FACTORS CONTRIBUTING TO THE PRESENCE OF *ESCHERICHIA COLI* O157:H7 AND O157:NM IN FEEDLOTS AND FEEDLOT CATTLE

A Thesis

by

PAPHAPIT UNGKURAPHINUNT

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2003

Major Subject: Food Science and Technology

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ABSTRACT

Factors Contributing to the Presence of *Escherichia coli* O157:H7 and O157:NM in Feedlots and Feedlot Cattle. (August 2003)

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Environmental sources within 5 feedlots were sampled for *E. coli* O157:H7 and O157:NM to determine the prevalence of this pathogen with a view to minimize or control its spread in the feedlot environment. Monthly samples were taken from the feedlots in the Panhandle and South Plains of Texas over a nine-month period. Samples were examined by an immunomagnetic bead separation, followed by plating onto CT-SMAC and CHROMagar™ O157 media. Sorbitol-negative colonies were tested using ImmunoCard Stat! *E. coli* O157:H7 Plus and confirmed as *E. coli* O157:H7, using biochemical (Vitek system) and serological tests (latex agglutination). Additionally, one hundred sponge samples were collected from the hides of stunned cattle at the slaughter plant. All isolates were subjected to rep-PCR DNA fingerprinting and antimicrobial profiling.

E. coli O157 was isolated from hide (56%) and environmental samples (4%). *E. coli* O157 was isolated from all environmental sources, with peak prevalence during November (9%) and March (10%). At least one sample from each feedlot was positive 42% of the time. The most contaminated sites were the chute area (6%) and sludge from

waste water ponds (6%). Positive samples were most frequently found from feedlot 5 (7%) and the greatest variation in positive samples between feedlots (0-34%) occurred during March. A decrease in the presence of *E. coli* O157 in feedlots was observed during January (0%), when ambient, water, and pond sludge temperatures were consistently low. No correlation with other environmental factors was observed. Hide was a primary source of *E. coli* O157 on carcasses with an overall prevalence of 56%. Of two sampling days, the number of positive hide samples varied from 14% for the first day to 98% for the second day. The total positive samples collected (environmental (47); hide (56)) were 64% H7, and 36% NM. The environmental isolates showed similar antibiotic resistance patterns, regardless of the source. Most *E. coli* O157 isolates from the feedlots and hides showed a high level of resistance to cephalothin (45%) and sulfisoxazole (56%). *E. coli* O157 isolates from feedlots were resistant to more than 10 antibiotics (9/317). All of the isolates appeared highly similar, with an average similarity of 53% by rep-PCR DNA fingerprinting.

DEDICATION

This thesis is dedicated to my parents, Parinya and Yupa, my family, Jariya Unkuraphinunt, Parinda Ungkuraphinunt, and Panaikhorn Ungkuraphinunt, my friends, Siriporn Thamwongsin and Piyada Huatcharoen, and my dogs, Leo and Mochi, in appreciation of their encouragement, love and support.

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INTRODUCTION

Serious human illnesses associated with *Escherichia coli* O157:H7 have increased since first reported in 1982. The infection can range from self-limited watery diarrhea to life-threatening manifestations including hemorrhagic colitis, and the diarrhea-associated form of the hemolytic-uremic syndrome. The increase in *E. coli* O157:H7 illnesses have lead to more clinical awareness and improved techniques for the isolation and identification of *E. coli* O157:H7. However, there is an increase in the incidence of infections presumably due to proliferation of the organism and increased exposure of the human population to *E. coli* O157:H7. Due to the direct and indirect link to bovine products in outbreaks, cattle have been implicated as the primary reservoir of this organism.

Environmental sources may serve as a vehicle for the transmission of *E. coli* O157:H7 within and between farms. The incidence of this organism has been found to be very low; therefore, a tremendous number of cattle samples would be necessary to evaluate transmission. Monitoring of this pathogen by collecting environmental samples at the feedlot is a preferable and more practical approach. Furthermore, this approach may be useful for determining if there is an association of certain factors of *E. coli* O157:H7 contamination on the cattle.

Detection of *E. coli* O157:H7 can be difficult because of high levels of other sorbital non-fermenting bacteria in the samples, as well as the lack of sensitivity and

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This thesis follows the style and format of Applied and Environmental Microbiology.

specificity of the sampling methodology. Recent studies have revealed a higher prevalence of *E. coli* O157:H7 than originally estimated as a result of a new technique based on immuno-magnetic bead capture. Immunomagnetic separation has increased the recoverability due to an antibody-based concentration procedure which uses magnetic beads coated with an antibody against *E. coli* O157:H7.

Intensive use of antimicrobial agents in animals is considered the main factor causing selection resistance in pathogens. The development of antibiotic resistance in human pathogens has been closely associated with the use of antibiotics for therapy, diseases prevention, growth promotion, and control of diseases in the animals in modern production systems. These conditions assist the spread and persistence of antimicrobial-resistant pathogens, including as *E. coli* O157:H7 which poses a public health threat, due to the higher risk of treatment failures in human. Furthermore, the spread of antimicrobial resistance through an acquired transmissible genetic element may be related to the ability of the organism to colonize an animal host, persist on the farm or in a food processing environment.

Repetitive element sequence-based PCR (rep-PCR) is the common method used to generate DNA fingerprints that allow discrimination between bacterial strains. The genomic relatedness among the *E. coli* O157:H7 isolates can be detected by rep-PCR which involves the use of oligonucleotide primers based on short repetitive sequence elements.

The objectives of this study were (A) to determine the prevalence of *E. coli* O157:H7 and O157:NM in the environment of selected feedlots and on hides from beef

cattle presented for slaughter at selected plants in Texas, (B) to evaluate the effect of extrinsic factors on the presence of *E. coli* O157:H7 and O157:NM in the feedlot environment, and (C) to measure the *in vitro* susceptibility of *E. coli* O157:H7 and O157:NM isolates to antimicrobial agents and analyze DNA relatedness using rep-PCR to identify the possible sources of isolates.

LITERATURE REVIEW

Human illness

Escherichia coli O157:H7 first gained public recognition as an important human foodborne pathogen in the United States in 1982 following two unusual hemorrhagic colitis outbreaks in the states of Oregon and Michigan caused by consumption of improperly undercooked hamburgers from a fast food restaurant chain. (98). Since 1982, the number of *E. coli* O157:H7 outbreaks and sporadic cases have increased, and more than 100 outbreaks have been documented. The largest foodborne outbreak of *E. coli* O157:H7 ever reported occurred in the spring of 1993 in several western states (19).

The severity of infection with *E. coli* O157:H7 and O157: NM (nonmotile) can range from self-limited watery diarrhea to life-threatening manifestations, such as hemolytic uremic syndrome (HUS) or thrombotic thrombocytopenic purpura (TTP) (62, 63). Children under 5 years of age, elderly, and immunocompromised individuals are in the highest risk group for infection by this organism (24, 25). In 1999, the Centers for Disease Control and Prevention (CDC) estimated that 73,000 illnesses and 60 deaths per year in U.S. were caused by *E. coli* O157:H7, while there were 37,000 estimated cases of illness caused by non-O157 Shiga toxin-producing *E. coli* (STEC) (75). There are more than 100 deaths annually due to STEC infections. *E. coli* O157:H7 is responsible for 85-95% of HUS cases worldwide and the primary cause of hemorrhagic colitis (HC) and HUS in the United States, Canada, Great Britain and Europe (47).

The Advisory Committee on the Microbiological Safety of Food (2) and Bolton et al. (11) suggest that the infectious dose of *E. coli* O157:H7 can be as low as 100 cells or less. However, the infectious dose may vary depending on the host.

Tarr (110) identified symptoms and onset of *E. coli* O157:H7 infection. There are three principal manifestations, including HC, HUS, and TTP. The sudden onset of HC usually occurs within 1-2 days after consumption of contaminated foods; however, longer periods (3-5 days) have been reported. The initial gastrointestinal symptoms begin with mild, non-bloody and watery diarrhea which later becomes grossly bloody, described as "all blood and no stool" (88), and is sometimes followed by a period of abdominal pain. Vomiting may occur as well as a short-lived fever. The intensity of diarrhea increases in the next 24-48 h up to 4-10 days, including overtly bloody diarrhea with severe abdominal pain and moderate dehydration.

Ten percent of patients infected with *E. coli* O157:H7 develop HUS. HUS is the leading cause of acute renal failure in patients, especially in children (under 5 years of age) (88). The symptoms of HUS typically occur one week after initial onset. Approximately 50% of HUS patients require dialysis and blood transfusions. Siegler et al. (104) reported that approximately 15% of cases can result in early development of chronic kidney failure, and consequently follow with persisting insulin-dependent diabetes in HUS patients.

According to Boyce et al. (13), TTP conditions primarily develop in adults, generally cause less renal damage than HUS and are involved in significant neurological conditions such as central nervous system deterioration, seizures, and strokes. Symptoms

usually consist of microangiopathic hemolytic anemia, acute thrombocytopenia, fluctuating neurologic signs, fever, and mild azotemia. Frequently, patients develop blood clots in the brain, resulting in death. Other unusual clinical manifestations of *E. coli* O157:H7 illnesses include hemorrhagic cystitis and balanitis, convulsions, sepsis with another organism, and anemia (88).

The mode of transmission of *E. coli* O157:H7

E. coli O157:H7 infections are transmitted via three primary routes: 1) directly from animals (such as farm animals and domestic pets such as deer (64, 95), sheep (67), pigs (23, 24) horses, dogs (53, 115), and wild birds (51, 64, 95, 120); 2) person-to-person such as in day-care centers and nursing homes (8, 109); 3) via contaminated foods, especially undercooked ground beef and unpasteurized milk (48).

Animals used for food, such as cattle, pigs, and sheep, may carry *E. coli* O157:H7 in the 'normal' gut flora which causes a potential risk of infection to humans. Routes of infection include fecal-oral route from animals to humans during rearing processes, fecal contamination of food crops with untreated or poorly treated manure used as a fertilizer, and fecal contamination of carcasses during slaughter and evisceration processes due to poor hygienic practices (6).

Contaminated food is the principle mode of transmission of *E. coli* O157:H7 infections (4, 108, 125). Willshaw et al. (125) reported that the infectious dose of *E. coli* O157:H7 could be as low as 10 bacteria in a meat sample, depending on variables including stomach pH, food composition and the host susceptibility (17, 43). Contaminated and improperly cooked ground beef has been epidemiologically

implicated as the food most often associated with outbreaks during 1992-1993 that affected more than 500 individuals in the western United States (5). In addition, undercooked ground beef was suspected as the vehicle of transmission in two major outbreaks in 1982, an outbreak at an Ontario nursing home in 1982 (107), a Nebraska nursing home in 1984 (99), an Alberta nursing home in 1986 (58), and in a community in 1986 (49) and 1990 (18). The World Health Organization (WHO) (128) has reported that more than 50% of *E. coli* O157:H7 outbreaks have been attributed or linked to foods associated with cattle.

E. coli O157:H7 is transferred to beef likely via fecal contamination of carcasses during slaughter and processing (25, 36). In beef processing, grinding may inoculate the bacteria throughout ground beef patties where *E. coli* O157:H7 is more likely to survive inadequate cooking (13, 74). Ground beef is normally made from trimmings, thus the possibility exists for contamination in a large quantity of ground beef produced from a few contaminated carcasses (13). If non-bovine products are incriminated, cross-contamination by bovine products is usually suspected (20, 21, 27, 48). Studies by Le Saux et al. (69) and Pai et al. (89) showed that ground beef has also served as a risk factor in sporadic infection cases. Dry-cured salami was the cause of an *E. coli* O157:H7 outbreak in the western United States, which suggested *E. coli* O157:H7 can survive the acidic conditions of fermented meats and cause illness in humans (113).

Other varieties of foods have been linked with disease-causing *E. coli* O157:H7 worldwide, including cantaloupe (33), salad dressing containing mayonnaise (130), cooked ham (42) unpasteurized apple cider (9, 80), and ham, turkey, or cheese

sandwiches and turkey roll sandwiches (88). Cross-contamination with bovine products or contamination with feces of wild or domestic animals has been suspected in the majority of these outbreaks.

