An experimental model for edema disease (*Escherichia coli* enterotoxemia) in swine

by

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A Thesis Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
MASTER OF SCIENCE

Major: Veterinary Pathology

Approved:

Iowa State University
Ames, Iowa

1989

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INTRODUCTION

Diseases caused by the bacterium Escherichia coli, are economically important in pigs. Colibacillosis in pigs can be divided into neonatal diarrhea, postweaning diarrhea and edema disease, as gastrointestinal forms (Whipp 1989b) and agalactia, endocarditis, abortion and puerperal septicemia, as extraintestinal forms (Sojka 1965).

Edema disease was first described in Ireland (Shanks 1938) and shortly afterwards in Great Britain (Hudson 1938) and other parts of the world. The disease occurs with varying incidence throughout the world (Nielsen 1986). Bertschinger (1987) reported that edema disease was diagnosed in 13-21% of the swine necropsied during the preceding 10 years in Zurich, Switzerland. Weikl (1959) diagnosed edema disease in 39% of 2,393 diseased piglets examined in Germany. The incidence of edema disease may be lower in North America, but there are no specific figures available. The disease is not reportable and can be diagnosed readily without the help of specialized laboratories. A survey by a private company [Swine Consultant, Jan. 1989 (Norden Laboratories, Lincoln, NE)] showed that edema disease was diagnosed infrequently by most practitioners and the incidence seemed to be decreasing in the United States.

The pathogenesis of edema disease is not completely understood. It is widely accepted, however, that bacteria adhering to the intestinal epithelium produce a toxin which is absorbed into the circulation (Bachmann 1988, Bertschinger 1987, Clugston et al. 1974a, Timoney 1950). Small blood vessels, especially arterioles, are thought to be damaged by the toxin. Vascular damage apparently leads to edema in the affected organs (Clugston et al. 1974b, Drommer 1976, Kurtz et al. 1969).

The objective of the research reported in this thesis was to establish a model for the experimental reproduction of edema disease in 3-week-old piglets. A model is needed for further research on edema disease--pathogenesis and prevention strategies.

An unpublished pilot study was performed by Dr. H. W. Moon, National Animal Disease Center, Ames, IA, and was used as a basis for the research reported here. In the pilot study, 18 healthy pigs were inoculated with a Shiga-like toxin II variant (SLT-IIv)-producing E. coli strain (NADC #2228) 1 day after weaning at three weeks of age. Two pigs, necropsied 2 days post-inoculation, had no gross or microscopic lesions observed. Fifteen of the 16 remaining pigs had diarrhea and 7 pigs had clinical signs of edema disease. Of the 7 pigs with clinical edema disease, 1 pig died and others were depressed

or showed various signs of neurological dysfunction ranging from walking in circles to twisting their heads sideways. Of the 4 pigs with clinical edema disease that were killed for necropsy, 1 pig had edema in the stomach wall, 3 pigs had lacy fibrin strands free in the peritoneal cavity and 3 pigs had swollen and congested ileal lymph nodes. There was froth in the trachea of 1 pig. Hemolytic E. coli was the predominant fecal flora in 18/18 pigs 1 day post-inoculation and in 13/16 pigs 5 days later. The experiment was terminated at 14 days post-inoculation, and all 16 pigs that were necropsied later than 2 days post-inoculation had necrosis of myocytes of arteries and arterioles in various organs. Microscopic lesions were most consistently found in the brains of affected pigs. Results from the pilot study were used to develop the model described in this thesis.

This project was reviewed and approved by the National Animal Disease Center - National Veterinary Services Laboratories Animal Care and Use Committee. Fabian Martin Kausche, the candidate for the Master of Science, was the principal investigator in the study.

LITERATURE REVIEW

Introduction

Edema disease occurs as a contagious disease of pigs mainly in the early postweaning period (Nielsen 1986, Timoney 1950). It was first described in Ireland in 1938 (Shanks 1938) and shortly afterwards in Great Britain (Hudson 1938). The disease syndrome is widespread in Europe, Africa, Asia and America. Its incidence seems to be higher in Europe than in North America (Nielsen 1986). Because the disease is not reportable and can be diagnosed easily without the help of specialized laboratories (Moon, personal communication), the economic impact of this disease can only be estimated. In 1959 edema disease was diagnosed in 39% of 2,393 piglets necropsied during a 5 year period in a specialized laboratory in southern Germany (Weikl 1959). Thirty percent of all pigs necropsied in 1964 in a federal institute in Germany were diagnosed as having edema disease (Brack 1965). Bertschinger (1987) reported edema disease as the diagnosis in 13-21% of the swine necropsy material presented during the preceding 10 years in Zurich, Switzerland. No incidence data are available for the United States.

Edema disease is associated with enterotoxemia subsequent to enteric infection with certain Escherichia coli serotypes. Other names coined for this disease are "Escherichia coli enterotoxemia", "bowel edema" and "gut edema" (Nielsen 1986). Edema disease is part of the postweaning enteric colibacillosis syndrome. Enteric diseases caused by E. coli can be divided into enterotoxic, enterotoxemic, locally invasive and septicemic forms (Moon 1974). A division of diarrheagenic E. coli was made by Levine (1987). He identified 5 main categories in humans: (1) enterotoxigenic, (2) enteroinvasive, (3) enteropathogenic, (4) enterohemorrhagic and (5) enteroadherent E. coli. Enterotoxigenic strains cause disease by production of enterotoxins, and enteroinvasive strains cause disease by invasion of intestinal epithelium. Strains that cause enteric disease but do not produce classical enterotoxins and are not invasive are classified as enteropathogenic (Okerman 1987). Enterohemorrhagic strains cause hemorrhagic colitis in humans (Okerman 1987). Enteroadherent strains clearly adhere to intestinal epithelial cells and cause diarrheal disease, but they do not belong to any other category. The grouping of strains into this class is preliminary, pending further information on their virulence mechanisms (Levine 1987). Only enterotoxigenic (ETEC) and enteropathogenic (EPEC) E. coli are

described by Okerman (1987) as being prevalent in animals. Okerman (1987) interprets attaching and effacing E. coli (AEEC) and solely verotoxigenic E. coli (VTEC) as subgroups of EPEC. AEEC strains adhere intimately to intestinal epithelial cells and efface microvilli (Moon et al. 1983). VTEC strains produce cytotoxins that are toxic to Vero cells, an African green monkey kidney cell line. Edema disease is caused by porcine E. coli that produce a Vero toxin (VTEC) (Okerman 1987).

Epidemiology

Edema disease occurs most often in young pigs 1 to 2 weeks after weaning but may also affect suckling and older pigs (Austvoll 1957, Brack 1965, Kernkamp et al. 1965, Nielsen 1986, Timoney 1950). Morbidity in an affected herd varies but is usually around 15% (Brack 1965, Kernkamp et al. 1965, Nielsen 1986). High morbidity rates are reported in affected litters, (Bertschinger et al. 1986, Nielsen 1986, Timoney 1950), and some sows produce several affected litters (Kernkamp et al. 1965). Bertschinger (1984b, 1986) hypothesized that there was a genetic influence of the boar on the susceptibility of the pigs. His observations on 5,307 piglets over a 5 year period, revealed a significant influence of the boar on mortality due to edema disease. He

hypothesized that the genetic trait for susceptibility may be due to a recessive gene expressing with incomplete penetrance. Expression of susceptibility is highly influenced by the environment. Such genetically controlled susceptibility would be comparable to the genetically controlled intestinal receptor described for the K88 pili of diarrheagenic E. coli (Bertschinger et al. 1986, Nielsen 1986). The idea of a genetic influence on susceptibility of pigs to edema disease is also suggested by Nielsen (1986) and Smith and Halls (1968), who report varying degrees of susceptibility to experimental edema disease in piglets from different herds.

The mortality in clinically affected pigs usually ranges from 50 to 90% (Nielsen 1986) but may reach 100% (Brack 1965, Timoney 1950). Kernkamp et al. (1965) reported 10% mortality in an affected herd, but also stated that only 36% of pigs that develop clinical edema disease recover. The disease does not recur in a pig that has been affected once and recovered (Bertschinger et al. 1984a, Kernkamp et al. 1965, Nielsen 1986, Smith and Halls 1968).

Edema disease occurs most frequently in spring and late summer/early fall (Brack 1965, Kernkamp et al. 1965, Timoney 1950). The duration of the disease in affected herds is approximately 1 week (Kernkamp et al. 1965, Nielsen 1986). Litters are often affected clinically only for 3 days (Sweeney

1976 as cited from Nielsen 1986) and the disease ends as abruptly as it starts (Kernkamp et al. 1965, Nielsen 1986, Timoney 1950).

Clinical Signs

Clinical signs associated with outbreaks of edema disease are characteristic, and vary from peracute death without obvious clinical signs to moderate neurologic disorders (Nielsen 1986, Timoney 1950). Often the most thrifty pigs in the pen are affected (Brack 1965, Kernkamp et al. 1965, Nielsen 1986, Schofield and Robertson 1955, Weikl 1959), but Timoney (1950) and Bertschinger et al. (1984a) also report cases in unthrifty pigs. Clinically affected pigs in the same pen in which fatal disease occurred can show neurological signs ranging from a swaying or staggering gait to complete recumbency (Nielsen 1986, Timoney 1950). Pigs with muscular incoordination may knuckle over at the fetlocks of the forelimbs (Nielsen 1986), or may have fine muscular tremors sometimes progressing to clonic convulsions. Pigs at the terminal stage of the disease can be found lying on their side and making "paddling" movements with their legs (Kernkamp et al. 1965). Affected pigs often appear constipated (Nielsen 1986, Timoney 1950) or, if the E. coli strain also produces enterotoxin, may have diarrhea (Bertschinger and Pohlenz 1983,

Gannon et al. 1988, Nielsen 1986, Schofield and Robertson 1955, Schulz and Reichel 1964, Smith and Halls 1968). Timoney (1950) described a characteristic hoarse squeal in affected pigs. Due to laryngeal edema (Nielsen 1986) or nervous dysfunction (Timoney 1950), the squeal becomes hoarse and often resembles a bark. Clinically, a majority of affected pigs present with visible edema of the eyelids (Nielsen 1986, Timoney 1950). Some pigs are reported as being semiconscious (Kernkamp et al. 1965) or comatose (Kurtz et al. 1969). The body temperature in most cases is not elevated and may be subnormal, but fever may occur early in the course of the disease (Brack 1965, Nielsen 1986, Timoney 1950). Death occurs in most pigs within the first 24 hours after the onset of clinical signs, but may occur any time from minutes to several days after the first signs are seen (Nielsen 1986).

Pathological Changes

Macroscopic lesions

Pigs affected by clinical edema disease present with characteristic lesions at necropsy which include edema of the eyelids and forehead (Brack 1965, Kurtz et al. 1969, Nielsen 1986, Timoney 1950, Weikl 1959) and irregular reddening of the ventral trunk (Nielsen 1986). There may be effusion in abdominal, pleural and pericardial cavities (Nielsen 1986,

Timoney 1950) and fine fibrin strands on viscera (Timoney 1950).

Submucosal edema in various abdominal organs is common. The organs usually affected include the cardiac region of the stomach (Brack 1965, Clugston et al. 1974b, Kurtz et al. 1969, Nielsen 1986, Weikl 1959), the submucosa and mesentery of the small and large intestine (Brack 1965, Timoney 1950), and intestinal lymph nodes (Kurtz et al. 1969, Timoney 1950). These nodes may be dark red (Brack 1965, Nielsen 1986, Timoney 1950) and may have petechia (Brack 1965). The stomach of affected pigs is usually full of relatively dry feed while the small intestine is empty (Brack 1965, Nielsen 1986, Smith and Halls 1968). Lesions typical for postweaning diarrhea attributable to E. coli may be present additionally if the E. coli strain causing the edema disease lesions also produces enterotoxins (Nielsen 1986).

