiosis in a snake. Veterinary Medicine and Small Animal Clinician 79:96-98.

304. Tarwid, J.N.; Cawthorn, R.J.; Riddell, C. (1985). Cryptosporidiosis in the respiratory tract of turkeys in Saskatchewan. Avian Diseases 29:528-532.

305. Taylor, D.N.; Echeverria, P. (1986). When does Cryptosporidium cause diarrhoea? Lancet 1:320.

306. Taylor, J.P.; Perdue, J.N.; Dingley, D.; Gustafson, T.L.; Patterson, M.; Reed, L.A. (1985). Cryptosporidiosis outbreak in a day-care center. American Journal of Diseases of Children 139:1023-1025.

307. Tewari, S.G.; Tandon, B.N. (1974). Functional and histological changes of the small bowel in patients with Giardia lamblia infections. Indian Journal of Medical Research 62:689-695.

308. Tham, V.L.; Kniesberg, S.; Dixon, B.R. (1982). Cryptosporidiosis in quails. Avian Pathology 11:619-626.

309. Triffit, M.J. (1925). Observations on 2 new sp. of coccidia

parasitic in snakes. Protozoology 1:19-26.

310. Trotti, G.C.; Quesada, A. (1983). Primo reperto di Cryptosporidium sp. in bufali italiani (Bubalus bubalis). Atti della Societa Italiana delle Scienze Veterinarie 37:737-740.

311. Tsai, S.S.; Ho, L.F.; Chang, C.F.; Chu, R.M. (1983). Cryptosporidiosis in domestic birds. Chinese Journal of Microbiology and Immunology 16:307-313.

312. Tyzzer, E.E. (1907). A sporozoan found in the peptic glands of the common mouse. Proceedings of the Society for Experimental Biology and Medicine 5:12-13.

313. Tyzzer, E.E. (1910). An extracellular coccidium, Cryptosporidium muris (Gen. et sp. nov.) of the gastric glands of the common mouse. Journal of Medical Research 23:487-509.

314. Tyzzer, E.E. (1912). Cryptosporidium parvum (sp. nov.), a coccidium found in the small intestine of the common mouse. Archiv fur Protistenkunde 26:394-418.

315. Tyzzer, E.E. (1929). Coccidiosis in gallinaceous birds. American Journal of Hygiene 10:269-383.

316. Tzipori, S. (1983). Cryptosporidiosis in animals and humans. Microbiological Reviews 47:84-96.

317. Tzipori, S. (1985). Cryptosporidium: Notes on epidemiology and pathogenesis. Parasitology Today 1:159-165.

318. Tzipori, S. (1985). The relative importance of enteric pathogens affecting neonates of domestic animals. In: Advances in Veterinary Science and Comparative Medicine, Cornelious, C.F. and Simpson, C.F.; eds. 29:103-206.

319. Tzipori, S.; Angus, K.W. (1981). Cryptosporidiosis in calves: significance within the enteritis syndrome and diagnosis of infection. In: Laboratory diagnosis in neonatal calf and pig diarrhoea.(Current topics in veterinary medicine and animal science) De leeuw, P.W.; Guinee, P.A.M.; eds. 13:196-198.

320. Tzipori, S.; Angus, K.W.; Campbell, I.; Clerihew, L.W. (1981). Diarrhea due to Cryptosporidium infection in artifically reared lambs. Journal of Clinical Microbiology 14: 100-105.

321. Tzipori, S.; Angus, K.W.; Campbell, I.; Gray, E.W. (1980). Cryptosporidium: Evidence for a single-species genus. Infection and Immunity 30:884-886.

322. Tzipori, S.; Angus, K.W.; Campbell, I.; Gray, E.W. (1982). Experimental infection of lambs with Cryptosporidium isolated from a human patient with diarrhoea. Gut 23:71-74.

323. Tzipori, S.; Angus, K.W.; Campbell, I.; Sherwood, D. (1981). Diarrhea in young red deer associated with infection with Cryptosporidium. Journal of Infectious Diseases 144:170-175.