Cattle as a reservoir of *E. coli* O157:H7

Cattle have been implicated as a reservoir of *E. coli* O157:H7 since 1982 as a result of the *E. coli* O157:H7 outbreaks associated with undercooked ground beef and raw milk (98, 122). Several investigations from previous outbreaks determined that *E. coli* O157:H7 was frequently excreted in cattle feces, raising suspicion that contaminated bovine products might have been the source of infection in humans. Because of the common link to bovine products, cattle have been implicated as the primary reservoir of *E. coli* O157:H7 and other verotoxin-producing *E. coli* (53, 86, 116, 117). Prevalence of *E. coli* O157:H7 fecal excretion varies significantly among *E. coli* O157 positive herds (53, 132). Hancock et al. (53) found the prevalence of *E. coli* O157:H7 was 0.28% in dairy cattle and 0.71% in beef cattle, with a herd prevalence of 8.3% in dairy cattle and 16% in beef cattle. Previous studies revealed a low prevalence of *E. coli* O157:H7 in cattle feces (37, 53). However, recent research has shown higher levels of *E. coli* O157:H7.

Chapman et al. (23) isolated 15.7% *E. coli* O157:H7 from cattle (752/4800) over a year-long period. The monthly prevalence ranged from 4.8% to 36.8%. In addition, Elder et al. (36) recently revealed 28% *E. coli* O157:H7 prevalence in fecal samples (91/327) isolated from slaughter cattle during July and August. *E. coli* O157:H7 was most prevalent in spring and late summer (23). Van Donkersgoed et al. (119) reported

E. coli O157:H7 in 7.5% of fecal samples collected from cattle at slaughter. The prevalence of *E. coli* O157:H7 in fecal samples was higher in yearling cattle (12.4%) than in cull cows (2.0%). In addition, Zhao et al. (132) found that young animals tended to carry *E. coli* O157:H7 more frequently than adult animals. Two primary U.S. investigations, Zhao et al, (132) and USDA/APHIS (117) indicated that 3.2% of dairy calves and 1.6% of feedlot cattle were *E. coli* O157:H7 positive, and 0.4% of feedlot cattle were *E. coli* O157:NM positive. Zhao et al. (132) found that *E. coli* O157:H7 levels in calf feces ranged from less than 10^2 to 10^5 CFU/g.

E. coli O157:H7 shedding fluctuates and varies in duration from several weeks to months (14, 29). *E. coli* O157:H7 strains with the same pulsed field gel electrophoresis (PFGE) genomic DNA profiles can be isolated from calves in different states or farms. However, different *E. coli* O157:H7 strains can be isolated from the feces of the same animal or different animals within the same herd (37, 79).

A number of investigations have shown that cattle are asymptomatic carriers of *E. coli* O157 and that the bacteria can be isolated from healthy beef and dairy cattle (53, 123, 132). Brown et al. (14) and Cray and Moon (29) indicated *E. coli* O157:H7 is not pathogenic to calves at an inoculum level of 10^{10} CFU/ ml. *E. coli* O157:H7, excreted in feces, dramatically dropped during the first 14 days after inoculation, from 10^4-10^6 CFU/ g feces at 48 h to 5-10^2 CFU/g feces at 14 days. In some cases, the level of *E. coli* O157 excreted in the feces greatly increased, but not in all animals. The pattern of *E. coli* O157 shedding by cattle tends to be short periods with a relatively high prevalence of excretion separated by longer periods of reduced or undetectable shedding (10, 123).

Recent studies have reported that prevalence among cattle varies extensively, partially due to the differences in sensitivity of procedures used for detecting *E. coli* O157:H7. Chapman et al. (26) reported a 10- to 100-fold increase in sensitivity of detection of *E. coli* O157:H7 using immunomagnetic separation techniques for bovine fecal samples. Dargatz et al. (30) and Elder et al. (36) reported that *E. coli* O157:H7 prevalence ranged from 63% to 100% in feedlot surveys in the United States. In addition, prevalence rates of *E. coli* O157 in cattle have ranged between 1.0% and 27.8% and up to 68% in heifers (23, 28, 36, 76).

Distribution of *E. coli* O157:H7 is widespread in cattle operations; however, the presence of *E. coli* O157 is subject to factors such as seasonal variation (52, 76, 124). In addition, higher prevalence of *E. coli* O157 may be influenced by geographic location, season, number, frequency and timing of sampling to transport and storage of samples (36, 119). Chapman (22) reported higher prevalence of *E. coli* O157 in the northwestern part of the USA and in northern England more than in other areas. Ostroff et al. (87) indicated a similar seasonal pattern between of *E. coli* O157 shedding and *E. coli* O157 foodborne illness in humans.

Contamination of cattle hides

Cattle hides are a known source for *E. coli* O157 contamination. Elder et al. (36) reported a positive correlation between fecal and hide prevalence of *E. coli* O157:H7 and subsequent contamination of carcasses with these bacteria during slaughter and processing.

A number of studies (7, 36, 59, 93) have reported contamination from the hide to the carcass surface during dehiding. Reid, et al. (93) assessed the prevalence of *E. coli* O157 on the rump, flank and brisket of cattle hides immediately after slaughter to evaluate the potential risk of hide-to-carcass contamination during the slaughter processing. The brisket area on the cattle hide was frequently the most contaminated site (22.2% prevalence on average); therefore, it was the most likely area to cross-contaminate the carcass during the de-hiding process. The brisket hide area was most contaminated when animals lie down on contaminated ground/floor on the farm, during transportation, in lairage, and/or within the stunning box. The rump area was the least contaminated area on the cattle hides (3.3%) (93).

Several factors influence the level of hide contamination on animals presented for slaughter, and directly affect the microbial load on the carcass (32). The level of visible contamination on cattle hides has been shown to subsequently affect the level of contamination on carcasses. In addition, visibly clean hides may not necessarily be pathogen free and offer a potential hazard for cross-contamination of *E. coli* O157 (73, 84, 97). The spreading pattern from this bacteria from one animal to other during transportation and lairaging was likely to be either directly via carcass-to-carcass contact, especially flank and rump areas, or indirectly via contact with contaminated floors surfaces, especially in the brisket area.

Environmental sources as a reservoir of *E. coli* O157:H7

Many researchers have reported that the initial sources of or exposure to *E. coli* O157:H7 in cattle might possibly occur in the feedlot environment. *E. coli* O157:H7

generally appeared in most cattle feedlot operations, but the proliferation or prevalence of this organism varied (52, 55). The excretion of *E. coli* O157:H7 in herds fluctuated, showing up in only a few samples for months, followed by an increase in the number of positive samples isolated from the same herds. Hancock et al. (51) suggested that the unpredictable presence of *E. coli* O157:H7 is due to an external reservoir. Environmental sources, such as feed and water may play a vital role in transmission of *E. coli* O157:H7 within and among feedlots (53, 126).

Recent research has pointed to environmental sources as possible reservoirs of *E. coli* O157:H7 in the farm environment, including manure piles, ponds, dams, wells, barns, calf hutches, straw and other bedding, feed and feed troughs, water and water troughs, farm equipment, and ground pasture. *E. coli* O157:H7 can grow in water, feed and soil, and can survive in feces for prolonged periods, depending on temperature and moisture conditions (60, 72, 119, 121). Once *E. coli* O157:H7 occurs in the farm environment, transmission of the bacteria to other sites occurs via rainwater, wind, removal and spreading of manure, and also by animals and humans (61). In recent studies, Wallace et al. (120) showed the potential for seabirds to be an *E. coli* O157 carrier, reporting that 0.9-2.9 % of fecal samples were positive for *E. coli* O157. If found on farms, birds can cycle these pathogens through the agricultural environment. Shere et al. (103) isolated 6.3% *E. coli* O157:H7 from feed samples obtained from a dairy farm in Wisconsin. Lynn et al. (72) studied growth of *E. coli* O157:H7 in a variety of wet grain mixtures and some silage-based mixtures *in vitro* at similar temperatures reached during the summer months. Research has shown replication of *E. coli* O157:H7

in several types of feed, therefore increasing the possibility of further dissemination of *E. coli* O157:H7 on the farm. Several studies isolated *E. coli* O157:H7 from animal drinking water (15, 16, 37, 51). Faith et al. (37) suggested water as a vehicle for transmitting *E. coli* O157:H7 among cattle. LeJeune et al. (71) demonstrated water troughs as long-term environmental sources of *E. coli* O157:H7 and a possible cause to subsequent cattle infection.

Antimicrobial resistance

Antimicrobials are used therapeutically and prophylactically in human and veterinary medicine, and also to promote growth in animal production. Antibiotics with similar structure are being applied in medical and veterinary practice.

Initially, *E. coli* O157:H7 was found to be susceptible to many antibiotics (12, 92). However, several recent studies have shown increasing antimicrobial resistance in *E. coli* O157 strains isolated from humans and animals (1, 38, 41, 65, 78, 101, 129). Resistance to tetracycline, sulfamethoxazole, cephalothin, and ampicillin has been reported in a number of studies (21, 78, 129). In addition, many O157 and non-O157 *E. coli* strains have developed multiple resistances to antimicrobials commonly applied in human and veterinary medicine (38, 46, 101).

The correlation between intensive use of antibiotics and development of bacterial resistance is well documented for pathogens (112). Currently, there is speculation regarding the role therapeutic and subtherapeutic use of antimicrobials in animals played in accelerating the development and dissemination of antimicrobial-resistant bacterial pathogens (1, 114, 127). Witte (127) reported medical consequences of antibiotic use in

agriculture where the selection for antimicrobial resistance among commensals in the intestinal tracts of food animals may create a public health threat. For example, food animals, especially mature cattle, may be asymptomatic carriers of *E. coli* O157, including STEC, and may serve as a reservoir of antimicrobial-resistant bacteria when exposed to antimicrobial agents in the animal production environment (77, 111, 127). Schroeder et al. (102) suggested that the initial impact on the selection of antimicrobial resistance in STEC and non-STEC O157 was imposed by the use of tetracycline derivatives, sulfa drugs, cephalosporins, and penicillins for therapeutic use in human and veterinary medicine and/or as prophylaxis in the animal production environment.

Multiple antimicrobial resistances in STEC and non-STEC strains may have been partially caused by spreading of genetic elements such as plasmids, transposons, and integrons (129). Acquisition of mobile genetic elements such as integrons, that encode multiple antibiotic resistance genes, are thought to play a major part in the evolution of multiple resistant bacteria (50). Integrons not only associate with multiple antibiotic resistances, but also may play a significant role in the dissemination of resistance genes on STEC strains. Zhao et al. (129) studied the characterization of antimicrobial susceptibility patterns among STEC strains, including *E. coli* O157 and non-O157 STEC isolated from cattle, ground beef, and humans and determined that the observed resistance phenotypes could be attributed to the acquisition of integrons encoding resistance gene cassettes. The study showed 79% of STEC isolates developed resistance to multiple classes of antimicrobials. The most frequently observed multiple resistances were to streptomycin, sulfamethoxazole, and tetracycline. The integrons identified

among O157:H7 isolates possessed an *aadA* gene and can be transferred via conjugation to another strain of *E. coli* O157.

Sáenz et al. (100) investigated antibiotic resistance of isolates from animal feces, human feces, and food products of animal origin. The study revealed that 88%, 38%, and, 40% of the bacteria isolated from broilers were resistant to nalidixic acid, ciprofloxacin and gentamicin, respectively, while 53%, 13%, and 17% were from foods. In addition, these data showed high levels of trimethoprim-sulphamethoxazole and tetracycline resistance in organisms isolated from broilers, pigs, and foods.