Kurtz et al. (1969) describe occasional macroscopic malacia in the brain which often is bilaterally symmetric; in other cases it is unilateral and segmental. Malacia can extend from the medulla oblongata proximal through the caudate nucleus. Timoney (1950) and Brack (1965) describe petechia in the heart, and Brack (1965) and Weikl (1959) state that the heart muscle often appears focally pale. The lungs may have

grossly visible edema of the interstitium and froth may be present in major bronchi as a sign of alveolar edema (Brack 1965, Johannsen 1974, Nielsen 1986, Timoney 1950).

In addition to the characteristic lesions of edema disease described above, many authors mention lesions usually associated with endotoxemia in pigs that died of edema disease. Edema of the gall bladder (Nielsen 1986), degeneration of the kidney with marked hyperemia, intertubular hemorrhages and hyaline casts in tubules (Nagy et al. 1968) and petechial hemorrhages beneath serous membranes of the mesentery (Nagy et al. 1968) have been reported.

Microscopic lesions

The microscopic lesions of edema disease are characterized by degenerative alterations in small arteries and arterioles in various organ systems. Lesions range from perivascular edema and endothelial swelling (Brack 1965, Clugston et al. 1974b, Drommer 1976, Nielsen 1986) to myocyte necrosis and hyalinization of vessel walls. Arteries and arterioles acutely affected by edema disease have karyorrhexis and pyknosis of nuclei and hyaline changes of the cytoplasm in myocytes of the vessel wall. In more chronic cases, proliferation of perivascular adventitial cells is the major change (Clugston et al. 1974b, Kurtz and Quast 1976, Kurtz et

al. 1969, Nielsen 1986). The characteristic vascular lesion of edema disease described by Kurtz et al. (1969) was necrotizing panarteritis and fibrinoid necrosis of the tunica media. Thrombosis is not a prominent feature of this disease (Kurtz et al. 1969, Nielsen 1986).

Kurtz et al. (1969) reported focal necrosis of the brain parenchyma as the most obvious brain lesion. Malacia was present in 42 of 69 pigs that died of edema disease and were examined. It tended to be bilaterally symmetrical and was confined to the brain stem (Kurtz et al. 1969). In necrotic areas there was vacuolation and the neuropil was arranged loosely. Neurons were shrunken and eosinophilic. Other reports of acute cases emphasize the presence of non-inflammatory perivascular edema (Brack 1965, Clugston et al. 1974, Nielsen 1986) with fibrinoid necrosis of vessel walls in affected areas (Kurtz et al. 1969). The angiopathy described above seems to be the cause for the encephalomalacia (Kurtz et al. 1969). Although affected vessels are more common in the brain stem, this angiopathy also occurs in other areas of the brain (Kurtz et al. 1969). Harding (1966) described cerebrospinal angiopathy in pigs which is associated with weaning and may be due to "a sub-clinical attack of some enterotoxemia similar to oedema disease". The lesions were characterized by thickening of the walls of small arterioles,

swelling and degeneration of cells in the tunica media, subendothelial hyaline material, and perivascular eosinophilic droplets. These droplets of unknown composition also occur in experimental edema disease caused by inoculation with preparations of SLT-IIv (Gannon 1987, Gannon et al. 1989).

In pigs acutely ill with edema disease, rod-shaped bacteria can be seen adhering to the brush borders of the epithelium in the small intestine. This colonization is present from 2 to 7 days post-inoculation (Methiyapun et al. 1984). Methiyapun et al. (1984) and Bertschinger and Pohlenz (1983) found the heaviest colonization in the mid jejunum and ileum of affected pigs. Colonization by these bacteria does not have a significant influence on the morphology of the intestinal mucosa (Bertschinger and Pohlenz 1983).

Hemorrhages occur in the mucosa of the stomach (cardiac region) (Bertschinger and Pohlenz 1983), small intestine (Bertschinger and Pohlenz 1983, Timoney 1950) and large intestine (Bertschinger and Pohlenz 1983, Gannon 1987, Gannon et al. 1989, Timoney 1950). Erosions and ulcerations in the mucosa of the stomach were mentioned by Bertschinger and Pohlenz (1983), Nielsen and Clugston (1971), and Clugston et al. (1974b). Nielsen (1986) attributed mucosal hyperemia and hemorrhages to the action of an E. coli enterotoxin produced by some strains associated with edema disease.

In pigs with edema disease the vascular lesions of the intestine are characterized by endothelial swelling with necrosis and hyalinization of the tunica media (Bertschinger and Pohlenz 1983, Methiyapun et al. 1984). Electron dense material interpreted as fibrin was seen in subendothelial locations by Methiyapun et al. (1984). Perivascular edema was noted by various authors in natural and experimental disease (Clugston et al. 1974c, Methiyapun et al. 1984, Nielsen 1986).

Vascular lesions characterized as fibrinoid necrosis have also been observed in kidney, liver, spleen, lungs, skeletal muscle, salivary gland, urinary bladder and other tissues of clinically affected pigs (Kurtz et al. 1969). Drommer (1976) suggested that the morphologic lesions and neurologic signs of edema disease do not preferentially occur in one organ but are due to systemic vascular injury.

Pathogenesis of Edema Disease

Although Shanks (1938) noted in the first report on edema disease that he was not able to prove the presence of enterotoxemia, this concept of pathogenesis is now favored by most investigators. It is well established, that pathogenic strains of E. coli adhere to and colonize the small intestine (Bertschinger and Pohlenz 1983, Methiyapun 1984, Nielsen 1986,

Smith and Halls 1968). It is assumed that these adhering bacteria release a biologically active principle which is absorbed into the circulation (Nielsen 1986). This principle [edema disease principle (Clugston and Nielsen 1974)] is a cytotoxin believed to cause the lesions in small arteries and arterioles (Nielsen 1986). Recently, strong evidence has accumulated that edema disease principle and a variant of Shiga-like toxin II (SLT-IIv) are the same toxin. SLT-II, a toxin produced by certain E. coli, is similar to toxins produced by Shigella dysenteriae (Dobrescu 1983, Gannon 1987, Gannon et al. 1989, Gyles et al. 1988, Marques et al. 1987, O'Brien and Holmes 1987, Smith et al. 1983, Weinstein et al. 1988). SLT-IIv is thought to inhibit protein synthesis in affected cells by specific RNA cleavage (Saxena et al. 1989). Parenteral injection of SLT-IIv causes lesions resembling those of edema disease (Gannon 1987, Gannon et al. 1989); however, it has not been proven whether SLT-IIv produced by E. coli colonizing the small intestine is the toxin responsible for the pathogenesis of the disease.

Characteristics of bacteria associated with edema disease

Escherichia coli bacteria cause a wide variety of diseases in animals and man. In humans, traveller's diarrhea, food intoxication, infant diarrhea, hemorrhagic colitis and

hemolytic uremic syndrome are associated with intestinal E. coli infections (Gannon 1987, Okerman 1987). The 3 most common manifestations of intestinal colibacillosis in pigs are neonatal diarrhea, postweaning diarrhea and edema disease (Whipp 1989b). Sojka (1965) lists abortion, agalactia, endocarditis and puerperal septicemia as extra-intestinal infections in swine.

Pathogenic E. coli strains causing specific diseases in a large variety of animals must have "an armada of virulence attributes" (Gannon 1987). These virulence attributes of pathogenic strains include mechanisms of adhesion, invasion, toxin production, and a capacity to evade host defenses. Specific bacterial surface molecules and structures (fimbriae, pili) mediate adhesion to host membranes. Some strains damage mucosal surfaces by invading epithelial cells, whereas other strains elaborate specific toxins (Gannon 1987). Enteroinvasive strains, which are well known in human Shigella-like dysentery, have not been recognized in animal disease (Okerman 1987).

Colonization factor antigens, fimbriae

The ability of pathogenic E. coli to establish an intestinal infection depends on the capability to adhere to and colonize the intestinal epithelium (Isaacson 1987).

Colonization factor antigens (CFA), facilitating bacterial adherence to intestinal epithelial cells are well known virulence factors in pathogenic strains (Moon 1989). The term CFA was originally coined for adhesive structures (fimbriae, pili) on enterotoxigenic E. coli in humans, but the term is also used for E. coli fimbriae involved in diarrheal disease in calves and pigs (Moon 1989). Seven different fimbrial antigens are known in enterotoxigenic E. coli. Three of these, K88 (F4), K99 (F5) and 987P (F6), are important for colonization of small intestines in pigs. Wild type strains possessing one of these antigens are capable of colonizing small intestines, whereas isogeneic mutants lacking the gene for the fimbriae are non-adherent (Moon 1989).

Bertschinger and Pohlenz (1983) demonstrated that the small intestine is intensively colonized in pigs experimentally infected with a strain of serogroup O139 and which were ill with edema disease. The pattern of colonization was the same as in an infection with enterotoxigenic E. coli. Colonization of the small intestine in pigs as a feature of edema disease is also mentioned by Smith and Halls (1968). They found high numbers of bacteria in wall scrapings from the small intestines of affected pigs inoculated with strains of serotype O141 and O138. Both

groups noted greater numbers of bacteria in the distal jejunum and proximal ileum than in other areas of the intestine.

Recently Bertschinger et al. (1988) and Bachmann (1988) described an E. coli strain isolated from a pig with clinical edema disease which produces fimbriae in vitro that are antigenically different from K88, K99, 987P and F41. This strain was described as a mutant of a wild-type strain which was used for experimental infection in the same pig. Antibodies raised against the mutant and absorbed with the wild-type strain grown in vitro reacted with the wild-type strain grown in vivo. It was concluded that the mutant expresses a fimbrial antigen in vitro which is expressed by the wild-type strain only when grown in vivo.

Oral vaccination of piglets and gilts with a revertant of a streptomycin-resistant mutant of E. coli O139:K12(B):H1 gave inconsistent results in field studies (Bertschinger et al. 1984a). Residual virulence of the vaccine strain and insufficient immunity were mentioned as possible causes for inconsistent results. However, experimentally vaccinated pigs challenged orally with a virulent strain 12 to 14 days later, had reduced shedding of the challenge strain (Bertschinger et al. 1984a). Bertschinger et al. (1984a) speculated that the reduced shedding was due to acquired immunity against common adhesive fimbriae expressed by both the vaccine strain and

wild type strains because cross immunity between edema disease strains of heterogenous serotypes could be observed.

Hemolysins

Hemolysins, toxins that lyse cell membranes of erythrocytes and leukocytes by mechanisms not clearly defined, are produced by many pathogenic E. coli. Lysis of leukocyte membranes may be a virulence mechanism used against host defense (Wood and Davis 1980). Smith (1963) found two different hemolysins in E. coli isolated from animals and man. One (α-hemolysin) is antigenic and can be detected in the filtrate from bacterial cells. The other hemolysin (β-hemolysin) is not antigenic and cannot be separated from bacterial cells. The α-hemolysin is lethal when injected into mice (Smith 1963).

Large numbers of hemolytic E. coli can often be isolated from pigs clinically ill with edema disease (Schofield and Davis 1955, Sojka 1965, Sojka et al. 1957). The disease has been reproduced experimentally with hemolytic strains (Bertschinger and Pohlenz 1983, Methiyapun et al. 1984, Smith and Halls 1968), however, McAllister et al. (1979) stated that hemolytic E. coli can also be isolated from healthy piglets and the number of hemolytic E. coli in pigs with edema disease is not consistently great. Proliferation of hemolytic E. coli

in intestines of pigs immediately after weaning is not necessarily accompanied by clinical disease. Because McAllister et al. (1986) were unable to define an intestinal segment as a "focus of colonization of hemolytic E. coli", they characterized the large numbers as a periodic overgrowth. Smith (1963) doubted that the α-hemolysin was an important virulence factor in edema disease in pigs because culture supernatants from hemolytic E. coli strains associated with edema disease were no more hemolytic than those from non-pathogenic hemolytic strains. Non-hemolytic strains have been isolated from cases of edema disease. In addition, there is no nervous involvement and no edema in the large intestine of laboratory animals injected with α-hemolysin (Smith 1963). Strains of E. coli having lost the α-hemolysin gene located on a plasmid show no difference in ability to proliferate in the small intestine of pigs or to cause edema disease or diarrhea (Smith and Linggood 1971). It is unknown why so many E. coli strains isolated from cases of edema disease bear the trait for α-hemolysin production (Smith and Linggood 1971).