324. Tzipori, S.; Angus, K.W.; Gray, E.W.; Campbell, I. (1980). Vomiting and diarrhea associated with cryptosporidial infection New England Journal of Medicine 303:818.

325. Tzipori, S.; Angus, K.W.; Gray, E.W.; Campbell, I.; Allan, F. (1981). Diarrhea in lambs experimentally infected with Cryptosporidium isolated from calves. American Journal of Veterinary Research 42:1400-1404.

326. Tzipori, S.; Campbell, I. (1981). Prevalence of Cryptopsporidium antibodies in 10 animal species. Journal of Clinical Microbiology 14: 455-456

327. Tzipori, S.; Campbell, I.; Angus, K.W. (1982). The therapeutic effect of 16 antimicrobial agents on Cryptosporidium infection in mice. Australian Journal of Experimental Biology and Medical Science 60:187-190.

328. Tzipori, S.; Campbell, I,; Sherwood, D.; Snodgrass, D.R.; Whitelaw, A. (1980). An outbreak of calf diarrhoea attributed to cryptosporidial infection. Veterinary Record 107:579-580.

329. Tzipori, S.; Chandler, D.; Makin, T.; Smith, M. (1980). Escherichia coli and rotavirus infections in four-week-old gnotobiotic piglets fed milk or dry food. Australian Veterinary Journal 56:279-284.

330. Tzipori, S.; Larsen, J.; Smith, M.; Luefl, R. (1982). Diarrhoea in goat kids attributed to Cryptosporidium infection. Veterinary Record 111:35-36.

331. Tzipori, S.; Makin, T.J.; Smith, M.L.; Krautil, F.L. (1981). Clinical manifestations of diarrhea in calves infected with rotavirus and enterotoxigenic Escherichia coli. Journal of Clinical Microbiology 13:1011-1017.

332. Tzipori, S.; Makin, T.J.; Smith, M.L.; Krautil, F.L. (1982). Enteritis in foals induced by rotavirus and enterotoxigenic Escherichia coli. Australian Veterinary Journal 58:20-23.

333. Tzipori, S.; McCartney, E.; Lawson, G.H.K.; Rowland, A.C.; Campbell, I. (1981). Experimental infection of piglet with Cryptosporidium. Research in Veterinary Science 31:358-368.

334. Tzipori, S.; Sherwood, D.; Angus, K.W.; Campbell,I.; Gordon,

M. (1981). Diarrhea in lambs: experimental infections with enterotoxigenic Escherichia coli rotavirus, and Cryptosporidium sp. Infection and Immunity 33:401-406.

335. Tzipori, S.; Smith, M.; Birch, C.; Barnes, G.; Bishop, R. (1983). Cryptosporidiosis in hospital patients with gastroenteritis. American Journal of Tropical Medicine and Hygiene 32:931-934.

336. Tzipori, S.; Smith, M.; Halpin, C.; Angus, K.W.; Sherwood, D.; Campbell, I. (1983). Experimental cryptosporidiosis in calves: Clinical manifestations and pathological findings. Veterinary Record 112:116-120.

337. Tzipori,S.; Smith, M.; Makin, T.; Halpin, C. (1982). Enterocolitis in piglets caused by Cryptosporidium sp. purified from calf faeces. Veterinary Parasitology 11:121-126.

338. Ungar, B.L.P; Soave, R.; Fayer, R.; Nash, T.E. (1986). Enzyme Immunoassay Detection of Ig M or G antibody to Cryptosporidium in immunocompetant and immunocompromized individuals. Journal of Infectious Diseases 153:570-579.

339. Upton, S.J.; Current, W.L. (1985). The species of Cryptosporidium (Apicomplexa: Cryptosporidiidae) infecting mammals. Journal of Parasitology 71:625-629.

340. Van Winkle, T.J.; 1985. Cryptosporidiosis in young artiodactyls. Journal of the American Veterinary Medical Association 187:1170-1178.

341. Veldhuyzen Van Zanten, S.J.O.; Lange, J.M.A.; Sauerwein, H.P.; Rijpstra, A.C.; Laarman, J.J.; Rietra, P.J.G.M.; Danner, S.A. (1984). Amprolium for coccidiosis in AIDS. [Correspondence]. Lancet ii:345-346.