Antibiotic resistant strains of *E. coli* O157:H7 could possibly be transmitted to humans through the food chain if animal food products are improperly cooked or mishandled. Other sources of contamination could include contact via occupational exposure, or waste runoff from animal production facilities (118, 127). Different antibiotic resistances were frequently detected in *E. coli* O157 isolates from different sources, including humans, broilers, pigs, pets, and feed. The difference may come from the specific use of antibiotics in each group. Schroeder et al. (102) found it difficult to identify the origin of observed antimicrobial resistance because the microbial ecosystems of humans, swine, cattle, and food are closely connected.

MATERIALS AND METHODS

Preliminary testing

Ice chest studies. Preliminary studies were carried out to determine if the shipping container would adequately insulate samples during overnight transportation. In this study, samples were shipped in an ice chest (47.6 X 30.1 X 31.1 cm ThermoSafe® Mutipurpose Insulated Bio-Polyfoam Shipper; Model 494, Polyfoam Packers Corp., Wheeling, Ill.). The samples were collected in 150-ml Oxford specimen cups (International BioProducts, Inc., Redmond, Wash.). The ice chests were packed with varying combinations of sample cups, plastic racks and 360-ml or 1500-ml refrigerant packs (VWR International, Suwanee, Ga.) to simulate possible arrangements. Each sample cup was filled with distilled water and placed inside a Nasco 710-ml Whirl-Pak® sampling bag (VWR). To monitor the internal temperature of the ice chest at 30-min intervals over a 48-h period at room temperature, 5 thermocouples (Type K; Pico Technology Limited, St. Neots, Cambridgeshire, UK) were placed inside the ice chest and connected to the thermocouple data logger (Pico). Four of the thermocouples were placed in the corners of the ice chest without touching the refrigerants, and the fifth was placed in one of the plastic cups to measure the temperature of the water in the container. The data logger was then connected to a computer and analyzed with the supplied software (Pico) which provided a macro to collect data directly onto an Excel spreadsheet (Microsoft Excel; Microsoft, Bellevue, Wash.). After 48 h, the thermocouples were removed and the data was analyzed to establish which combination effectively lowered the temperature of the ice chest to approximately 0-5°C after 7 h.

Standard Operating Procedure (SOP)

A SOP document containing written instructions and photographs was sent in advance to the sample collector, Mr. Kevin McBride, at the Texas Agricultural Extension Station, Amarillo, Tex., to enable familiarization with the protocol prior to the sampling period. Supplies were packed into five 47.6 X 30.1 X 31.1-cm ice chests and sent to Amarillo on a monthly basis. Each ice chest was loaded according to Table 1.

Sampling design

Samples were collected from 5 commercial feedlots that handled more than 40,000 cattle in and areas around Amarillo**,** Tex. The potential sources of *E. coli* O157:H7 to be sampled included: A) surface water from the runoff holding pond, B) sludge from the bottom of the runoff holding pond, C) water and sludge runoff from the drainage trenches and the collection ponds in the pens, and D) surface area of the cattle handling chute. The surface water samples and sludge samples were collected from locations north, south, east, and west of the runoff pond just under the surface of the water. Five surface area samples from the back right, front right, back left, front left and front were collected around the chute areas. In addition, 5 runoff areas were sampled to evaluate the possibility of contaminated drainage at the feedlot. However, the number of runoff samples mainly depended on the design of each feedlot.

Sampling occurred on a monthly basis for an 8-month period from July 2001 to March 2002. During that time, several environmental factors were monitored for their effect on the presence of *E. coli* O157:H7 in feedlots. These included: A) ambient temperature,

TABLE 1. Sample collecting supplies provided in each ice chest for each sample trip

Items	Number
24 X 30-inch Biohazard Plastic bags (VWR)	2
Nasco 710-ml Whirl-Pak ® sampling bags	38
A set of labels for each feedlot	1
Oxford 150-ml specimen cup (International BioProducts)	38
Bottle of 25 ml of sterile Butterfield's buffer (International BioProducts)	7
BioPro sponge/glove sampling systems (International BioProducts)	7
Large latex gloves (International BioProducts)	9
Medium latex gloves (International BioProducts)	8
360-ml U-Tex refrigerant packs (VWR)	20
1500-ml U-Tex refrigerant packs (VWR)	3

Note: In addition, 4 boxes of 15.25 X 2.0-cm wooden tongue depressors (International BioProducts), a case of 100 individual disposable plastic USDA Template 100™ (International BioProducts) for a 10 X 10-cm^2 sampling area, OAKTON® TDSTestr™ 4 water conductivity (OAKTON; distributed by VWR), and Magellan GPS 315™ global positioning system (Magellan, San Dimas, Calif.) were supplied at the beginning of the experiment.

B) dew point, C) electrical conductivity, D) liquid levels in the pond, E) sludge temperature, F) relative humidity, G) station pressure, H) temperature dry bulb, I) temperature wet bulb, and J)water temperature of the runoff pond.

Additionally, 100 randomly selected hide samples were obtained from stunned adult cattle that originated from each of the 5 commercial feedlots, at a commercial slaughter plant, located in Amarillo, Tex. during August, 2002.

Sample collection

Waste water samples. Water samples were collected using an Oxford 150-ml specimen cup. Prior to collection, the specimen cups were rinsed 2-3 times with the liquid being collected. At least 100 ml of the runoff pond water was collected just under the water surface and then the exterior of the specimen cup was wiped and dried using a paper towel. The water sample cup was then placed into a Nasco 750-ml Whirl-Pak® sampling bag to provide an additional leakage barrier for transport.

Pond sludge samples. The sludge samples were collected from the bottom of the runoff ponds using a spade. The sample was thoroughly mixed using a 15.25 X 2.0-cm wooden tongue depressor (International BioProducts) to ensure uniformity**,** and placed in an Oxford 150-ml specimen cup. The specimen cup was placed in a Nasco 710-ml Whirl-Pak ® sampling bag to provide an additional leakage barrier for transport.

Pen drainage samples. The number of drainage samples and retention pond samples varied between feedlots. Semi-dry material samples were handled and sampled in a manner similar to that described for the sludge samples. The liquid samples were treated the same as the water samples and were handled as previously described.

Chute samples. Samples were collected from 5 surface areas of the chutes used for animal treatments using 25 ml of sterile Butterfield's buffer (International BioProducts) and a BioPro sponge/glove sampling system consisting of the top compartment of a 540-ml resealable sample bag which contained a dry, sterile, biocide-free 4 X 8-cm sponge, and the bottom compartment which held a pair of sterile polyethylene disposable gloves (International BioProducts). Chute samples were collected from the front and back area of both sides of the chute, including the head restraint area. The sponge was pre-moistened with 25 ml of sterile Butterfield's buffer (International BioProducts) immediately prior to sampling and hand-massaged from the outside of the bag to ensure it was fully hydrated. Excess liquid was squeezed out and the sponge was removed using the sterile gloves. An approximate area of 100 cm^2 was swabbed for each chute using a 10 X 10-cm disposable plastic USDA Template 100™. In some cases, it was impossible to use the template so an approximate 100-cm^2 area was estimated. The chute area was sampled with the hydrated sponge 10 times in a vertical direction then 10 times in a horizontal direction, inverting the sponge before changing direction. The sponges were then placed into sterile sample bags, excess air was released, and the bag was resealed using wire tabs. Sample bags were immediately refrigerated in an insulated container.

Conductivity determination. The conductivity of the water samples was measured immediately before the samples were collected with an OAKTON® TDSTestr™ 4 (VWR) by immersing the electrode (OAKTON® Model 316, VWR) into the water of the runoff pond. The TDSTestr™4 was calibrated according to

manufacturer's specifications using a standard between 3 mS and 19.90 mS before the conductivity meter was used.

Hide samples. One-hundred hide samples were obtained from hides of cattle at a slaughter plant receiving cattle from the 5 feedlots. Samples were collected immediately after stunning by swabbing 450 cm^2 of surface area near the ventral brisket using sterile 4 X 8-cm^2 sponge pads (International BioProducts) dampened with 25 ml of sterile Butterfield's buffer as described for chute samples (Fig. 1).

Storage and transportation. Once all the samples were collected, the ice refrigerant packs and samples were arranged in the ice chest as described in the SOP. Samples were transported from the feedlots to Texas A&M University by overnight shipping in an insulated container with sixteen 360-ml and two 1500-ml U-Tex refrigerant packs to keep the samples refrigerated (0-5°C) during transportation. Based on the results obtained from the preliminary study, the following was determined to be the best arrangement to consistently and reliably drop the temperature to 0-5°C after 10 h and maintain the temperature for 48 h (Fig. 2A-2F): six 360-ml ice refrigerants were placed on the first layer (Fig. 2A). The second layer consisted of 2 plastic racks (Fig. 2B). The samples were immediately arranged in the third layer which was surrounded by 10 360-ml ice refrigerants (Fig. 2C). Two 1500-ml-refrigerants were placed on top of the samples (Fig. 2D). Two sheets of newspaper and 2 24 X 30-inch Biohazard bags (VWR) were used to cover the samples before closing the ice chest and sealing with packaging tape (Fig. 2E and 2F).

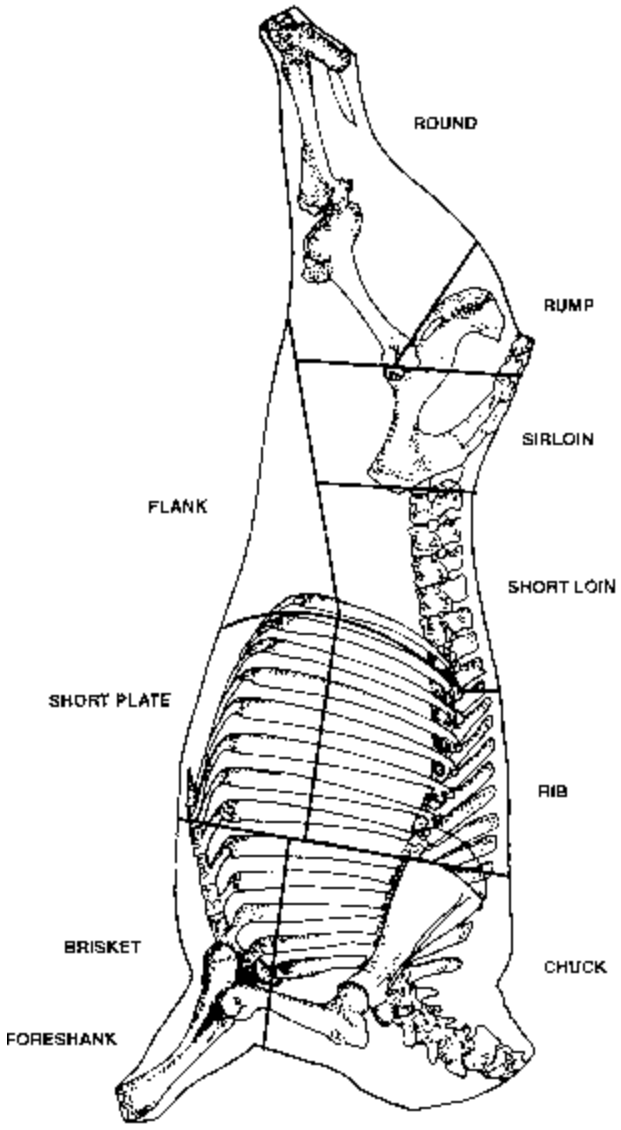

FIG. 1. The ventral brisket area of beef carcass for hide samples.

A: 6 360-ml ice refrigerants

B: 2 plastic racks

C: samples were surrounded by 10 360-ml ice refrigerants

D: 2 1500-ml-refrigerant

E: 2 sheets of newspaper and 2 plastic bags

F: cover of ice chest

FIG. 2. Standard arrangement of samples in ice chest for transport to the laboratory.