Escherichia coli toxins

Strains of E. coli associated with edema disease produce cytotoxins similar to toxins of Shigella species, and some strains also produce enterotoxins (Moon et al. 1986).

Enterotoxins There are 3 different families of enterotoxins which cause secretion of water and electrolytes in the intestinal lumen (Guerrant et al. 1985). The cholera-like toxin, LT, is heat-labile and causes diarrhea by activation of adenylate cyclase in mammalian cells (Guerrant et al. 1985). Toxins in the 2 other families are heat stable (ST), but elicit their toxic action by very different mechanisms. STa specifically activates guanylate cyclase in intestinal cells, whereas STb causes secretion of anions and fluids by a cyclase independent pathway (Guerrant et al. 1985).

Heat-labile enterotoxins (LT) Two antigenically distinct types of LT are described in this family of enterotoxins. LT-I was isolated from E. coli in humans and piglets and is antigenically similar to cholera toxin (Holmes et al. 1986). LT-II was isolated from a water buffalo and resembles LT-I in its biologic activities, but is antigenically different (Holmes et al. 1986). LT-II is now frequently identified in E. coli from cattle and buffalo, but only rarely in strains from cases of human diarrhea (Seriwatana et al. 1988). Both LT-I and LT-II are proteins composed of five B subunits and one A subunit. Both toxins have a molecular weight of 84,000 to 86,000 daltons (Guerrant et al. 1985, Holmes et al. 1986). LT binds to GM1 ganglioside

receptors of cells via B subunits and releases the A subunit into the cell. The A subunit is enzymatically active in ADP ribosylating the regulatory G_s subunit of adenylate cyclase. This mechanism activates the cyclase system irreversibly, and leads to an intracellular rise in cAMP, which mediates the biological effects of LT (Guerrant et al. 1985, Holmes et al. 1986). LT was detected in 11% of edema disease strains tested for four different enterotoxins. It was always present in conjunction with STb (Moon et al. 1986).

Heat stable toxins (STa and STb) The two types of heat stable enterotoxins of E. coli induce diarrhea by different mechanisms (Guerrant et al. 1985). STa causes diarrhea in suckling mice and pigs, whereas STb causes intestinal secretion in pigs but not in suckling mice (Guerrant et al. 1985, Moon et al. 1986). Neither of the heat stable enterotoxins is antigenic (Guerrant et al. 1985). STa is the name for a family of polypeptides with toxic activity and a molecular weight of 1,500 to 4,000 daltons (Weikel et al. 1986). STa causes secretion by activation of guanylate cyclase restricted to intestinal epithelium. Guanylate cyclase activation induces rising cGMP levels (Guerrant et al. 1985). The mechanism of activation at the molecular level is unknown (Guerrant et al. 1985). Two distinct genes have been identified for the production of STa, one from an ETEC strain

from pigs and the other from an ETEC strain from a human case. Genes that hybridize with the gene isolated from the porcine strain are called STaP; the ones hybridizing with the gene from the human strain are called STaH (Moon et al. 1986).

The mechanism of action of STb is unknown. It acts on intestinal cells by a nucleotide independent pathway (Weikel et al. 1986). Its specificity for pigs may be related with its susceptibility to trypsin-like protease cleavage. Consistent results in pigs could be achieved, if trypsin activity was blocked in intestinal loops in pigs (Whipp 1987). STb is also active in other species if trypsin activity is blocked. There are, however, species differences in sensitivity (Whipp 1989a). It is not known whether pigs have less trypsin or whether this enzyme is compartmentalized in pig intestines in a way that it cannot cleave STb near the epithelial surface.

Moon et al. (1986) examined a collection of E. coli from pigs for the presence of the enterotoxin genes encoding LT, STaP, STaH and STb. Nineteen of 37 isolates from cases of edema disease contained at least one enterotoxin gene. Of the isolates with at least one enterotoxin gene, all encoded the gene for STb and 74% contained the gene for STaP.

Shiga-like toxins (SLT) Gregory (1960b) stated that E. coli strains isolated from pigs with edema disease produce

a toxin which is responsible for the lesions of edema disease. He reported that the toxin is a protein and its biological effects closely resemble those of Shigella dysenteriae (Shiga) neurotoxin (Gregory 1960b). Konowalchuk et al. (1977) discovered that various E. coli strains produce cytotoxins, which destroy Vero cells (a line of African green-monkey kidney cells). These cytotoxins are called verotoxins (VT). Neutralization experiments indicate there may be more than one antigenic type of VT (Konowalchuk et al. 1977). The cytotoxic effect of VT is different from the cytotonic effect of LT in that VT does not increase intracellular cAMP levels (Konowalchuk et al. 1977). O'Brien et al. (1983) coined the term Shiga-like toxin for E. coli cytotoxins that are toxic for Vero and HeLa cells and resemble toxins produced by Shigella dysenteriae in their biologic and antigenic behavior (O'Brien et al. 1983). In this thesis the terms VT and SLT will be used interchangeably. Research on VT/SLT of E. coli was intensified when enterohemorrhagic strains of the serogroup O157:H7 were found to produce VT/SLT. Strains of the serogroup O157:H7 are associated with outbreaks of hemorrhagic colitis and hemorrhagic uremic syndrome in humans (O'Brien and Holmes 1987, Scotland et al. 1985) and produce Shiga-like toxin (SLT) (O'Brien et al. 1983). Recently Scotland et al. (1985) confirmed that there are two

antigenically distinct VT produced by O157:H7 E. coli, both of which are cytotoxic for Vero and HeLa cells. It was possible to neutralize one VT with anti-Shiga-toxin antiserum. The VT neutralized was termed VT 1; the second one, which was not neutralized, was called VT 2. The designations VT 1 and VT 2 are equivalent to SLT-I and SLT-II, respectively (O'Brien and Holmes 1987).

SLT-I and SLT-II are complex toxins consisting of one A subunit and multiple B subunits. It appears that there is one A subunit bound to five B subunits (O'Brien and Holmes 1987). The mechanism of action for SLT may be similar to that of Shiga-toxin and the plant toxin ricin (Saxena et al. 1989). The toxin binds to glycolipid cell surface receptors via the B subunit and is internalized in clathrin coated pits. After lysosome-vesicle fusion and acidification, an A_1 fragment is proteolytically cleaved from the A subunit and released into the cytoplasm. This fragment specifically removes an adenine residue ($adenine_{3732}$) via N-glycosidasic cleavage from the 28S rRNA, when microinjected into Xenopus oocysts. The base $adenine_{3732}$ is located near the 3' end of the 28S rRNA and its removal inhibits nonsecretory protein synthesis (Saxena et al. 1989).

In 1974, Clugston and Nielsen prepared an active principle from cultures of E. coli strains which were isolated

from pigs clinically ill with edema disease. The freeze-thaw lysate obtained was capable of inducing typical edema disease lesions in pigs if inoculated intravenously. This toxin was heat labile and could be precipitated with ammonium sulfate. It was named edema disease principle (EDP) (Clugston and Nielsen 1974). Dobrescu (1983) discovered that EDP is cytotoxic for Vero cells. Her preparation was not neutralized by antiserum against a VT from a strain isolated from a human. It was not specified which VT (SLT) was used for the production of the antiserum (Dobrescu 1983) as the distinction between the two antigenic types of SLT had not yet been made. It can be assumed that anti-SLT-I serum was used, as this does not neutralize SLT-II cytotoxicity (Scotland et al. 1985). In a recent report, 81 strains isolated from pigs with edema disease were tested for cytotoxic activity (Marques et al. 1987). Seventy-two of these strains expressed cytotoxicity on Vero cells but not on HeLa cells. The cytotoxic effect was neutralized only with antiserum against crude SLT-II, but not with antiserum against SLT-I. These findings indicate that the toxin expressed by E. coli that cause edema disease is related, but not identical to SLT-II. Unlike SLT-II, edema disease toxin is markedly more cytotoxic for Vero cells than for HeLa cells. It has been proposed that SLT-IIv has specific cell receptors different from those of SLT-II (Saxena

et al. 1989, Weinstein et al. 1988). This variation might account for the observed difference in cytotoxicity for HeLa cells (Marques et al. 1987). Weinstein et al. (1988) observed considerable homology between A and B subunits of SLT-II and SLT-IIv, respectively (93% homology in amino acid sequence in A subunits, 84% in B subunits). Gyles et al. (1988) cloned the genes determining SLT-IIv production in a porcine edema disease E. coli isolate and found 91% nucleotide sequence homology between SLT-II and SLT-IIv. The homology in the amino acid sequence they deducted from these genetic studies is identical to that found by Weinstein et al. (1988).

Recently a second variant of SLT-II was detected in a strain isolated from a human patient with hemolytic uremic syndrome (HUS) (Oku et al. 1989). Although different in its physicochemical properties (Oku et al. 1989), this variant is closely related to the SLT-IIv isolated from porcine edema disease strains in its toxicity for Vero cells.

Smith et al. (1983) examined 519 E. coli strains of animal and human origin. They found 61 SLT-positive strains, most of which were of porcine origin. The porcine strains belonged to serogroups O138, O139 and O141. A major difference between VT (SLT) produced by strains of human origin and those produced by strains of porcine origin was, that VT (SLT) in human strains is phage mediated, whereas in

porcine strains it is not. Smith et al. (1983) state that SLT-IIv may be plasmid encoded, as SLT production could be transferred by conjugation. Conversely Weinstein et al. (1988) suggest a chromosomal location for the gene because plasmid DNA did not hybridize with the SLT-IIv gene probe.

Smith et al. (1988) and Linggood and Thompson (1987) examined collections of E. coli for SLT production. In the work of Smith et al. all 14 porcine strains and none of 20 strains from other species produced VT 2 (SLT-II), but not VT 1 (SLT-I), and 6 of the VT 2 producing strains belonged to serogroup O138, O139, and O141 (Smith et al. 1988). Linggood and Thompson (1987) found that all strains isolated from pigs with edema disease produced a heat labile VT (SLT), which was antigenically different from "human verotoxins". The VT (SLT) positive strains belonged to serogroup O138, O139 and O141. Additional strains were found that belonged to these 3 serogroups, but did not produce SLT. Three additional strains producing SLT could not be typed. It is noteworthy that none of the SLT negative strains were associated with clinical outbreaks of edema disease (Linggood and Thompson 1988). Linggood and Thompson conclude that the SLT detected in the strains isolated from cases of edema disease is identical to the edema disease principle described earlier. Gannon et al. (1988) examined 668 E. coli strains of porcine origin for VT

production and found that VT production is common among the 3 serogroups O138, O139 and O141. SLT producing strains were isolated in the same frequency from healthy weaned pigs as from diseased weaned pigs. SLT producing strains were not isolated from unweaned piglets. Therefore, Gannon et al. (1988) concluded that VT positive E. coli strains may be a normal component of the intestinal flora of weaned pigs.

Purified SLT-IIv (Gannon et al. 1989) and purified SLT-II (Barrett et al. 1989) caused characteristic lesions in pigs and rabbits. Gannon et al. (1989) compared the lesions induced by purified SLT-I, SLT-II and SLT-IIv in pigs inoculated intravenously. Pigs inoculated with SLT-IIv developed characteristic clinical edema and histopathological lesions of edema disease. The only differences between lesions in these experiments and the natural disease was the absence of edema and lesions in the stomach wall and brain stem, and the presence of hemorrhage in meninges, cerebellum, retina and spiral colon. Preparations of SLT-I or SLT-II induced lesions similar to those from SLT-IIv in the vasculature of most organs, but neither toxin induced lesions in the gastrointestinal tract. SLT-I and SLT-II induced kidney lesions (cortical edema and hemorrhage and tubular necrosis) in addition to the lesions caused by SLT-IIv inoculation (Gannon et al. 1989).