342. Vetterling, J.M.; Jervis, H.R.; Merrill, T.G.; Sprinz, H. (1971). Cryptosporidium wrairi sp. n. from guinea pigs Cavia porcellus with an emendation of the genus. Journal of Protozoology 18:243-247.

343. Viring, S.; Bornstein, S.; Jacobsson, S.O.; Strom, C.H. (1985). Cryptosporidier pavisade vid ett besattningsutbrott av kalvdiarre. Svensk Veterinartidning 37:24-26.

344. Visvesvara, G.S.; Smith, P.D.; Healy, G.R.; Brown, W.R. (1980). An immunofluorescence test to detect serum antibodies to Giardia lamblia. Annals of Internal Medicine 93:802-805.

345. Vitovec, J. (1982). Aspekty morfologicke identifikace kryptosporidii ve strevech telat no urovni svetelne a

radkovaci elektronove mikroskopie. Veterinarni Medicini 27:419-424.

346. Vitovec, J. (1984). Enterokolitida pri spontannich kryptosporidiovych infekcich telat. Veterinarni Medicina 29:411-417.

347. Walsh, J.A.; Warren, K.S. (1979). Selective primary health care. New England Journal of Medicine 301:967-974.

348. Weber, J. (1985). Sexually acquired parasitic infections in homosexual men. Parasitology Today 1:93-95.

349. Weikel, C.S.; Johnston, L.I.; Auxiliadora de Sousa, M.; Guerrant, R.L. (1985). Cryptosporidiosis in northeastern Brazil: Association with sporadic diarrhea. Journal of Infectious Diseases 151:963-965.

350. Weinstein, L.; Edelstein, S.M.; Madara, J.L.; Falchuk, R.; McManus, B.M.; Trier, J.S. (1981). Intestinal cryptosporidiosis complicated by disseminated cytomegalovirus infection. Gastroenterology 81:584-591.

351. Weisburger, W.R.; Hutcheon, D.F.; Yardley, J.H.; Roche, J.C.; Hillis, W.D.; Charache, P. (1979). Cryptosporidiosis in an immunosuppressed renal transplant recipient with IgA deficinecy. American Journal of Clinical Pathology 72:473-478.

352. Wetzel, R. (1938). Ein neues coccid (Cryptosporidium vulpis sp. nov.) aus dem rotfuch. Archiv fur Wissenschaftliche und Praktische 74:39-40.

353. Whittington, R.J.; Wilson, J.M. (1985). Cryptosporidiosis of the respiratory tract in a pheasant. Australian Veterinary Journal 62:284-285.

354. Willson, P.J.; Acres, S.D. (1982). A comparison of dichromate flotation and fecal smears for diagnosis of cryptopsoridiosis in calves. Canadian Veterinary Journal 23:240-246.

355. Wilson, D.W.; Day, P.A.; Brummer, M.E.G. (1984). Diarrhea associated with Cryptosporidium spp. in juvenile macaques. Veterinary Pathology 21:447-450.

356. Wilson, R.B.; Holscher, M.A.; Lyle, S.J. (1983). Cryptosporidiosis in a pup. Journal of the American Veterinary Medical Association 183:1005-1006.

357. Wolfson, J.S.; Hopkins, C.C.; Weber, D.J.; Richter, J.M.; Waldron, M.A.; McCarthy, D.M. (1984). An association between Cryptosporidium and Giardia in stool. [Correspondence]. New

England Journal of Medicine 310:788.

358. Wolfson, J.S.; Richter, J.M.; Waldron, M.A.; Weber, D.J.; McCarthy, D.M.; Hopkins, C.C. (1985). Cryptosporidiosis in immunocompetent patients. New England Journal of Medicine 312:1278-1282.

359. Wyllie, A.S. (1984). Cryptosporidiosis. British Medical Journal 298:1383-1384.

360. Zar, F.; Geiseler, J.; Brown, V.A. (1985). Asymptomatic carriage of Cryptosporidium in stool of a patient with AIDS. Journal of Infectious Diseases 151:195.