Laboratory methods

Bacterial cultures. *E. coli* (ATCC 25922) and *E. coli* O157:H7 (ATCC 43895) cultures were purchased from the American Type Culture Collection (Rockville, Md.). *E. coli* O157:H7-positive fluorescent strain 465-97 (EC- 46597) was provided by Dr. Frankie J. Beacorn, at the Microbial Outbreaks and Special Projects Branch, FSIS, Athens, Georgia, USA. ATCC 25922 served as a negative control for LT toxin production while ATCC 43895 was used in this study as positive controls for Shiga-like toxin I and toxin II production. A second positive control culture, EC- 46597, FSIS stock cultures, was maintained as a freeze-dried culture in single vials. The culture pellets were rehydrated using 0.5 to 1.0 ml of Trypticase Soy Broth (TSB, Difco Laboratories, Detroit, Mich.). The culture suspensions were aseptically transferred and streaked to Trypticase Soy Agar slants or plates (TSA, Difco). The TSA slants or plates (Difco) were incubated at 37°C for 24 h.

Stock culture maintenance. Stock cultures were transferred monthly and maintained on TSA slants at room temperature. Prior to experiments, the cultures were transferred once on TSA and twice in TSB, incubated at 37°C for 24 h.

Waste water samples. For each sample, 25 ml of water was transferred into 25 ml of double strength GN pre-enrichment broth (Difco) containing vancomycin (16 mg/l; Sigma Chemical Co., St. Louis, Mo.), cefixime (1.0 mg/l; Dynal, Lake Success, N.Y.), and cefsuludin (20 mg/l; Sigma) (Fig. 3). The sample broth mixture was gradually hand-mixed for 1 min before being incubated for 6-18 h at 37°C. A 1-ml

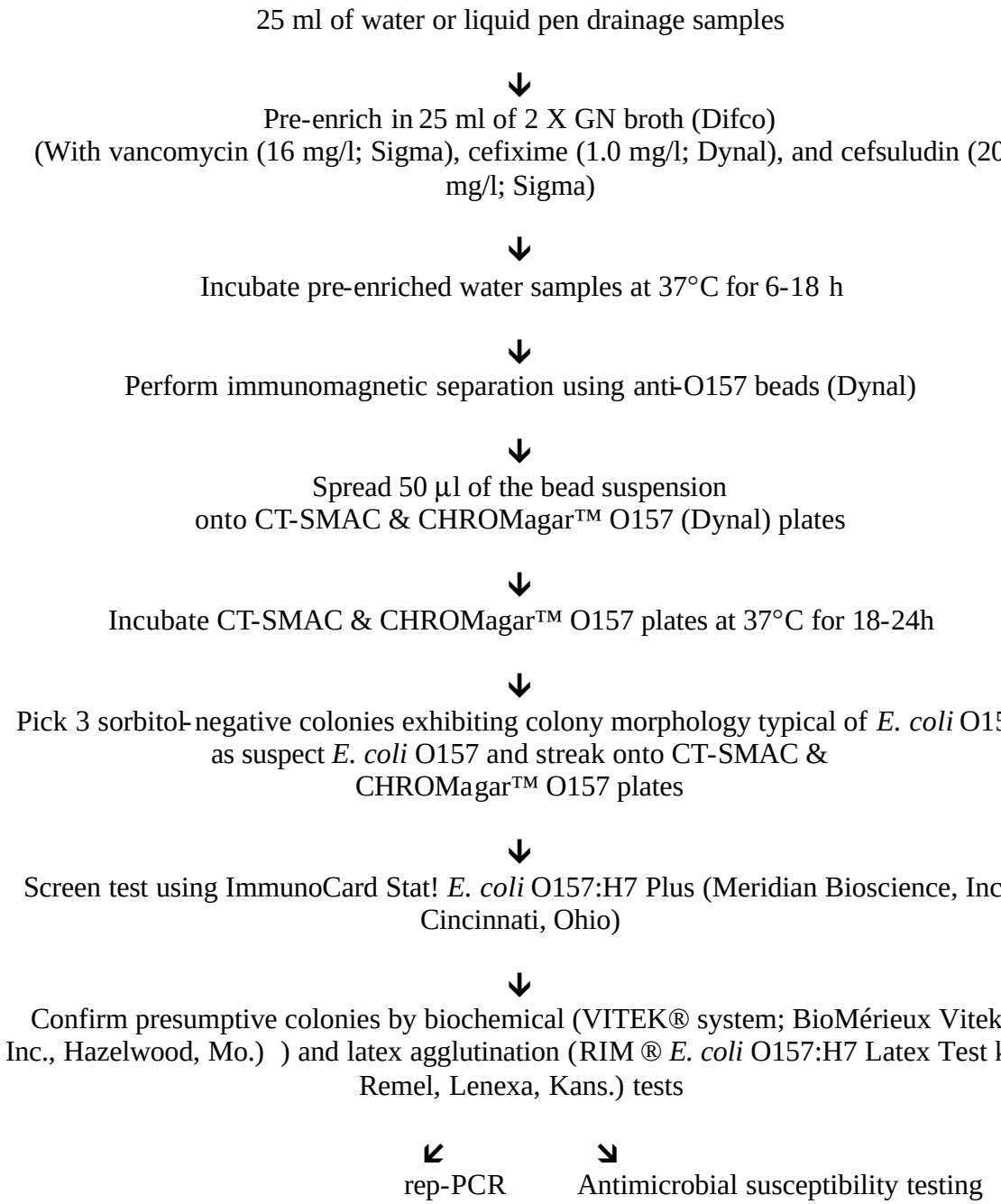

FIG. 3. Schematic representation of the isolation protocol for *E. coli* O157:H7 from water and liquid pen drainage samples.

aliquot of the pre-enriched sample was transferred to a 1.5-ml polypropylene microcentrifuge tube (International BioProducts), and 20 µl of Dynabeads anti-*E. coli* O157 (Dynal) were added. Immunomagnetic separation of the enriched bacteria on the Dynabeads was accomplished by incubating the immunomagetic bead suspension at 24°C for 10 min with gentle, continuous agitation (60 cycles/min) at 25°C using a Dynal Biotech® sample mixer (Model 10111, Dynal) to prevent the beads from settling. The beads were then washed 3 times with 1 ml of wash buffer (PBS-Tween containing 0.05% Tween 20, Sigma) on a magnetic separation rack (Dynal MPC-S; Dynal). Each wash was accomplished by: A) inverting the Dynal MPC-S rack for 1 min to concentrate the beads into a pellet on the magnetized side of the microcentrifuge tube, B) allowing 3 min for proper magnetic recovery of the beads from solution, and then C) carefully aspirating and discarding the sample supernatant in the tube. After the final wash, the Dynabead-bacteria complex was suspended in 100 µl of PBS-0.05% Tween 20 using a vortex mixer (Votex-Genie™; Scientific Industries Inc., Bohemia, N.Y.). Fifty µl of the resuspended beads were plated and streaked for isolation onto each of 2 petri plates containing MacConkey Sorbitol (SMAC, Difco) media supplemented with CT-supplement (Dynal) containing cefixime 0.5 mg/l and potassium tellurite 2.5 mg/l, and CHROMagar™ O157 (Dynal), and incubated at 35-37°C for 18-24 h. At least 3 sorbitol-negative colonies (colorless) on CT-SMAC media and ß-D-glucuronidase-negative colonies (pink-mauve color) on CHROMagar™ O157 exhibiting colony morphology typical of *E. coli* O157 were picked as suspect *E. coli* O157:H7 after 18-24

h incubation at 35-37°C. If there were no individual isolated colonies, growth was restreaked onto CT-SMAC media and CHROMagar™ O157. Suspect colonies were screened initially for detection of antigens from Shiga toxin-producing *E. coli* O157 using ImmunoCard Stat! *E. coli* O157:H7 Plus. Presumptive positive isolates were isolated by streaking on fresh CT-SMAC, and CHROMagar™ O157 plates, followed by incubation at 35-37°C for 18-24 h. A single presumptive colony was picked from each agar for further confirmation as described below.

Pond sludge samples. A 10-g sample of pond sludge was removed from each transport cup, homogenized and transferred into a Nasco 710-ml Whirl-Pak sampling bag using a 15.25 X 2.0-cm wooden tongue depressor. Ninety ml of GN broth containing vancomycin (8 mg/l), cefixime (0.5 mg/l), and cefsuludin (10 mg/l) was added to the sample. The samples were incubated, plated and processed as previously described for the water samples.

Chute and hide samples. Twenty ml of sterile 1.5X Brilliant Green Bile 2% (60 g/l, Difco) was added to bags containing the sponge samples followed by kneading for 1 min similar to procedures described by Elder et al. (36). The pre-enrichment samples were incubated, plated and processed as previously described for the water samples.

Pen drainage samples. Semi-dry samples were collected from cattle holding pens and were incubated, plated and processed as previously described for pond sludge samples. Liquid samples from the pens were incubated, plated and processed as previously described for the water samples.

Immunoassay test. ImmunoCard Stat! *E. coli* O157:H7 Plus is a rapid immunoassay used as a screening test to detect antigens of a Shiga toxin-producing *E. coli* O157. Testing was conducted using the procedure described by manufacture's instruction. In brief, suspect colonies on agar plates were diluted using 700-µl sample diluent (solution containing 0.094% sodium azide as a preservative) to a 5-ml sterile disposable culture tube (VWR). Suspect colonies were collected from both CT-SMAC, and CHROMagar™ O157 plates with a sterile cotton swab applicator (International BioProducts) sufficient to make a heavy suspension in the sample diluent. Each suspension was adjusted to match a 2-4 McFarland turbidity standard using a colorimeter (Model DR 100; HACH Company, Loveland, Co.). The suspension was mixed gently using a vortex mixer. Then 150 µl of the diluted specimen was added to the sample port of ImmunoCard Stat! *E. coli* O157:H7 Plus card using the transfer pipette provided, and incubated for 10 min at room temperature (21-27°C). During the incubation period, the sample was immobilized by gold particles coated with monoclonal antibody specific for the *E. coli* O157 lipopolysaccharide, and migrated along the membrane through the test and control zones. The test zone contained immobilized monoclonal antibodies specific for an epitope common to Shiga toxin-producing *E. coli* O157. After 10 min**,** the test and control zones were visualized for the presence or absence of red/purple lines across the membrane surface. A red/purple line in the test zone indicated a positive result. If a Shiga toxin-producing *E. coli* O157 was present in the sample, a complex was formed between the capture antibody, the Shiga toxin-producing *E. coli* O157 and the monoclonal antibody-gold conjugate. No

red/purple line appeared in the test zone in the case of a negative result. The control line, as a procedural control, ensured appropriate sample migration distance along the membrane.

Stock culture preservation. *E. coli* O157:H7 and O157:NM isolates were preserved as stock cultures using the following methods. Isolates were routinely transferred at 4-week intervals onto 10-ml TSA slants, incubated at 37°C overnight (24 h) and stored at room temperature (21-27°C). Cryogenic beads (Protect™Bacterial Preservers; Key Scientific Products, Round Rock, Tex.) were used as an alternative preservation method to maintain the characteristics of the isolates for long periods of time by avoiding freeze-drying or routine culture transferring. The storage system consists of 20-25 chemically treated porous ceramic beads suspended in a cryopreservative fluid containing TSB, glycerol and a hypertonic additive within a 1.5-ml sterile freezer vial. The isolate was inoculated onto Protect™ beads according to the manufacturer's specifications. Prior to stock culture preservation, each isolate was streaked for isolation onto TSA supplemented with 5% defibrinated sheep blood (Cleveland Scientific, Bath, Ohio) and incubated at 35°C for 18-24 h. Well-isolated colonies were selected from the blood agar plate using a sterile loop and transferred to a 1.5-ml sterile freezer vial until the mixture broth reached a minimum turbidity equivalent to a 3-4 McFarland standard using a colorimeter. The culture mixture broth was mixed using a sterile pipette. Then, the vial was inverted 6 times and was allowed to stand for 30 sec to allow the organisms to bind with the beads. The liquid culture was then discarded using a sterile pipette, leaving the beads as dry as possible.

All isolates were frozen at -70°C for long-term storage. For recovery, a frozen bead was removed from the vial using a sterilized bent hook (Key Scientific Products) and a sterile loop was used to streak onto TSA supplemented with 5% defibrinated sheep blood, which was then incubated at 35°C for 18-24 h.