Barrett et al. (1989) administered SLT-II to rabbits for 14 days by continuous infusion with mini osmotic pumps placed in the peritoneal cavity. Hemorrhage and submucosal edema in the cecum was visible in most animals. Kidneys had focal necrosis and mineralization of proximal convoluted tubules. Brain lesions consisted of scattered foci of hemorrhage and necrosis in the cerebellum and pyknotic nuclei in some vessels. Spinal cords had areas of patchy necrosis and hemorrhage (Barrett et al. 1989). These lesions closely resemble the lesions of hemorrhagic colitis and hemorrhagic uremic syndrome in humans.

Escherichia coli serogroups

Sojka et al. (1957) examined 77 E. coli strains isolated from cases of edema disease for their agglutination with antisera produced against 4 isolates from different edema disease outbreaks. Sixty-eight of these strains could be typed with two of the antisera, and one of the antisera agglutinated 61 strains (Sojka et al. 1957). In 1958, Ewing et al. (1958) typed 38 strains from edema disease outbreaks in the United States and Ireland and found that the majority belonged to either serogroup O138:K81 or O139:K82. In a survey on 2,321 strains isolated from pigs with various diseases Sojka et al. (1960) isolated serogroups O139:K82,

O138:K81 and O141:K? with high frequency from cases of edema disease and concluded that these serogroups are specific for pigs and edema disease (Sojka et al. 1960). In a recent survey on the distribution of E. coli serogroups in outbreaks of edema disease in Austria, Awad-Masalmeh et al. (1988) found a high prevalence of serogroup O139, followed by O138 and O141. Other serogroups isolated with some frequency were O147, O149, O45, and O8. The K88 antigen was not detected in any O139 strain, but was found in an average of 21% of the strains in the other serogroups. Agar diffusion tests with antisera against edema disease principle showed reactions with 89%, 85% and 83% of strains from serogroup O139, O138, and O141, respectively. When examining strains isolated from pigs in Ontario, Canada for VT (SLT) production and serotype, Gannon et al. (1988) found that expression of VT (SLT) was most prevalent in strains of serogroup O139:K82, O138:K81 and O141:K85. VT (SLT) was also detected in strains of serogroup O149:K91 and O157:K"V17". Serogroup O141 was isolated in 3 out of 6 edema disease cases in Southern Ontario.

Experimental Approaches to Reproduce the Disease

Inoculation with viable bacteria

Various authors have been unable to reproduce edema disease in pigs by using live bacteria as inocula (for

overview see Sojka 1965). Smith and Halls (1968) reproduced clinical edema disease by oral inoculation of piglets from a herd in which the disease occurred earlier. No clinical disease was seen when pigs from another herd were inoculated with the same isolate. Successful reproduction of clinical disease was also reported from Bertschinger and Pohlenz (1983) who also used pigs from a farm where edema disease occurred during previous years. The same pigs were used in the electron microscopic studies of Methiyapun et al. (1984).

Recently, Tzipori et al. (1988) and Francis et al. (1989) reported that experimental oral inoculations of gnotobiotic piglets resulted in edema disease-like symptoms and lesions. Both groups used E. coli strains of serogroup O157:H7 that produced Shiga-like toxins. Tzipori's strain was isolated from a fatal case of human hemorrhagic colitis. Francis et al. used various strains that were isolated from humans, pigs and hamburger. The pathogenic bacteria adhered to the mucosa of the large intestine in experimental pigs and attached to and effaced microvilli. This large intestinal adherence pattern with effaced microvilli was not described for edema disease strains (Bertschinger and Pohlenz 1983, Methiyapun et al. 1984).

Inoculation with intestinal contents or extracts of bacterial colonies

Intestinal contents Clinical signs and lesions of edema disease have been reproduced by the intravenous inoculation of supernatants of intestinal contents of pigs affected with the disease (Gregory 1960a, Schimmelpfennig 1970, Timoney 1950). Timoney (1950) was able to reproduce edema disease lesions by intravenous administration of supernatants of contents from various areas of the intestine of pigs that died from edema disease. He concluded from these experiments that edema disease is a toxemia, originating in the intestine (Timoney 1950). Gregory (1960a) produced typical signs in pigs and mice, when intestinal extracts of pigs that died from edema disease were used. His preparation differed from that used by Timoney (1950) in that he froze the intestinal contents before and after centrifugation. Schimmelpfennig (1970) used intestinal extracts to establish a toxicity unit in mice. One "mouse unit" is equal to one LD_{50} in mice and was determined in 40 animals (Schimmelpfennig 1970).

Not all experiments with intestinal contents were successful. Schofield and Robertson (1955) failed to reproduce signs of edema disease in pigs inoculated with

filtrates of intestinal contents obtained from pigs with clinical edema disease.

Freeze-thaw extracts Erskine et al. (1957) first reported the capacity of freeze-thaw extracts of E. coli to induce edema disease-like lesions in pigs. Cultures of E. coli strains isolated from pigs with edema disease were frozen, thawed and then centrifuged. The supernatants of these cultures produced clinical signs of edema disease when inoculated intravenously. Gregory (1960a) used the same approach and was also able to reproduce the signs and lesions of edema disease. Nielsen and Clugston (1971) and Clugston and Nielsen (1974) further purified the toxin obtained by freeze-thaw extraction. Isolates from pigs with clinical edema disease were cultured, frozen, thawed and then centrifuged. The supernatant was precipitated using ammonium sulfate, centrifuged and the pellet was dialyzed. The substance concentrated in this manner contained edema disease-inducing activity, and induced less severe endotoxic shock than untreated freeze-thaw lysate (Clugston and Nielsen 1974, Nielsen and Clugston 1971). Gannon et al. (1989) used ammonium sulfate to precipitate the supernatants of E. coli strains of human or porcine origin that produced a SLT or into which the gene for the production of a SLT was cloned. He was able to obtain preparations of SLT-I, SLT-II and SLT-IIv,

which induced lesions typical for each toxin. SLT-I and SLT-II induced renal lesions, which were not observed if SLT-IIv was used as inoculum. SLT-IIv produced the lesions most typical of edema disease.

When SLT-II was administered to rabbits via mini-osmotic pumps (Barrett et al. 1989), lesions developed in the kidneys, colon and cecum. Central nervous system lesions were more prominent in the spinal cord than in the brain stem.

The role of endotoxin in the pathogenesis of edema disease has been under discussion for several years (Johannsen 1974, Nagy et al. 1968, Nielsen and Clugston 1971). While Nagy et al. (1968) concluded from their experiments that endotoxic shock and edema disease are similar conditions, Nielsen and Clugston inferred from their comparison of the lesions induced by inoculation of pigs with endotoxin or edema disease principle that endotoxin is not involved in the pathogenesis of edema disease (Nielsen and Clugston 1971). It is interesting, though, that Johannsen (1974) states that endotoxic shock is the common pathogenic principle behind edema disease, circulatory and heart failure and the gastro-intestinal form of colibacillosis in pigs. He states that he was able to reproduce lesions of edema disease, gastroenteritis and heart failure by application of E. coli endotoxin.

The literature review has identified several significant gaps in our understanding of the pathogenesis of edema disease in swine. The E. coli strains and the toxin (SLT-IIv) believed to be responsible for the vascular lesions of edema disease have been well characterized. However, the mechanisms of colonization and toxin production in vivo by edema disease-producing strains of E. coli are poorly understood. An experimental model for the consistent production of edema disease in swine is badly needed for studies of complex and interrelated events of pathogenesis.

In the following study, pigs were raised and inoculated with E. coli according to a model of postweaning diarrhea developed previously at the National Animal Disease Center. Pigs were inoculated with an SLT-IIv-positive strain of E. coli isolated from a pig with clinical edema disease. An original plan to use a strain of E. coli containing streptomycin-resistance as a valuable marker was discarded when the strain proved to be neither virulent nor isogeneic to the virulent parent strain. Stressors of delayed access to feed postweaning and treatment with pharmacological agents were used to predispose pigs to edema disease. The purpose of the research reported here was to establish a reliable model for experimental edema disease in swine.

MATERIALS AND METHODS

The research reported in this thesis is based on 2 experiments with a total of 70 pigs. In the first experiment, 2 groups of 11 pigs each were inoculated with either the SLT-IIv-positive E. coli strain NADC #2228 (principal group) or the non-pathogenic E. coli strain NADC #123 (control group). In the second experiment 48 pigs were randomly assigned to 4 groups of 12 pigs each. Pigs in 2 groups (24 pigs total) were inoculated with E. coli strain #2228 (principal groups) and pigs in two other groups (24 pigs total) were inoculated with E. coli strain #123 (control groups). Pigs in one control and one principal group were subjected to additional stressors (see below and Tables 1 and 2).

Experimental Animals and Management

Pregnant gilts were obtained from a closed sow herd. New genetic material was introduced into the herd only through purchase of boars. Pigs used in the pilot experiment performed by Dr. H. W. Moon were purchased from this herd. Gilts were vaccinated by the producer with an E. coli vaccine containing pilus antigens (Littergard LT^R, Norden Laboratories, Lincoln, NE). No other vaccines were used. Gilts were delivered to the NADC on the 96th to 98th day of

Table 1. Experimental design (pig numbers)

Group	Number of pigs inoculated	Number of pigs necropsied 2 days PI	Number of pigs necropsied while having clinical signs	Number of pigs remaining 14 days PI	Number of pigs necropsied 14 days PI
EXPERIMENT I					
Principal[a]	11	4	0	7	4
Control[b]	11	4	0	7	4
EXPERIMENT II[c]					
Principal 1	12	4	0	8	4
Control 1	12	4	0	8	4
Principal 2 (with stress)[d]	12	4	1	7	4
Control 2 (with stress)	12	4	0	8	4
TOTAL					
Principals	35	12	1	22	12
Controls	35	12	0	23	12

[a]Pigs in principal groups were inoculated with the SLT-IIv-positive E. coli strain NADC # 2228.

[b]Pigs in control groups were inoculated with the non-pathogenic E. coli strain NADC # 123.

[c]Pigs in the second experiment were housed on concrete floors at an ambient temperature of 20 ± 2°C.

[d]Pigs subjected to stress were treated with atropine and ranitidine and fasted 24 hours prior to inoculation.

Table 2. Experimental design (treatment)

Group	*E. coli* strain inoculated[a]	Housing[b]	Ambient temperature[c]	Fasting, atropine and ranitidine treatment[d]
EXPERIMENT I				
Principal	#2228	raised	warm	no
Control	#123	raised	warm	no
EXPERIMENT II				
Principal 1	#2228	floor	cold	no
Control 1	#123	floor	cold	no
Principal 2 (with stress)[e]	#2228	floor	cold	yes
Control 2 (with stress)	#123	floor	cold	yes

[a]Pigs were inoculated one day after weaning either with a SLT-IIv-producing E. coli strain isolated from a pig with edema disease (#2228) or with a non-pathogenic E. coli control strain (#123).

[b]Pigs were either kept in pens with raised plastic covered metal grid floors (raised) or in pens with solid concrete floors (floor).

[c]Ambient temperature was either uncontrolled and around 27 ^{o}C (warm) or ambient temperature was maintained at 20 $\pm$ 2 ^{o}C (cold).

[d]In groups marked "yes" pigs were treated with atropine at a dose of 5 mg/kg, and with ranitidine at a dose of 10 mg/pig. Therapeutic levels of both drugs were maintained for at least 12 hours post-inoculation.