Characterization of isolates

Suspect *E. coli* O157:H7 isolates were subjected to biochemical and serological characterization. The isolates were transferred to TSA plates supplemented with 5% defibrinated sheep blood and incubated at 35-37°C for 18-24 h. Prior to diagnostic testing, isolates were checked for gram reaction and morphology. Isolates proving to be gram-negative rods were subjected to further confirmation.

Biochemical confirmation. Biochemical characterization was determined using a Vitek Gram-Negative Identification+ card for *in vitro* diagnostic use (GNI+ card; BioMérieux Vitek, Inc.) in conjunction with a VITEK® system for automated identification of *E. coli* O157:H7 and O157:NM according to the manufacturer's specifications. The GNI+ card was composed of 30 wells of 28 biochemical broths, 1 negative control broth and 1 growth control broth. The identification scheme employed was based on biochemical methods established by Edwards and Ewing (35), Gilardic (44, 45), and Oberhofer et al. (85). The test procedure required 4-12 h in the Vitek Reader/Incubator. Then using a Vitek programmed computer, each well was determined to be positive or negative by an optical scanner which measured light attenuation. Bacterial identification patterns were automatically analyzed and printed for each card in the Reader/Incubator at the completed incubation cycle. Prior to biochemical

identification, isolates were streaked on TSA supplemented with 5% defibrinated sheep blood and incubated overnight at 37°C. In addition, a gram stain and cytochrome oxidase test was performed on the isolate. The gram-negative rod and oxidase-negative organisms were further examined through biochemical identification. Using a cotton swab applicator, 4 to 5 well-isolated colonies of a morphologically identical type were selected from the blood plate to prepare the inoculum. The selected colonies were suspended into 2.0 ml of 0.45% sterile saline solution (Baxter Healthcare, Deerfield, Ill.) in a 12 X 75-mm clear sterile disposable test tube. To standardize the inoculum density, the suspension turbidity was visually measured in the blue zone (67-77%) of the colorimeter to the equivalent of a McFarland No. 1 standard. A transfer tube was aseptically inserted into the GNI+ Card Port at the bend section and rotated 180 degrees so that the end of the tube was pointing away from the notches on the card. The mated card/transfer tube unit was placed onto the Vitek filling stand in conjunction with the long part of the transfer tube, and was subsequently inserted into the test tube. The Vitek filling stands were then arranged onto the filling rack and placed into the filling module. The cards were filled with culture suspension in the filling module which pulled a vacuum to remove air in the cards and then released the vacuum to replace the void with the inoculum. The filled cards were sealed at the card port. The cards were placed into the reader/incubator tray where biochemical tests were automatically evaluated over a 2-12 h incubation cycle.

Serological confirmation. Serological characterizations of isolates were performed using an *E. coli* O157:H7 latex test agglutination kit according to the

manufacturer's directions. The latex test was used to determine whether colorless colonies on CT-SMAC agar plates belong to O157 and/or H7 serogroups, and were therefore a potential verocytotoxin-producing strain. This presumptive identification of *E. coli* O157:H7 was based on latex reagents coated with specific antibodies either for anti-O157 somatic or anti-H7 flagellar antigens. Each latex reagent was coated with a different antibody: a) an antibody against *E. coli* serotype O157, b) an antibody against *E. coli* serotype H7, and c) normal rabbit globulin as a control latex. In brief, a minimum of 5 well-isolated colonies of the same morphological type were selected from a 5% sheep blood agar surface after overnight incubation. When mixed with latex particles, fresh colonies of O157 and/or H7 grown on blood agar more readily illustrated antigen presence, because blood agar enhances flagella production. Prior to the study, the latex reagents were allowed to cool to room temperature, and then each reagent was suspended using a vortex mixer. First, a drop of *E. coli* O157 and *E. coli* control test latex was dispensed on a separate well of the latex test slide. Then the suspect non-sorbitol fermenting colonies (NSFC) were selected from CT-SMAC using the provided plastic stick. Colonies belonging to O157 and/or H7 serogroups appeared colorless, but others showed pink on CT-SMAC agar plates. *E. coli* O157 and control latex suspensions were mixed thoroughly on the slide, and the plastic sticks were changed between suspensions. The latex cards were rotated carefully using complete circular motions for 1 min, or until agglutination was observed. Isolates fitting the biochemical profile of *E. coli* and serologically positive for somatic (O) 157, flagellar (H) 7, or both antigens were classified as confirmed *E. coli* O157.

Antimicrobial susceptibility testing

Antimicrobial resistance profiles of *E. coli* O157:H7 and O157:NM isolates were determined via the agar disk diffusion technique in accordance with the National Committee for Clinical Laboratory Standards (NCCLS) (81, 82, 83) and previously published literature focusing on antibiotic susceptibility of *E. coli* O157 isolates from animals (21, 78, 100). The Sensi-Disc™ Susceptibility Test System (BBL, Becton Dickinson Microbiology Systems, Cockeyville, Md.), including Bacto Müeller-Hinton agar plates (Difco) and Sensi-Disc™ Antimicrobial Susceptibility Test Discs (BBL), 12 discs Sensi-Disc™ Designer Dispenser (BBL), and the recommended quality control organisms were used to measure the susceptibility or resistance of the isolates to antimicrobial agents and interpreted according to the NCCLS (81, 82, 83). *E. coli* ATCC 25922 and ATCC 35218, *Enterococcus faecalis* ATCC 29212, *Staphyloccoccus aureus* ATCC 29213, and *Pseudomonas aeruginosa* ATCC 27853 were used as control microorganisms in antimicrobial minimal inhibitory concentration (MIC) determinations of *E. coli* O157 isolates. The following antimicrobial agents were included in determination of antimicrobial resistance: Amikacin, Amoxicillin-clavulanic acid, Ampicillin, Cefazolin, Cefotetan, Cefoxitin, Ceftazidime, Ceftiofur, Ceftriaxone, Cefuroxime, Cephalothin, Chloramphenicol, Ciprofloxacin, Gentamicin, Imipenem, Kanamycin, Nalidixic acid, Streptomycin, Sulfisoxazole, Tetracycline, Ticarcillin, Tobramycin, and Trimethoprim-sulfamethoxazole (BBL). Susceptibility tests followed NCCLS breakpoints (81, 82, 83). The twenty-three antibiotics were tested and their resistance criteria, as recommended by the NCCLS, are shown in Table 2.

TABLE 2. Antibiotics used and their resistance criteria based on inhibition zone diameters

Antibiotic	**Abbreviation**	**Disk concentration (㎎g)**	**Criteria[a] (mm)**		
			R	**I**	**S**
Amikacin	An	30	=12	13-14	≥15
Amoxicillin/ clavulanic acid	AMC	20/10	=13	14-17	≥18
Ampicillin	AM	10	=13	14-16	≥17
Cefazolin	CZ	30	=14	15-17	≥18
Cefotetan	CTT	30	=12	13-15	≥16
Cefoxitin	FOX	30	=14	15-17	≥18
Ceftazidime	CAZ	30	=14	15-17	≥18
Ceftiofur	XNL	30	=17	18-20	≥21
Ceftriaxone	CRO	30	=13	14-20	≥21
Cefuroxime	CXM	30	=14	15-22	≥23
Cephalothin	CF	30	=14	15-22	≥23
Chloramphenicol	C	30	=14	15-17	≥18
Ciprofloxacin	CIP	5	=15	16-20	≥21
Gentamicin	GM	10	=12	13-14	≥15
Imipenem	IPM	10	=13	14-15	≥16
Kanamycin	K	30	=13	14-17	≥18
Nalidixic acid	NA	30	=13	14-18	≥19
Streptomycin	S	10	=11	12-14	≥15
Sulfisoxazole	G	250	=12	13-16	≥17
Tetracycline	TE	30	=14	15-18	≥19
Ticarcillin	TIC	75	=14	15-19	≥20
Tobramycin	NN	10	=12	13-14	≥15
Trimethoprim-Sulfamethoxazole	SXT	1.25/23.75	=10	11-15	≥16

R= Resistant; **I=** Intermediate; **S=** Susceptibility

[a] MIC Breakpoints less than the indicated value indicate the bacterial strain was resistant according to NCCLS guidelines.

Isolates were streaked for isolation onto TSA agar plates supplement with 5% defibrinated sheep blood (Cleveland Scientific) and incubated overnight (18-24 h) at 35°C. A minimum of 3-5 well-isolated colonies on the agar plate culture were selected and transferred into 5 ml TSB. The turbidity was adjusted until it reached a minimum turbidity equivalent to a 0.5 McFarland standard using a 0.45% sterile saline solution and a colorimeter. The 150-mm Müeller-Hinton agar plates were streaked on the entire surface using a sterile cotton swab applicator emerged with the culture suspension. The plates were swabbed, rotating the plate approximately 60° twice to ensure an even distribution of inoculum, and finally the rim of the plates was swabbed. The agar plates were allowed to absorb excess inoculum on the surface for maximum 15 min before applying the discs. Twenty-three antibiotic discs were placed firmly onto the surface of inoculated agar plates using a 12 disc Sensi-Disc™ Designer Dispenser ensuring the discs were in contact with the agar surface. The discs were dispensed evenly and not distributed closer than 24 mm between each center of antibiotic discs in order to prevent any complications of multiple antibiotic diffusions. The agar plates were inverted and incubated at 37°C for 16-18 h. After incubation, the diameter of the inhibition zone of the inoculated agar plates was measured (including the 6-mm disc diameter) using a caliper (DialMax 150 mm, Scienceware, Bel-art-Products, Pequannock, N.J.). The diameters of the complete inhibition zones were examined by placing the caliper on the back of the inverted petri disks. The apparent zones of inhibition were measured as the margin area which displayed no obvious or visible growth. Results were reported as

resistant (R), intermediate (I), and susceptible (S) on the basis of NCCLS guidelines (81, 82, 83).

rep-PCR

Genetic profiling of isolates for *E. coli* O157 markers were completed by repetitive sequence-based polymerase chain reaction (rep-PCR). A single pair of rep-PCR primers, Uprime-B1 or BOX A1R (5'-CTACGGCAAGGCGAC GCTGAC G-3') and Uprime-RI, which compose of primers REP 1R (5'-III ICGICGICATCI GGC-3') and REP 2I (5'-ICG ICTTATCIGGCCTAC-3'), was used to generate potential toxigenicity information and simultaneously identify the toxin type of each *E. coli* O157 isolate. The result of this testing was a complex rep-PCR DNA banding pattern (fingerprint). Further analysis was performed using computer-based analyses of digitized images of fingerprints. The computer-based analyses generated dendrograms that made it possible to visually discriminate *E. coli* O157 isolates to a specific strain or substrain level for comparison, and determine the relatedness of isolates from various sources. rep-PCR was performed by Bacterial BarCodes, Inc. (Houston, Tex.). The rep-PCR procedure was accomplished by a) extracting genomic DNA from purified *E. coli* O157, b) preparing a master mix using reagents provided in the rep_{PRO} kit (Bacterial BarCodes, Inc.) with *Taq* DNA polymerase (Ampli*Taq*® DNA Polymerase, Applied Biosystems, Foster City, Calif.) and *E. coli* O157 DNA, c) followed by rep-PCR amplification in a thermal cycler 9700 with a 96-well heat block (Applied Biosystems), d) electrophoresis and staining of amplified DNA on an agarose gel to visualize the

DNA fingerprints, and e) capture of an image of the fingerprints for analysis of their relatedness and possible source.

Data analysis

All data, including antimicrobial resistance information, were initially entered into computer spreadsheets. The software was used to generate descriptive statistics including graphs and charts of *E. coli* O157:H7 and *E. coli* O157:NM isolates. Prevalence of *E. coli* O157:H7 and *E. coli* O157:NM were computed as the number of samples with *E. coli* O157:H7 and *E. coli* O157:NM divided by the number of samples. In addition, rep-PCR DNA fingerprints of *E. coli* O157:H7 and *E. coli* O157:NM were analyzed using Bionumerics software (Bacterial BarCodes). Pearson correlation was used for determine the significance of comparisons.

RESULTS AND DISCUSSION

Prevalence of *E. coli* O157 in environmental feedlots

A total of 1125 environmental samples were collected from feedlots in the Texas Panhandle from July 2001 to March 2002. These samples consisted of 229 chute samples, 399 waste water samples, 399 sludge samples, 55 drainage samples, and 23 retention pond water samples and 20 retention pond sludge samples.

The total prevalence of *E. coli* O157 in environmental samples each month ranged from 0%-10% (Table 3). Of the 1125 environmental samples collected from 5 commercial feedlots, overall 4% (47/1125) were found to be positive for *E. coli* O157 (Table 3). Cattle are reported as the major reservoir of *E. coli* O157 (25, 68), and in the current study, this pathogen was discovered frequently in the cattle feedlot environment. Although *E. coli* O157:H7 shedding in the feces from cattle may lead to contamination of the feedlot environment as the current study has shown, other non-bovine reservoirs (chutes, waste pond, and retention pond) of *E. coli* O157 may exist in same environment. A number of studies have suggested the possibility of reservoirs for *E. coli* O157 other than cattle (52, 55), and reported *E. coli* O157 in non-bovine species (67, 95). Furthermore, there was a possibility that the environmental sources identified in the current study may serve as *E. coli* O157 transmission routes within and/or between feedlot environments.

In the current survey, the maximum prevalence at any one visit (34%) was found in the environment of feedlot 3 in March. On 19 of 45 (42%) feedlot sampling

TABLE 3. Prevalence of *E. coli* O157 in each feedlot over nine-month sampling period

Feedlot	**Jul**[a]	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Total**
FL1[b]	1/29[c] (3%)	0/33 (0%)	0/23 (0%)	0/17 (0)%	2/30 (7%)	2/23 (9%)	0/29 (0%)	1/29 (3%)	0/29 (0%)	**6/242 (2%)**
FL2	0/31 (0%)	3/33 (9%)	1/25 (4%)	0/25 (0)%	5/29 (17%)	0/33 (0%)	0/31 (0%)	1/29 (3%)	2/30 (7%)	**12/266 (5%)**
FL3	0/33 (0%)	1/33 (3%)	2/34 (6%)	0/35 (0)%	0/34 (0%)	0/33 (0%)	0/29 (0%)	0/29 (0%)	10/29 (34%)	**13/289 (4%)**
FL4	4/25 (16%)	0/23 (0%)	0/23 (0%)	0/17 (0)%	2/23 (9%)	0/23 (0%)	0/17 (0%)	0/17 (0%)	0/17 (0%)	**6/185 (3%)**
FL5	2/12 (17%)	4/19 (21%)	0/18 (0%)	1/17 (6%)	2/13 (15%)	0/13 (0%)	0/17 (0%)	1/17 (6%)	0/17 (0%)	**10/143 (7%)**
Total	**7/130 (5%)**	**8/141 (6%)**	**3/123 (2%)**	**1/111 (1%)**	**11/129 (9%)**	**2/125 (2%)**	**0/123 (0%)**	**3/121 (2%)**	**12/122 (10%)**	**47/1125 (4%)**

[a] Jul: July; Aug: August; Sep: September; Oct: October; Nov: November; Dec: December; Jan: January; Feb: February; Mar: March.
[b] FL1-5; Feedlot 1-5.
[c] O157 Positive/Total Sampled.

occasions at least one environmental sample was found positive for *E. coli* O157 (Table 3). This result is lower than that previously reported by Hancock et al. (52) who found 61% of environmental samples taken from 100 feedlots were positive for *E. coli* O157. The difference in prevalence may be explained by the different sampling and isolation protocols employed in the current study and that of Hancock et al. (52), who collected fecal samples in order to estimate the feedlot prevalence of *E. coli* O157. Armstrong et al. (4) reported that estimation of the prevalence of *E. coli* O157 may vary widely due to the diagnostic method employed, the number of samples collected, and the type of samples collected.

Prevalence by month

Over the 9 month sampling period, January was the only month in which *E. coli* O157 was not detected from any of the environmental sources (n=123) (Table 3). Positive *E. coli* O157 environmental samples were most frequently detected in November and March (9 and 10%, respectively) (Table 3). The widest range of prevalence of *E. coli* O157 (0-34%) was displayed in feedlot 3 (Table 3), and the prevalence of *E. coli* O157 varied most between feedlots (0-34%) during the month of March (Table 3). *E. coli* O157 was most frequently detected in environmental samples during the spring (not including April and May) and summer (July-August), where 5.5 and 10% respectively of samples were positive. In this study, the prevalence of *E. coli* O157 between each feedlot over 9 months did not differ remarkably between the five feedlots (2-7%) (Table 3). Seasonal factors may explain the different results obtained in

this study; however, samples were collected over a nine-month period only, and so results may not accurately reflect seasonal trends.

Peak prevalence of *E. coli* O157 was demonstrated in late summer and early fall in studies involving North American cattle which is the same period in which the Hancock et al. (52) study was conducted. The effect of time variables may influence the outcome of *E. coli* O157 prevalence in feedlot environment. Hancock et al. (52) predicted that the level of the contamination would drop dramatically at other times during the year based on the apparent correlation between positive *E. coli* O157 cattle feces and carcass contamination. A number of studies (23, 25, 52, 55) showed a seasonal peak of *E. coli* O157 presence in late spring and early summer. The addition of different types of cattle (lactating cows, non-lactating cows, calves, and heifers) in the feedlot for each month in the feedlots may be one of the reasons for differences in prevalence rates in this study. Mechie et al. (76) have reported all cattle groups had varied excretion rates during the survey with a similar seasonal pattern. For example, excretion rates of *E. coli* O157 were significantly lower in lactating cows than other groups, but lactating cows showed the highest *E. coli* O157 excretion during the first month after calving. This level fell during lactation and rose to its peak at 7 months postpartum.

Prevalence by feedlot

Of 45 sample sets tested, the range of overall prevalence of *E. coli* O157 in feedlot 1 to feedlot 5 was 6 (2%), 12 (5%), 13 (4%), 6 (3%), and 10 (7%) (Table 4).

TABLE 4. *E. coli* O157 isolation from environmental sources in five cattle feedlots in the Texas Panhandle

	Sources of sample						
Feedlot	Chutes	Water	Sludge	Drainage	RT[a]	Total positive	**Prevalence**
FL1[b]	0/45[c] (0%)	2/96 (2%)	4/96 (4%)	0/5 (0%)	NS[d]	6/242	**2%**
FL2	4/45 (9%)	0/102 (0)	7/102 (7%)	1/17 (6%)	NS	12/266	**5%**
FL3	3/45 (7%)	6/108 (5%)	4/108 (4%)	0/28 (0%)	NS	13/289	**4%**
FL4	1/45 (2%)	0/69 (0%)	5/69 (7%)	0/2 (0%)	NS	6/185	**3%**
FL5	6/49 (12%)	1/24 (4%)	2/24 (8%)	0/3 (0%)	1/43 (2%)	10/143	**7%**
Total	**14/229 (6%)**	**9/399 (2%)**	**22/399 (6%)**	**1/55 (2%)**	**1/43 (2%)**	**47/1125 (4%)**	**4%**

[a] RT: retention pond.
[b] FL1-5: Feedlot 1-5.
[c] O157 Positive/Total Sampled.
[d] NS: not sampled.

Similarly, Hancock et al. (52, 53, 54, 55) presented data that showed *E. coli* O157 existed in most cattle operations, but prevalence was highly variable among herds. In the work reported here, *E. coli* O157 was most often isolated from environmental samples from feedlot 5 (7%) (Table 4); however, there was little difference in the prevalence of *E. coli* O157 between feedlots. In the current study, feedlots were selected without prior knowledge of the O157 status of cattle previously tested in the same feedlots. Although, the prevalence of *E. coli* O157 in the feedlot environment did not differ extensively between feedlots, it did vary widely within feedlots (0-34%) (Table 4). In the current study, there was no available information on bovine characteristics or conditions of each feedlot which may influence *E. coli* O157 presence in the feedlot environment. The wide distribution of *E. coli* O157 after cattle arrive at the large feedlots possibly occurs during certain seasons. New incoming cattle to feedlots have been identified to be at a greater risk for shedding *E. coli* O157 than cattle on feed (30). Smith et al. (106) showed the prevalence of cattle shedding *E. coli* O157:H7 varied greatly between pens in each feedlot. *E. coli* O157:H7 should be considered common to cattle grouped together in pens, and the condition of the pen floor in the feedlots may affect the prevalence of cattle shedding the organism. In addition, the differences in *E. coli* O157 prevalence rate in any feedlot in any month (0-34%) may result from the differences in excretion rates of cattle in the feedlots. The causes of different excretion rates are not clearly known, but may result from differences in ruminal development, diet, or specific immunity to infection (76). Reid et al. (93) demonstrated that characteristics of the cattle and conditions of each pen in the feedlot may affect the

prevalence of *E. coli* O157 shedding in the feedlot environment. Park et al. (90) revealed fecal shedding of *E. coli* O157:H7 persisted longer in calves than in adult cattle and the type of feed consumed by cattle may influence the prevalence and acid resistance of this pathogen.

The results of the current study have shown that *E. coli* O157 were present in every environmental source in each feedlot (Table 4). The presence of *E. coli* O157 in different environmental sources ranged from 2-6%.

There may be varied exposure and transmission within and between feedlots. The means of sustaining and transmitting *E. coli* O157 in the feedlot environment is unknown; however, Hancock et al. (51) presented three possible models that account for prevalence of *E. coli* O157 in feedlots and dairy farms, including a) multiple reservoir species of *E. coli* O157, b) the ability of *E. coli* O157 to transiently colonize many species (but at least one species serve as the reservoir), and c) environmental reservoir, such as the sedimentary layer of water-troughs. Furthermore, Hancock et al. (51) suggested a possibility that a reservoir other than cattle may exist in feedlots and dairy farms. Besser et al. (10) have shown that *E. coli* O157 seems to colonize only transiently in cattle and long term carriers have not been found. The shedding of *E. coli* O157 in herds of cattle is intermittent to the level that the organism cannot be detected on the majority of sampling visits (52, 55). Unfortunately, there was lack of prior information of *E. coli* O157 prevalence on cattle and other animals in the feedlots surveyed in this study and, therefore, no similar conclusions can be drawn.