[e]Pigs in groups subjected to stress were fasted 24 hrs prior to inoculation and treated with atropine and ranitidine at inoculation.

gestation, placed in quarantine for 2 weeks, and then placed in farrowing units on the 110th day of gestation. Farrowing was induced by intramuscular injection of 10 mg prostaglandin $F2_{\alpha}$ gilt (Lutalyse^{R}, The Upjohn Company, Kalamazoo, MI) at 112 days of gestation.

Sows and litters were housed on plastic covered metal grid floors (Dura-Trac^{R} Pads, ADA Enterprises, Freeborn, MN). Piglets had voluntary access to heat lamps and electrically heated floor pads, but they had no access to the sow's feed. All piglets were marked individually by ear notches on the second day of life. Piglets were weaned at 3 weeks of age and divided at random into principal and control groups. The groups were housed in separate barns under strict isolation. In Experiment I, weaned pigs were housed in pens with raised metal grid floors and had access to feed immediately after weaning. In Experiment II, the pigs were housed on concrete floors. Pigs in all groups had voluntary access to heat lamps and water. Pens were equipped with metal feed troughs and 2 watering nipples. A commercial corn based pig starter feed (Ames Feed and Supply, Ames, IA) was given daily ad libitum. It was composed of 18% protein, 75.5% carbohydrate, 3% crude fiber and 2.5% fat and contained 60% corn and 40% commercial products (30% Baby Mate Mixer^{R}, 5% Baby Mate Booster Pak^{R},

Protein Blenders Inc., Iowa City, IA and 5% Melk-O-Mix^{R}, Tri Foods Inc., Concordia, MO).

Stressors

In Experiment II stressors were added to the standard model to determine whether additional stress could increase the incidence of clinical edema disease (Table 2). Pigs in one principal and one control group were fasted for 24 hours prior to inoculation and all groups were kept in barns with an ambient temperature of 20 $\pm$ 2 °C. To inhibit the secretion and motility of the intestine each fasted pig was injected with 5 mg/kg atropine (Atropine Injectable L.A., Fort Dodge Laboratories, Inc., Ft. Dodge, IA). Stomach acid and pepsin secretion was impaired with the injection of 10 mg of the H_2-blocker ranitidine (Zantac^{R} Glaxo Inc., Research Triangle Park, NC) per pig one-half hour before inoculation with E. coli. The injections were repeated with 2.5 mg/kg atropine and 5 mg/pig ranitidine 6 hours post-inoculation to maintain therapeutic levels of both drugs for approximately 12 hours.

Bacteria and Inocula

Escherichia coli strains used for inoculation were E. coli strains #2228 and #123. E. coli strain #2228, originally designated as S1191 (Marques et al. 1987), was

isolated from a pig with clinical edema disease (Marques et al. 1987). E. coli strain #2228 was supplied by Dr. A. D. O'Brien, USUHS, Bethesda, MD. It produces SLT-IIv in vitro (A. O'Brien personal communication), and was positive for STb when tested by a colony hybridization technique previously described by Mainil et al. (1986) (T. Casey, National Animal Disease Center, Ames, IA, personal communication). E. coli strain #2228 belongs to the O139 serogroup (unpublished data).

E. coli Strain #123 belongs to the O43:K$^-$:H28 serogroup and was originally isolated from a healthy piglet (Bertschinger et al. 1972). It is a non-pathogenic, non-hemolytic E. coli strain (supplied by Dr. H. W. Moon, NADC, Ames, IA).

Inocula were prepared according to the method described by Sarmiento et al. (1988). Bacteria were grown overnight at 37°C on Trypticase^R soy agar (TSA) (MicroBioLogics, St. Cloud, MN). Single colonies were transferred to flasks containing 1 L Trypticase^R soy broth (TSB) (MicroBioLogics, St. Cloud, MN) and incubated overnight at 37°C in an agitating incubator. The resulting culture was centrifuged at 10,000 x g for 20 minutes and the resulting pellet was resuspended to a final concentration of 10^{10} colony forming units (CFU) bacteria per ml in half strength TSB containing 10% glycerol. This suspension was stored at -70 °C in 12 ml aliquots.

Immediately before inoculation, bacterial suspensions were thawed and 1 ml containing 10^{10} CFU of bacteria was added to 60 ml of half strength Trypticase^{R} soy broth (BBL Microbiology Systems Cockeysville, MD) containing 1.2% sodium bicarbonate to neutralize gastric acid (Sarmiento et al. 1988). Each pig in the principal groups was inoculated with 10^{10} CFU of E. coli strain #2228, whereas each pig in the control groups was inoculated with 10^{10} CFU of E. coli strain #123.

Inoculation, Clinical Observations and Necropsies

Pigs were inoculated via gavage 1 day after weaning. Pigs in all groups were observed for the presence of diarrhea, edema or neurologic disorders at least twice daily for 14 days post-inoculation (PI). Necropsies were done on 4 pigs per group 2 days PI and on another 4 pigs per group at the end of the experiment 14 days PI. All pigs that were obviously weak or reluctant to rise when the observer entered the pen were euthanatized and necropsied immediately. Pigs were killed by intravenous injection of a sodium pentobarbital overdose (Sleepaway^{R}, Fort Dodge Laboratories, Inc., Ft. Dodge, IA). Tissues were harvested for bacterial counts and histopathological examination. A 5 cm section of ileum, 1 m anterior to the ileocecal valve, was collected under aseptic conditions and used for bacterial counts. Samples taken for

bacterial counts were placed on dry ice immediately and kept frozen at -70 °C until further examination. Tissues collected for histopathology were the brain, a section of stomach (including cardia), a 15 cm section of small intestine adjacent to that collected for bacterial counts, the spiral apex of the colon and a sample of kidney.

Blood samples were collected and sera were sent to Dr. J. Samuel, USHUS, Bethesda, MD for serology. Dr. Samuel tested sera for the presence of antibodies against SLT-IIv. He made two-fold serial dilutions of sera and determined the highest dilution capable of neutralizing 20 CD_{50} of SLT-IIv on Vero cells. One CD_{50} of SLT-IIv was defined as the amount of SLT-IIv that destroyed 50% of Vero cells in a microtiter well cell culture.

During the first 7 days PI rectal swabs were collected from diarrheic pigs on the first day of diarrhea. Swabs were stored at -70 °C until processed. All pigs were weighed at the beginning of the experiment, and survivors were weighed again at the end of the experiment. Weight gain (W_G) was calculated as weight at the end of the experiment (W_E) minus weight at the time of inoculation (W_o) calculated as percentage of weight at the time of inoculation according to the following formula

$$W_G = \frac{W_E - W_o}{W_o} \times 100\%.$$

Histopathologic Examination

Specimens of brain, stomach, ileum, colon and kidney were fixed in 10% neutral buffered formalin immediately after collection. The ileal sample was ligated on both ends and slightly filled with formalin before being placed into the formalin solution. Fixed tissues were trimmed, dehydrated with graded alcohols, embedded in paraffin, and sectioned at 6 μm. Sections were stained with hematoxylin and eosin (HE) for light microscopy. A representative number of ileum and brain stem sections were stained with periodic acid Schiff's reagent (PAS) for carbohydrates. Stained slides with tissue samples were coded and examined. Bacterial layers in the intestine and necrosis in arterial and arteriolar walls in all organs were particularly searched for and recorded.

Microbiological Examination and Bacterial Counts

The frozen ileal samples obtained aseptically at necropsy were suspended in 25 ml of 3% peptone water and homogenized with a Virtis® tissue homogenizer (Virtis Company Inc., Gardine, NY). The homogenate was diluted in steps to final concentrations of 1:250, 1:25000 and 1:250,000. The 3 final dilutions were plated on TSA plates, blood agar plates containing 5% sheep blood (BAP) and MacConkey agar plates (MicroBioLogics, St. Cloud, MN). Plates were inoculated by a

spiral plater (Spiral Systems, Cincinnati, OH) and incubated 18 hours at 37 °C. Colonies resembling hemolytic E. coli were counted on a calibrated grid, and bacterial numbers were calculated as CFU of bacteria per 5 cm ileum. Ilea were considered to be colonized if bacterial numbers exceeded 10^7 CFU/5 cm ileum and bacterial layers were seen histologically.

Rectal swabs obtained from pigs with diarrhea during the first 7 days PI were thawed and streaked onto TSA, BAP, and MacConkey agar plates. Plates were incubated at 37 °C for 18 hours and examined for colonies resembling hemolytic E. coli. Swabs from at least 1 pig per group were examined for the presence of rotaviruses by transmission electron microscopy if E. coli shedding was negative or if only a few hemolytic colonies were present.

Statistical Analysis

Group means for calculated body weight gain were compared using the Student's t-test (Steel and Torrie 1980). Bacterial counts were calculated as geometric means for each group and were compared using the Student's t-test.

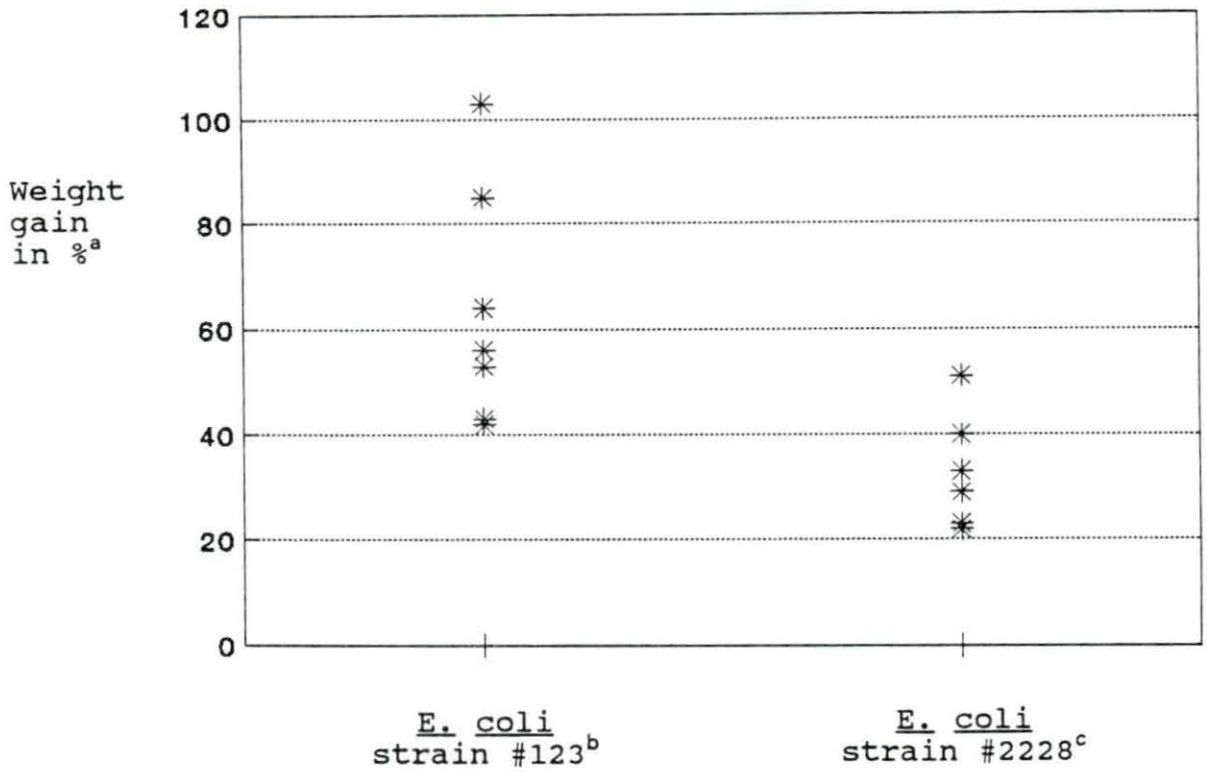
RESULTS

Pigs appeared normal at the time of inoculation and were of uniform size and weight. Weights at the time of inoculation were statistically similar ($P>0.10$) in all groups in both experiments. Pigs inoculated with *E. coli* strain #2228 in Experiment I had significantly less weight gain than pigs inoculated with the control *E. coli* strain #123 ($P<0.05$) (Figure 1). There was no significant difference in weight gain between groups in Experiment II ($P>0.5$).