Prevalence by environmental source

The results of this study suggest that the chute and sludge from the run-off pond are the main sources of *E. coli* O157 in the feedlot environment sampled as these sources had a higher prevalence of *E. coli* O157 (6%) (Table 5). A number of studies have shown that environmental persistence of *E. coli* O157 may play a key role in the epidemiology of *E. coli* O157 on farms. Dargatz et al. (30) and Elder et al. (36) indicated the widespread distribution of *E. coli* O157:H7 in cattle operations. In addition, LeJeune et al. (71) reported that environmental survival of *E. coli* O157 may play an important role in the persistence and dissemination of *E. coli* O157 on the farms. *E. coli* O157 was found in every source of the feedlot environment. However, there have been no previous reports of the chute and pond sludge as *E. coli* O157 reservoirs. The high *E. coli* O157 prevalence in the chute area may be due to direct contact of the chute with cattle hides when monitoring or medicating the cattle. Interestingly, *E. coli* O157 was recovered more frequently from sludge samples from the waste water pond than from the waste water itself. *E. coli* O157 may attach to the organic compounds in the water and settle down to the bottom of the pond where sludge was collected. Water trough sediments contaminated with feces from cattle excreting *E. coli* O157 may serve as a long-term reservoir of this organism on the farms and a source of infection for other cattle. Smith et al. (106) suggested that *E. coli* O157 may be common in feedlot cattle populations. *E. coli* O157:H7 exposure was widespread and most cattle were exposed to the bacteria before weaning (68). Smith et al. (106) found higher percentages of cattle

TABLE 5. Prevalence of *E. coli* O157 in feedlot environment by month and source

Month	Environmental source of isolates					**Total**
	Sludge	Water	Chute	RT[a]	Drainage	
July	3/45[b] (7%)	1/45 (2%)	3/27 (11%)	0/3 (0%)	0/10 (0%)	**7/130 (5%)**
August	2/48 (4%)	0/48 (0%)	5/26 (19%)	1/6 (17%)	0/13 (0%)	**8/141 (6%)**
September	2/42 (5%)	0/42 (0%)	0/26 (0%)	0/6 (0%)	1/7 (14%)	**3/123 (2%)**
October	0/36 (0%)	0/36 (0%)	1/25 (4%)	0/6 (0%)	0/8 (0%)	**1/111 (1%)**
November	8/48 (2%)	0/48 (0%)	3/25 (12%)	0/2 (0%)	0/6 (0%)	**11/129 (9%)**
December	1/45 (2%)	1/45 (2%)	0/25 (0%)	0/2 (0%)	0/8 (0%)	**2/125 (2%)**
January	0/45 (0%)	0/45 (0%)	0/25 (0%)	0/6 (0%)	0/2 (0%)	**0/123 (0%)**
February	2/45 (4%)	1/45 (2%)	0/25 (0%)	0/6 (0%)	NS[c]	**3/121 (2%)**
March	4/45 (9%)	6/45 (13%)	2/25 (8%)	0/6 (0%)	0/1 (0%)	**12/122 (10%)**
Total	**22/399 (6%)**	**9/399 (2%)**	**14/229 (6%)**	**1/43 (2%)**	**1/55 (2%)**	**47/1125 (4%)**

[a] RT: retention pond.
[b] FL1-5: Feedlot 1-5.
[c] NS: not sampled.

shedding *E. coli* O157:H7 from muddy pen conditions than cattle from pens in normal (dry) condition.

Relationship between environmental factors and *E. coli* O157 prevalence

Figures 4-13 illustrate ambient temperature (Fig. 4), dew point (Fig. 5), electrical conductivity (Fig. 6), liquid levels in the pond (Fig. 7), sludge temperature (Fig. 8), relative humidity (Fig. 9), station pressure (Fig. 10), temperature dry bulb (Fig. 11), temperature wet bulb (Fig. 12), and water temperature (Fig. 13), compared to *E. coli* O157 prevalence in each feedlot environment. These factors did not apparently correlate with prevalence of *E. coli* O157 in environmental samples. Similarly, Smith et al. (106) found no correlation between cattle held in pens shedding *E. coli* O157 and the temperature, pH, or cleanliness of water from the water tanks, pH of the feed, number of cattle held in the pen, mean body weight, or number of days in the feedlot. An association between the environmental condition of feedlots and prevalence of *E. coli* O157 seems biologically possible; however, the 0% prevalence found in January makes an obvious correlation more difficult. In January, the water, sludge, and ambient temperatures were consistently low, 0-8C°, 1-3C°, -1-8C°, respectively, compared with other months, where temperatures fluctuated (Fig. 5, 9, and 13). The minimum temperature for the growth of *E. coli* O157 is 8ºC, with an optimum at 37ºC and maximum of 44-45ºC (6). Freezing environmental conditions may affect the degree of injury and death, and influence the survival of *E. coli* O157. Previous exposure to stress conditions at feedlots may affect the absence of *E. coli* O157 in January. The apparent high prevalence of *E. coli* O157 in the feedlot environment in other months may result

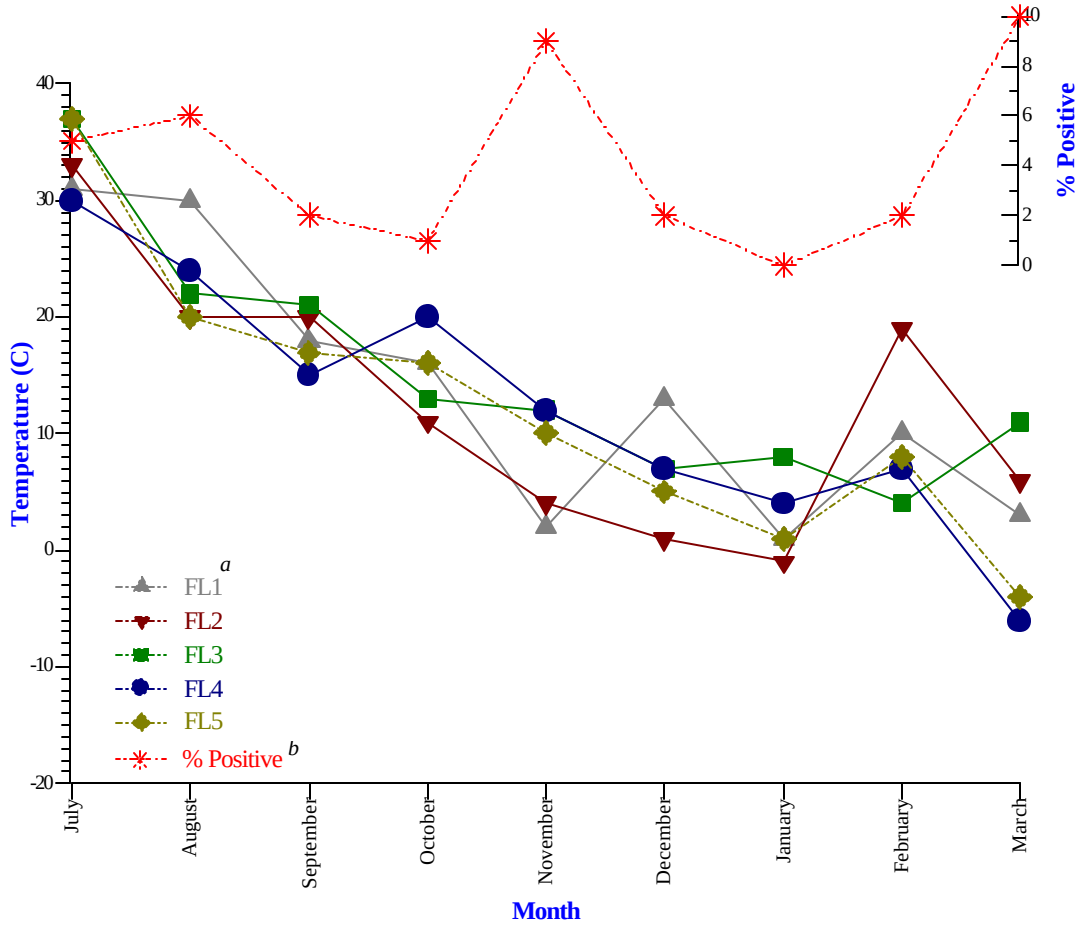

FIG. 4. Relationship between ambient temperature of the waste pond from July-March and prevalence of *E. coli* O157 in environmental samples.

[a] FL1-5: Feedlot 1-5.
[b] % total prevalence of *E. coli* O157.

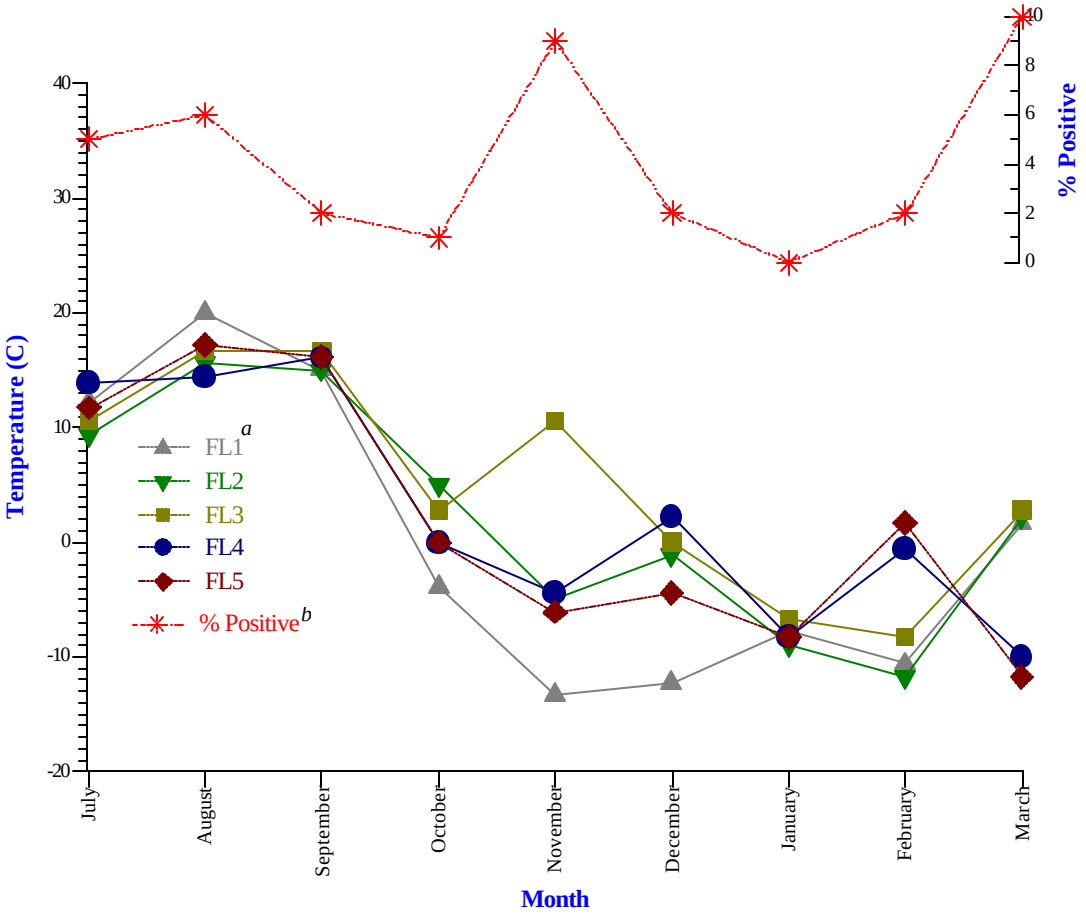

FIG. 5. Relationship between dew point from July-March and prevalence of *E. coli* O157 in environmental samples.

[a] FL1-5: Feedlot 1-5.
[b] % total prevalence of *E. coli* O157.

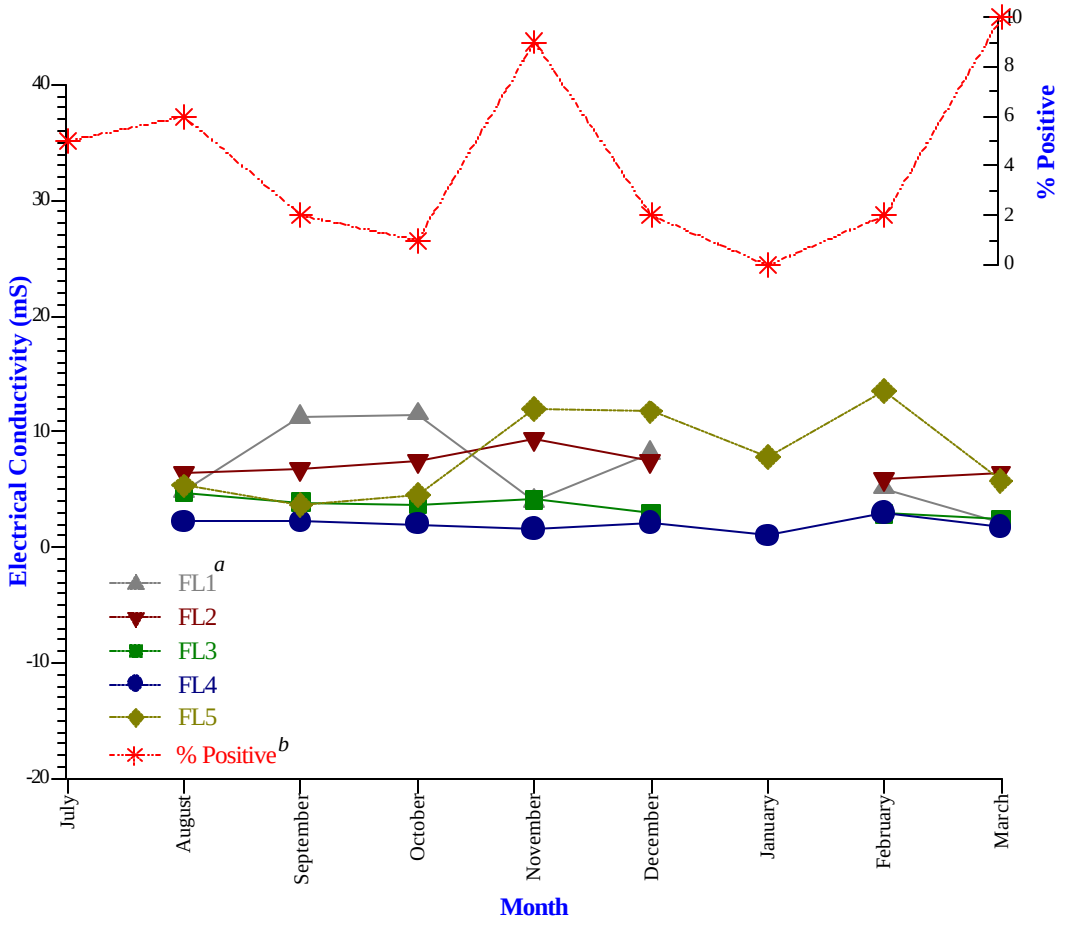

FIG. 6. Relationship between electrical conductivity of the waste pond from July-March and prevalence of *E. coli* O157 in environmental samples.