Clinical Observation

From the original 70 pigs in all experiments, 12 pigs were euthanatized in principal and control groups at 2 days PI. Therefore, after 2 days PI, only 23 pigs remaining in principal and control groups had the opportunity to develop edema disease (Table 1). One of 23 pigs inoculated with *E. coli* strain #2228 (principal groups) developed clinical edema disease, but none of 23 pigs from control groups developed edema disease. Diarrhea was a common clinical sign in pigs of principal and control groups of Experiment I and in pigs of the principal group with stress in Experiment II.

In Experiment I, no clinical signs of edema disease were observed in any of the 14 pigs remaining after 2 days PI.



[a]Weight gain (W_G) was calculated as weight at the end of the experiment (W_E) 14 days post-inoculation minus weight at the time of inoculation (W_o) calculated as percentage of weight at the time of inoculation according to the following formula

$$W_G = \frac{W_E - W_o}{W_o} \times 100\%.$$

[b]Non-pathogenic control strain.

[c]strain isolated from a pig with clinical edema disease.

Figure 1. Weight gain of individual pigs in Experiment I

Four of 7 pigs remaining at 2 days PI in the principal group developed diarrhea, whereas 6 of 7 pigs in the control group developed diarrhea. Regardless of the group, diarrhea started on days 2 to 7 PI. Two pigs from the principal group and 1 pig from the control group had diarrhea at the end of the experiment, 14 days PI.

In Experiment II, the pig that developed clinical edema disease was inoculated with E. coli strain #2228 and treated with a combination of atropine and ranitidine (principal group with stress). This pig had diarrhea 4 to 6 days PI, ataxia (swaying gait) at 7 to 8 days PI, and edema of the eyelids and forehead at 9 to 12 days PI. The pig was recumbent at 12 days PI and was euthanatized.

Three of 8 pigs remaining at 2 days PI in the principal group with stress developed diarrhea 3 to 5 days PI (including the pig which later developed clinical edema disease). No clinical signs of edema disease or diarrhea were observed in any pigs of the control groups or principal group without stress.

Postmortem Examination

None of the pigs necropsied 2 days PI had macroscopically visible lesions. The pig necropsied at the time of clinical signs in Experiment II was emaciated and had mild subcutaneous

edema of the forehead. All surviving pigs necropsied 14 days PI in both experiments were clinically and grossly normal.

Histopathological Examination

Myocyte necrosis and fibrinoid degeneration of arteriolar and arterial walls were seen in 12 of 13 pigs (both experiments) necropsied 12 or 14 days after inoculation with E. coli strain #2228. None of the 12 control pigs necropsied 14 days after inoculation with E. coli strain #123 had vascular lesions. None of the 24 pigs from either group necropsied 2 days PI had vascular lesions (Table 3). Vascular lesions consisted of segmental necrosis of myocytes in the tunica media of small arteries and arterioles. Karyorrhexis and pyknosis of scattered nuclei was prominent (Figures 2 to 6). Cytoplasm of necrotic and degenerating cells was homogenous eosinophilic. Small eosinophilic droplets, 5 to 20 μm in diameter, were occasionally seen in and between affected cells and around affected vessels (Figure 3). One pig had marked adventitial proliferation around arterioles in the submucosa of the ileum (Figure 6). Similar lesions in declining frequency were seen in ileum, brain stem, thalamus, cerebral cortex (and meninges), stomach, colon, and kidney (Table 3).

In coded, PAS-stained sections of ileum and brain stem, PAS-positive globules were found around small arteries and

Table 3. Incidence and organ distribution of microscopic vascular lesions in pigs necropsied 12 or 14 days after inoculation with E. coli strain #2228 (principals) or E. coli strain #123 (controls)

	Ileum	Brain stem	Brain cortex[b]	Thalamus	Stomach	Colon	Kidney	Total[a]
EXPERIMENT I								
Principal	4/4[c]	4/4	0/4	0/4	0/4	0/4	0/4	4/4
Control	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
EXPERIMENT II[d]								
Principal 1	4/4	3/4	2/4	2/4	1/4	1/4	0/4	4/4
Control 1	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
Principal 2 (with stress)[e]	4/5	4/5	4/5	4/5	2/5	1/5	1/4	4/5
Control 2 (with stress)	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
TOTAL								
Principals	12/13	11/13	6/13	6/13	3/13	2/13	1/13	12/13
Controls	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12

[a]Total number of pigs with lesions in any organ examined.

[b]Cerebral cortex with meningeal vessels.

[c]Number of pigs with lesions/number of pigs examined.

[d]Pigs in Experiment II were housed on concrete floors at an ambient temperature of 20 $\pm$ 2^{o}C.

[e]Pigs subjected to stress were treated with atropine and ranitidine and fasted 24 hours prior to inoculation.

arterioles of pigs from both principal and control groups. These PAS-positive globules did not correlate with vascular lesions seen in hematoxylin and eosin stained sections.

Ileal and cecal segments from 24 control pigs and 24 principal pigs were examined for bacterial layers along the mucosal surface. Bacterial layers were detected in only 1 pig. This pig, from Experiment II, was necropsied 2 days after inoculation with control E. coli strain #123. A few patchy layers of rod-shaped bacteria were seen in 1 section of ileum. The ileal segment of this pig contained 1.76×10^5 CFU of hemolytic E. coli. None of the 24 pigs inoculated with E. coli strain #2228 and necropsied 2 or 14 days PI had detectable bacterial layers in the intestine.

Bacterial Counts

Among pigs euthanatized 2 days PI, numbers of hemolytic E. coli per 5 cm segments of ileum varied from $< 5 \times 10^3$ to 2.4×10^8 CFU. In Experiment I, numbers of hemolytic E. coli were at least 1,000 x greater in pigs inoculated with E. coli strain #2228 (principal group) than in pigs inoculated with the control E. coli strain (Table 4). The coefficient of variation between groups in Experiment I was 7%.

In pigs euthanatized in Experiment II at 2 days PI, numbers of hemolytic E. coli per 5 cm of ileum varied greatly

Table 4. Colony-forming units (CFU) of hemolytic *E. coli* (geometric means) in 5 cm sections of ileum from pigs harvested 2 days after oral inoculation

	Experiment I		Experiment II	
E. coli strain	Number of Pigs	CFU of *E. coli*[a]	Number of Pigs	CFU of *E. coli*
#2228[b]	4	2.77×10^6 ($10^6 - 10^7$)[c]	8	1.95×10^6 ($10^4 - 10^8$)
#123[d]	4	$<5.0 \times 10^3$ (no range)	8	3.75×10^5 ($10^4 - 10^8$)

[a]CFU of hemolytic *E. coli* were determined in 5 cm sections of ileum homogenized with contents and plated on 5% sheep blood agar plates.

[b]SLT-IIv-positive strain isolated from a pig with clinical edema disease.

[c]Numbers in brackets represent range of numbers of hemolytic colonies resembling *E. coli*. Coefficient of variation in Experiment I was 7%, in Experiment II it was 70%.

[d]Non-pathogenic control strain.

among individual pigs and among treatment groups (Table 4). Regardless of the group, numbers of E. coli ranged from 10^4 to 10^8 CFU per 5 cm segment of ileum. The mean number of hemolytic E. coli in 8 pigs inoculated with E. coli strain #2228 was 1.95×10^6 compared with 3.75×10^5 for pigs inoculated with E. coli strain #123 (controls). The coefficient of variation was 70%.

None of the 23 pigs necropsied at 12 or 14 days PI had equal to or greater than 5×10^3 CFU of hemolytic E. coli per 5 cm of ileum. The pig that was euthanatized in a moribund state with edema disease at 12 days PI had $<5 \times 10^3$ CFU of hemolytic E. coli per 5 cm of ileum.

Bacterial culture of rectal swabs collected from pigs with diarrhea in Experiment I yielded small numbers of hemolytic E. coli. In contrast, rectal swabs collected from pigs with diarrhea in Experiment II (all from the principal group with stressors) yielded nearly pure cultures of hemolytic E. coli.

Electron microscopic examination of feces from 4 of the pigs with diarrhea failed to detect rotaviruses.

Serology

Serum antibodies to SLT-IIv were only detected in pigs inoculated with the SLT-IIv-positive E. coli strain #2228. In

Experiment I, 2 of 4 pigs necropsied 14 days PI had titers of 160 and 640, respectively. In Experiment II, antibodies to SLT-IIv titers were only detected in pigs inoculated with E. coli strain #2228 and subjected to additional stressors (housing on concrete floors, moderate cold stress, fasting and treatment with atropine and ranitidine). Three of 5 pigs that lived more than 12 days had SLT-IIv antibody titers of 80, 320 and 640. The pig which was clinically affected with edema disease was in this group and had a SLT-IIv antibody titer of 320.

Figure 2. Ileal submucosa from a pig necropsied 14 days after inoculation with SLT-IIv-positive E. coli strain #2228. There is severe necrosis of arteriolar wall with accumulation of eosinophilic material and cell debris perivascularly. (bar = 50 μm)

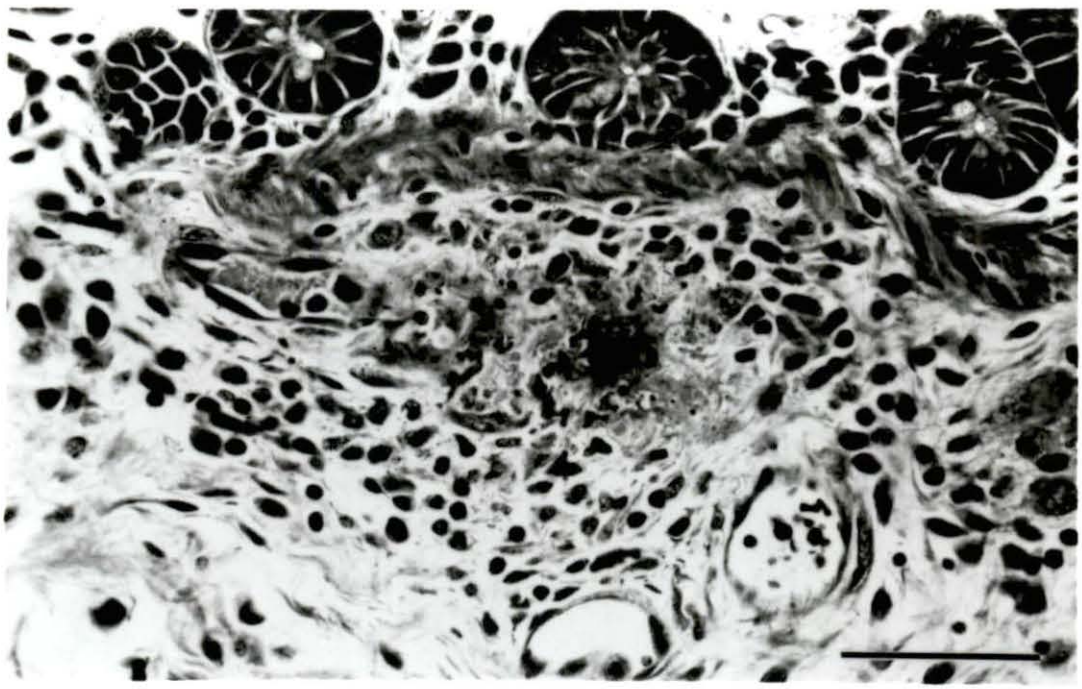


Figure 3. Ileal submucosa from a pig necropsied 14 days after inoculation with SLT-IIv-positive E. coli strain #2228. There is necrosis of myocytes of the tunica media of a small arteriole. Pyknotic nuclei (large arrows) and eosinophilic cytoplasmic droplets (small arrow) are present. (bar = 25 μm)

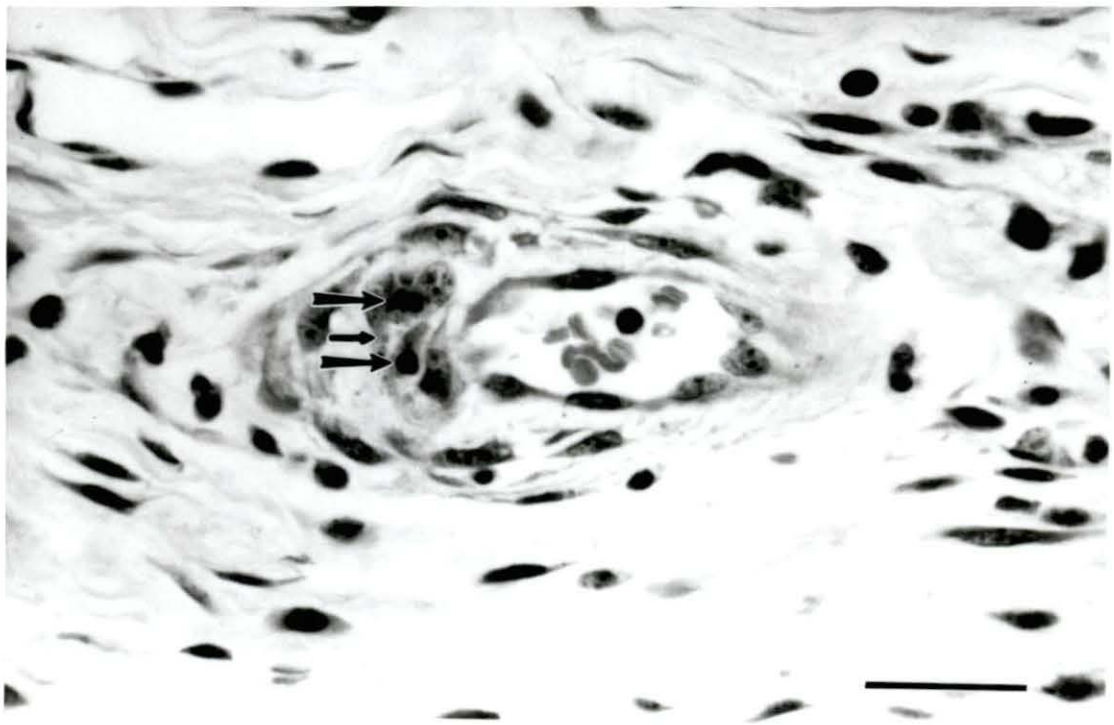


Figure 4. Ileal submucosa from a pig necropsied 14 days after inoculation with SLT-IIv-positive E. coli strain #2228. There is segmental myocyte necrosis of the tunica media of a small arteriole. Karyorrhectic nuclei (arrows) are present. (bar = 50 μm)

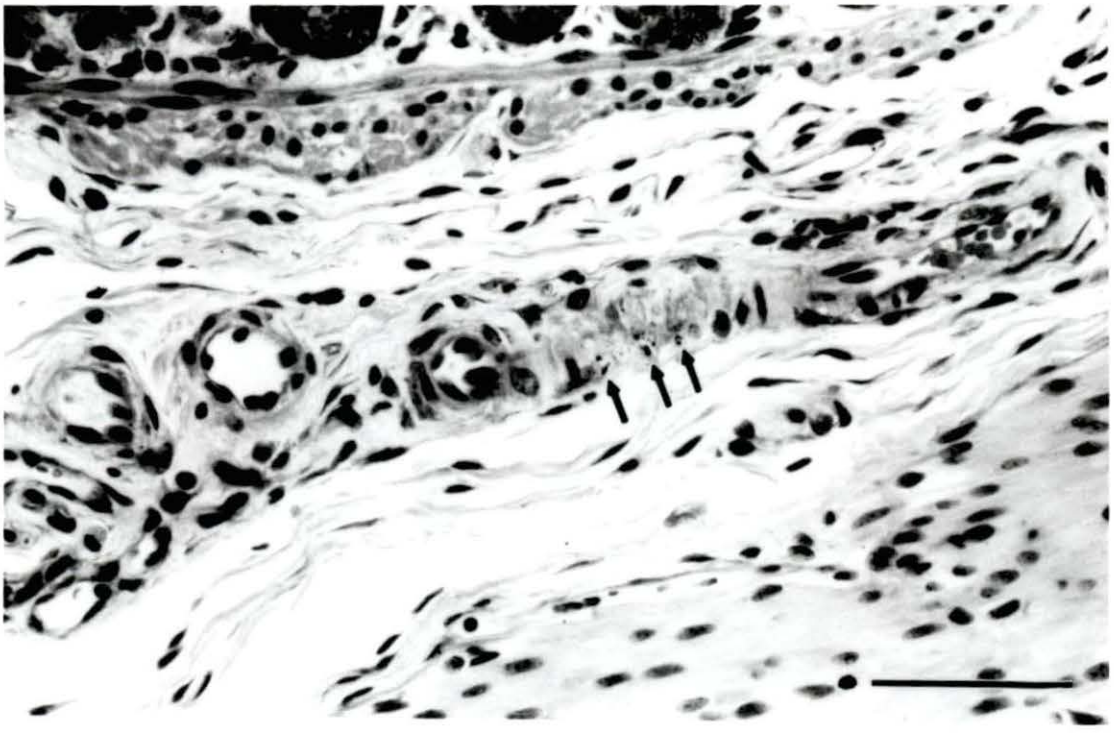


Figure 5. Arteriole in the brain stem from a pig necropsied 14 days after inoculation with SLT-IIv-positive E. coli strain #2228. There is karyorrhexis (small arrows) and pyknosis (large arrows) of individual myocyte nuclei. (bar = 50 μm)

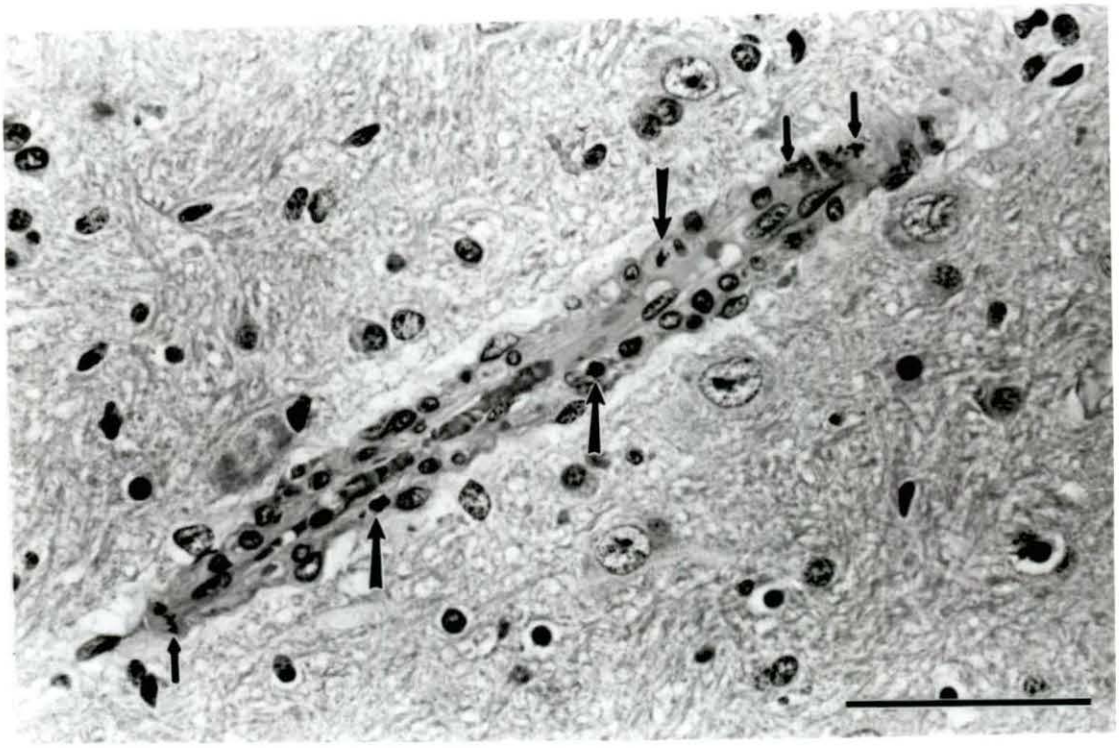
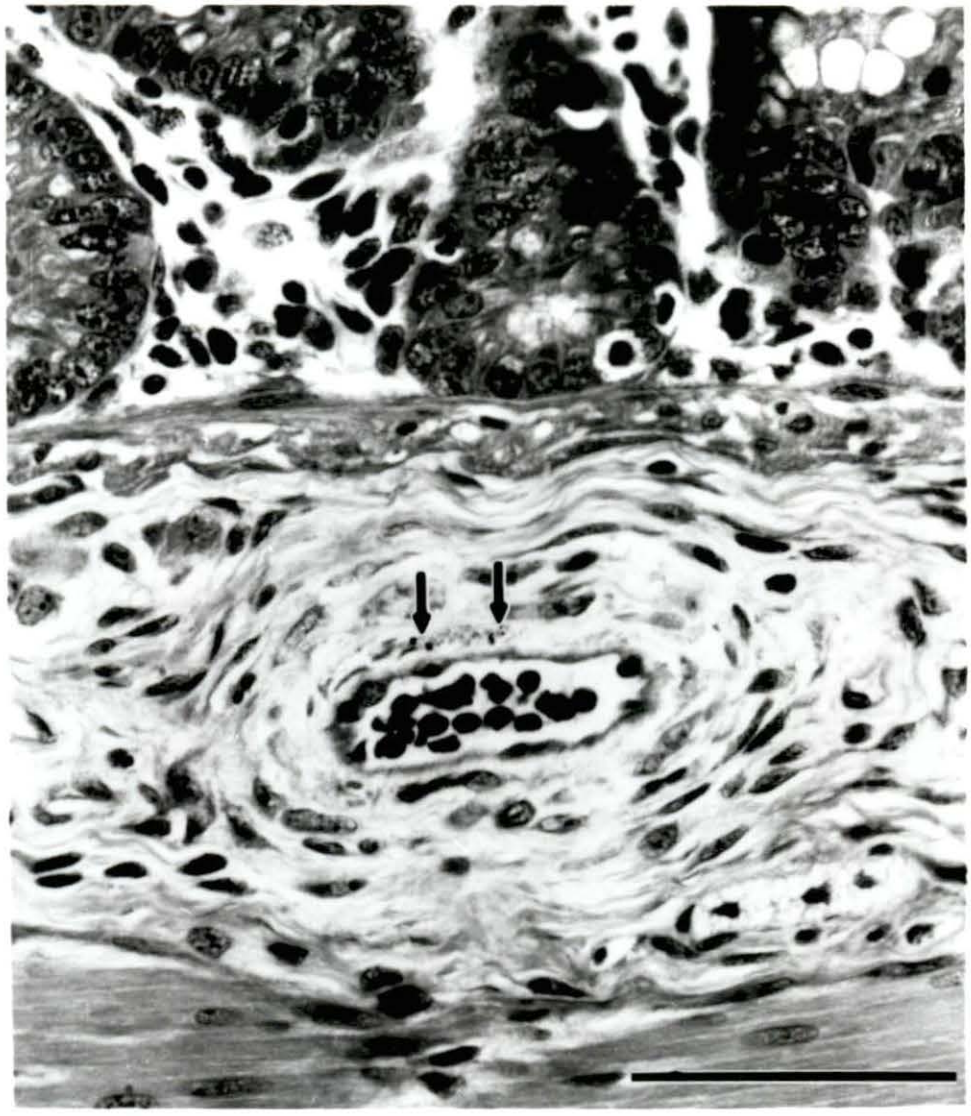


Figure 6. Ileal submucosa from a pig necropsied 14 days after inoculation with SLT-IIv-positive E. coli strain #2228. There is segmental myocyte necrosis (arrows) and diffuse proliferation of periarteriolar adventitial cells. (bar = 50 μm)



DISCUSSION

The data presented in this study provided evidence that histologic lesions of edema disease can be reproduced consistently in pigs in our model. Pigs were raised to 3 weeks of age, weaned and divided randomly into separate groups housed under strict isolation. Animals were then inoculated with E. coli strain #2228, 1 day after weaning, and examined 12 or 14 days PI. Lesions consistent with subclinical edema disease were detected in 12 of 13 pigs inoculated with strain E. coli #2228 and in none of 12 pigs inoculated with the control strain (E. coli strain #123). The lesions were comparable to those described for field cases of edema disease (Kurtz et al. 1969) and with those induced by intravenous injection of SLT-IIv (Gannon et al. 1989) or edema disease principle (Clugston et al. 1974b).

Schulz and Reichel (1964), Nakamura et al. (1982), and Nielsen (1986) reported that PAS-positive globules occur around vessels of the brain in pigs affected by acute edema disease or cerebrospinal angiopathy. PAS staining was used in the current study to facilitate detection of edema disease lesions in subclinically affected pigs. PAS-positive granules found in sections of brain stem and ileum of pigs in both the principal and control group were interpreted to be from

perivascular granulocytes. Therefore, staining with PAS did not increase the sensitivity of histopathologic examination for edema disease lesions.

In Experiment II, environmental stress and treatment with pharmacological agents were incorporated into the model to determine whether the incidence of clinical disease could be increased. Pigs were housed under moderate cold stress (ambient temperature of 20 $\pm$ 2 °C) on concrete floors with access to feces, and one principal and one control group were fasted 24 hours before inoculation. Atropine was injected into pigs in these groups for its anti-secretory and intestinal motility-suppressing capabilities. Ranitidine is a H_2-blocker which was given in addition to atropine to raise the stomach pH after inoculation. Both drugs were chosen to facilitate either stomach passage (ranitidine) or intestinal colonization (atropine) of inoculated bacteria. Moderate cold stress and treatment with atropine and ranitidine as well as housing on concrete floors did not increase the number of pigs with lesions. However, the only pig to develop clinical edema disease was from the principal group subjected to these stressors. It can be speculated that stressors might increase the incidence of clinical disease.

Pigs subjected to additional stressors or affected by clinical edema disease as in the pilot study tended to have

lesions in more organs than pigs without stressors (Table 3). However, pigs weaned without additional stressors and pharmacological treatment, when inoculated with E. coli strain #2228, had microscopic lesions consistently in ileum and brain stem vessels when necropsied 14 days PI.

Only 1 of a total of 23 pigs inoculated with the challenge strain and remaining in the experiment for more than 2 days PI developed clinical edema disease. This low incidence of clinical disease is in contrast to Dr. Moon's pilot study in which 7 of 16 pigs were clinically affected. The difference in clinical disease may be due to endogenous contamination with a virulent edema disease field strain in the pilot protocol; no control animals were present. Contamination with virulent field strains of E. coli can be virtually excluded for the subsequent experiments since no clinical or subclinical edema disease was seen in any control group in either experiment. Management of pigs and environmental stress factors such as housing on concrete floors and moderate cold stress, which were present in the pilot study, were repeated in the second experiment as closely as possible.

Successful reproduction of clinical edema disease was reported by various authors (Bertschinger and Pohlenz 1983, Methiyapun et al. 1984, Smith and Halls 1968). All of these

investigators used pigs from herds in which edema disease was present prior to the experiments. No cases of edema disease were reported in the herd that delivered pigs for the experiments reported here. However, pigs clinically affected in the pilot protocol came from the same herd as unaffected pigs in later experiments. Bertschinger et al. (1984b, 1986) suggested a genetic basis for the susceptibility to edema disease. Their study indicated an influence of the boar on the susceptibility of the offspring. Expression of this genetic trait was suggested to be highly influenced by the environment and it was proposed to have incomplete penetrance. A second possible explanation for the occurrence of clinical disease in 7 of 16 pigs in the pilot protocol and in only 1 of 35 pigs in the experiments reported here is that the pigs used for the experiments in this thesis may have been offspring of boars that bear the genetic trait for resistance to edema disease, whereas pigs in the pilot experiment might have inherited the trait for susceptibility.

Reports by Smith and Halls (1968) and Bertschinger and Pohlenz (1983) suggest that the intestine of pigs affected with edema disease is colonized by the virulent E. coli. Bertschinger et al. (1984b, 1986) speculated that the lack of intestinal receptors is responsible for the resistance to the disease in unaffected pigs. Histologic results obtained in

this study are consistent with this concept. Pigs were not colonized according to our criteria which were E. coli counts above 10^7 CFU in 5 cm ileal segments plus layers of bacteria adherent to villi. Vascular lesions seen in experimental pigs may have been caused by toxin present in the large number of E. coli used as inoculum.

Pigs in the pilot study shed almost pure cultures of hemolytic E. coli when sampled on Day 2 and Day 6 post-inoculation. In subsequent experiments, pigs with diarrhea were examined for shedding of hemolytic E. coli. Large numbers of hemolytic E. coli were detected only in 3 diarrheic pigs in the principal group subjected to the stressors of starvation and pharmacologic agents in Experiment II.

None of the animals with diarrhea in the first experiment shed large numbers of hemolytic E. coli on the first day of diarrhea. Rotavirus could not be detected in fecal samples of 4 pigs with diarrhea. The cause of diarrhea in the first experiment was not determined.

Bacterial counts obtained from 5 cm ileal samples were inconsistent in both experiments. Numbers of hemolytic E. coli were significantly greater in Experiment I in the group inoculated with E. coli strain #2228. This difference could not be reproduced in Experiment II and there was great variability in counts between individual pigs inoculated with

the same strain. The mean number of hemolytic E. coli in the control pigs was higher in the second experiment than in the first. It is not possible to explain these inconsistent results.

Pigs in the control group in Experiment II were kept on concrete floors with access to their own feces. This resulted in less favorable sanitary conditions, when compared to pigs in Experiment I. It is possible that the control pigs in the second experiment developed the previously described (McAllister et al. 1979) postweaning increase in numbers of hemolytic E. coli in the small intestine. The smaller numbers of hemolytic E. coli in pigs in the principal group in Experiment II as compared to Experiment I cannot be explained. One variable introduced in Experiment II was a newly grown inoculum of E. coli strain #2228.

It can be deduced from the results of the bacterial counts on ileal samples that the method for evaluating bacterial colonization of the small intestine should be revised for further experiments. One reason for inconsistent results may lie in the variable amounts of intestinal contents in the samples collected for bacterial counts. Flushing the ileal samples with sterile physiologic sodium chloride solution could probably decrease this variability.

In addition, a marker such as resistance to an antibiotic should be introduced into the challenge strain to facilitate detection in bacterial counts and fecal shedding. A streptomycin-resistant hemolytic E. coli strain, isolated from the same pig as E. coli strain #2228 and thought to be isogeneic with it (Dr. A. O'Brien, personal communication), was used in 2 unpublished experiments and neither colonized the intestines nor caused clinical disease nor consistent histologic lesions. The use of this strain was abandoned because it was not isogeneic with E. coli strain #2228 (T. Casey, personal communication) and because of the negative experimental results. A marked strain, however, should be used for colonization studies using this model of experimental edema disease.

Results from the first experiment indicated that pigs inoculated with E. coli strain #2228 gained significantly less weight than pigs inoculated with the control strain. Weight gain did not correlate with the inoculated strain or experimental group in the second experiment. Pigs in the control group in the first experiment gained the same weight as pigs in all groups in the second experiment. This inconsistency cannot be explained.

Antibodies against SLT-IIv were detected only in pigs inoculated with E. coli strain #2228. In the second

experiment antibodies were detected only in pigs that were subjected to additional stressors (Table 2). Stressors may be necessary for development of toxin levels sufficient to trigger the production of antibodies as well as for the development of clinical edema disease. This theory may be in accordance with the low antibody-titers found in the first experiment. Pigs in the principal group in Experiment I had lower weight gain than pigs in the control group, although diarrhea was present in both groups. Perhaps unknown stressors in Experiment I accounted for the antibody response. The presence of diarrhea in Experiment I can be considered as a stressor by itself.

In conclusion, a model for the consistent reproduction of subclinical edema disease in 3-week-old, recently weaned pigs is described in which necrosis of small arteries and arterioles is used as a criterion for evaluation.

SUMMARY AND CONCLUSIONS

Edema disease is part of the postweaning colibacillosis syndrome in pigs. Recent reports suggest a variant of Shiga-like toxin II (SLT-IIv) produced by some E. coli strains as the active principle of the disease.

Histopathologic lesions of edema disease were consistently induced in pigs by intragastric inoculation with a SLT-IIv-positive E. coli strain isolated from a pig with clinical edema disease. Pigs were born at the National Animal Disease Center and raised to 3 weeks of age. Pigs were weaned, divided at random into principal and control groups, and groups were housed under strict isolation. One day after weaning, pigs were inoculated with the SLT-IIv-positive strain or a non-SLT-IIv-producing control strain of E. coli isolated from a healthy pig.

The study was divided into 2 experiments. In the first experiment, pigs were housed on raised metal grid floors and had access to feed immediately after weaning. In the second experiment pigs in 1 group were fasted for 24 hours prior to inoculation and were additionally treated with 5 mg/kg atropine and 10 mg/pig ranitidine. In this experiment all pigs were housed on concrete floors with access to their own feces. Pigs in all experiments were observed daily and

necropsies were done on days 2 and 14 post-inoculation. One of 35 pigs inoculated with the SLT-IIv-positive strain developed clinical edema disease and 12/13 clinically normal pigs in that group necropsied on day 12 or 14 post-inoculation had vascular myocyte necrosis in the ileum (12/13 pigs) and brain stem (11/13 pigs). Lesions consisted of karyorrhexis and pyknosis of myocyte nuclei in small arteries and arterioles. None of the pigs inoculated with the non-pathogenic control strain developed clinical edema disease (0/35 pigs) or vascular lesions (0/12 pigs).

In conclusion, an experimental model for subclinical edema disease is described. Microscopic arteriolar necrosis is used as the end point for pathologic evaluation.

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ACKNOWLEDGEMENTS

I would like to thank my major professor, Dr. H. W. Moon, who generously invited me to work at the National Animal Disease Center in his group. His support and patience were greatly appreciated.

I wish to thank my co-major professor Dr. L. H. Arp, who is a great mentor and whose guidance and support deserve my special gratitude. I am especially thankful to Dr. E. A. Dean for her constant help with the day-to-day work in the laboratory. Her good advice and dedication in helping me through graduate school were very important for making this program successful.

I wish to thank Dr. H. T. Hill for his comments and suggestions.

The excellent technical support of Mayo Skartvedt, from whom I learned many skills in addition to maintenance of experimental animals, and the excellent help of the staff of the National Animal Disease Center were greatly appreciated.

Special thanks go to Paula Munns, for her friendship and constant help and support in preparation of this thesis.

Most of all, my appreciation is extended to my parents. Their love and confidence supported me through this work and stay abroad.

Ganz besonders bedanken möchte ich mich bei Prof. Dr. Pohlenz, Prof. Dr. Schwarz und Prof. Dr. Sallmann in Hannover, die mich bei der Planung und Realisation meines Auslandsaufenthaltes teilnehmend und ausdauernd unterstützten.

Ohne die grosszügige finanzielle und idelle Unterstützung der Studienstiftung des deutschen Volkes, die mir ein Stipendium für das erste Jahr meines Aufenthaltes in den USA gewährte, wäre dieses Projekt nicht möglich gewesen. Ich möchte mich für das in mich gesetzte Vertrauen herzlich bedanken.

Mein besonderer Dank gilt Evi, ohne deren Freundschaft und selbstlose Unterstützung dieses Projekt nicht realisiert worden wäre.