[a] FL1-5: Feedlot 1-5.
[b] % total prevalence of *E. coli* O157.
*Absence of point: no collected data.

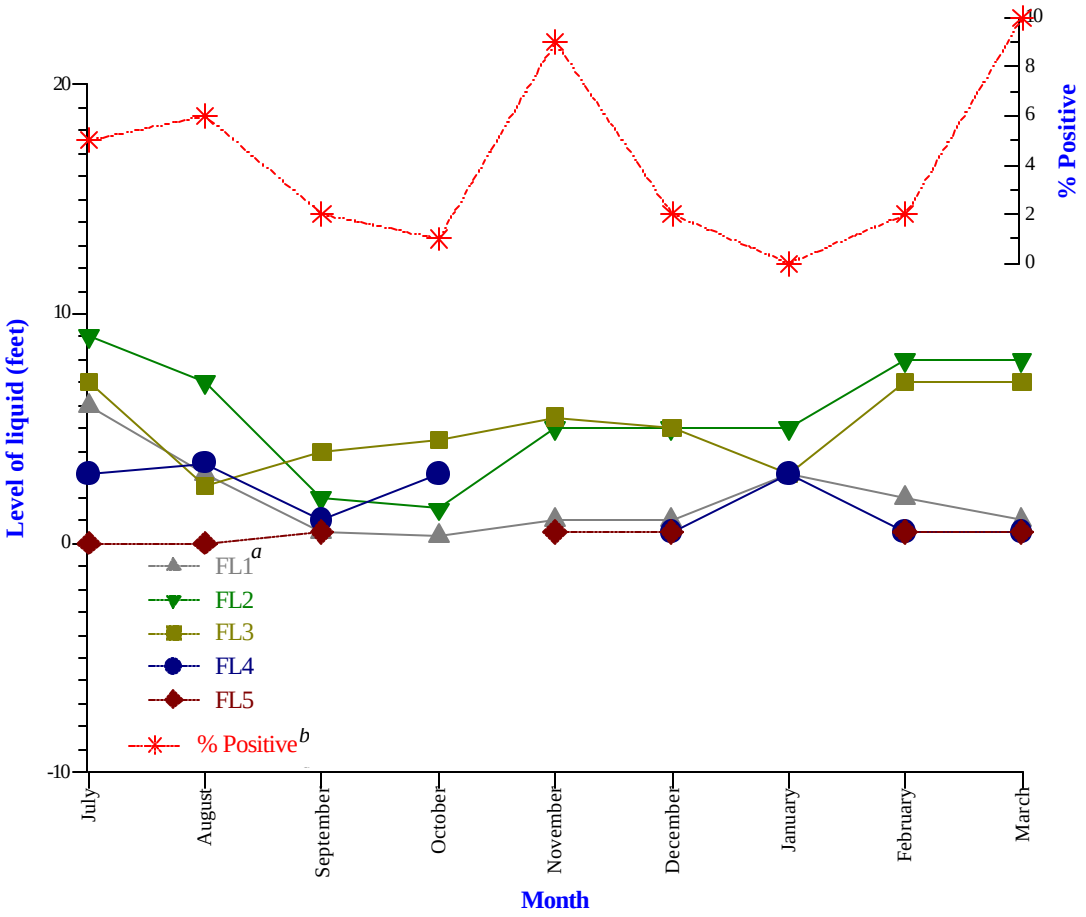

FIG. 7. Relationship between liquid levels in the waste pond from July-March and prevalence of *E. coli* O157 in environmental samples.

[a] FL1-5: Feedlot 1-5.
[b] % total prevalence of *E. coli* O157.
*Absence of point: no collected data.

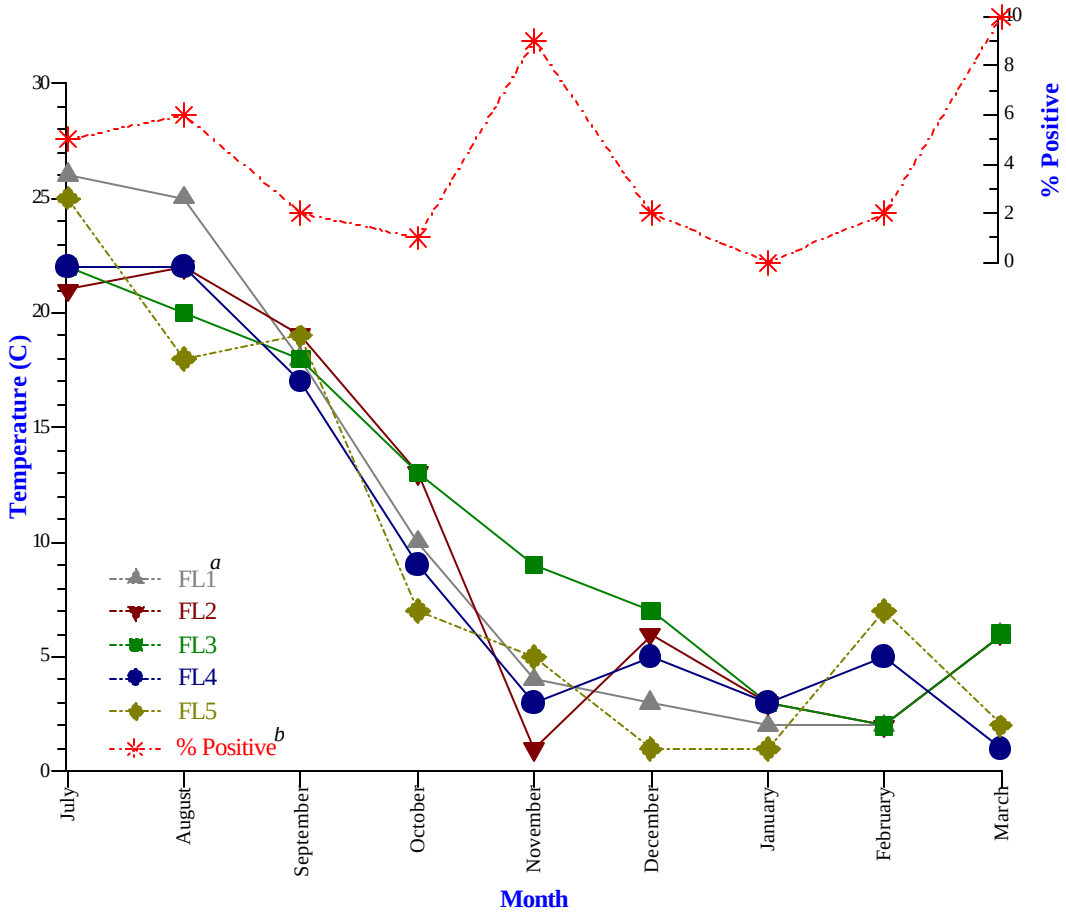

FIG. 8. Relationship between sludge temperature of the waste pond from July-March and prevalence of *E. coli* O157 in environmental samples.

[a] FL1-5: Feedlot 1-5.
[b] % total prevalence of *E. coli* O157.
* Absence of point: no collected data.

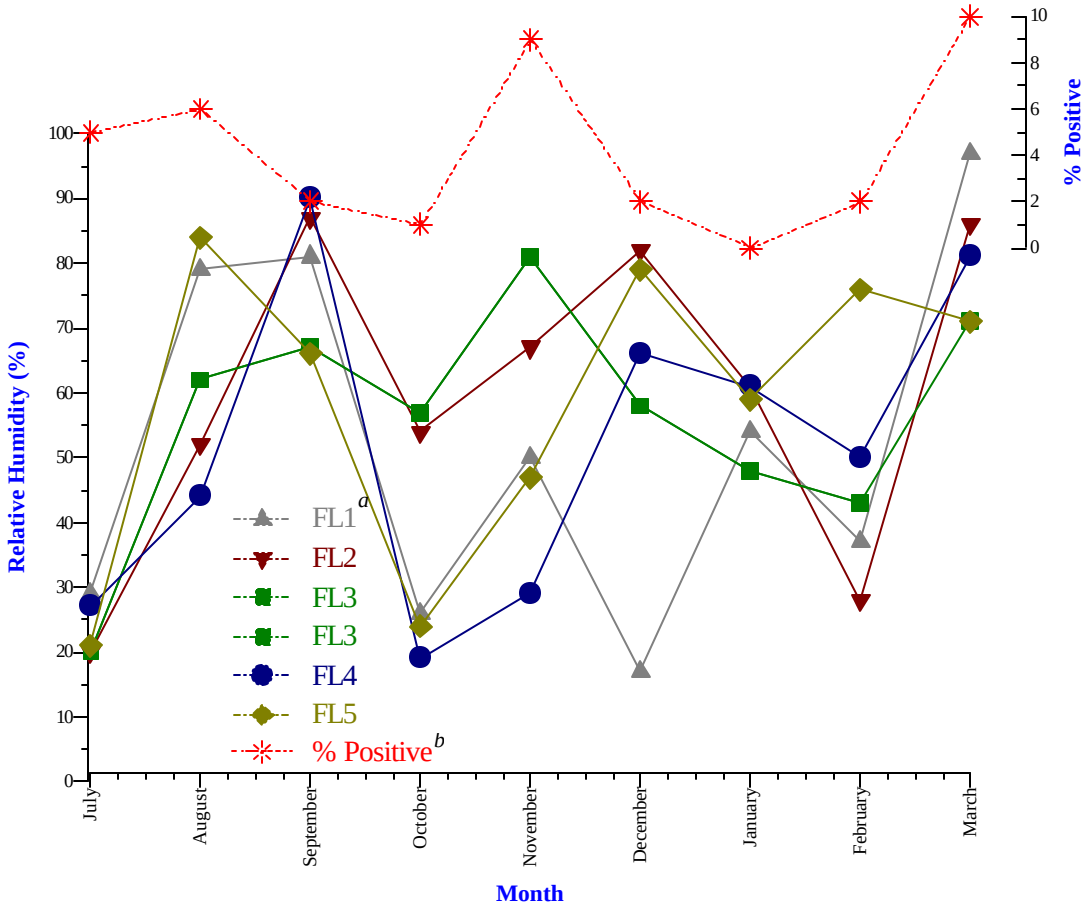

FIG. 9. Relationship between relative humidity of the waste pond from July-March and prevalence of *E. coli* O157 in environmental samples.

[a] FL1-5: Feedlot 1-5.
[b] % total prevalence of *E. coli* O157.

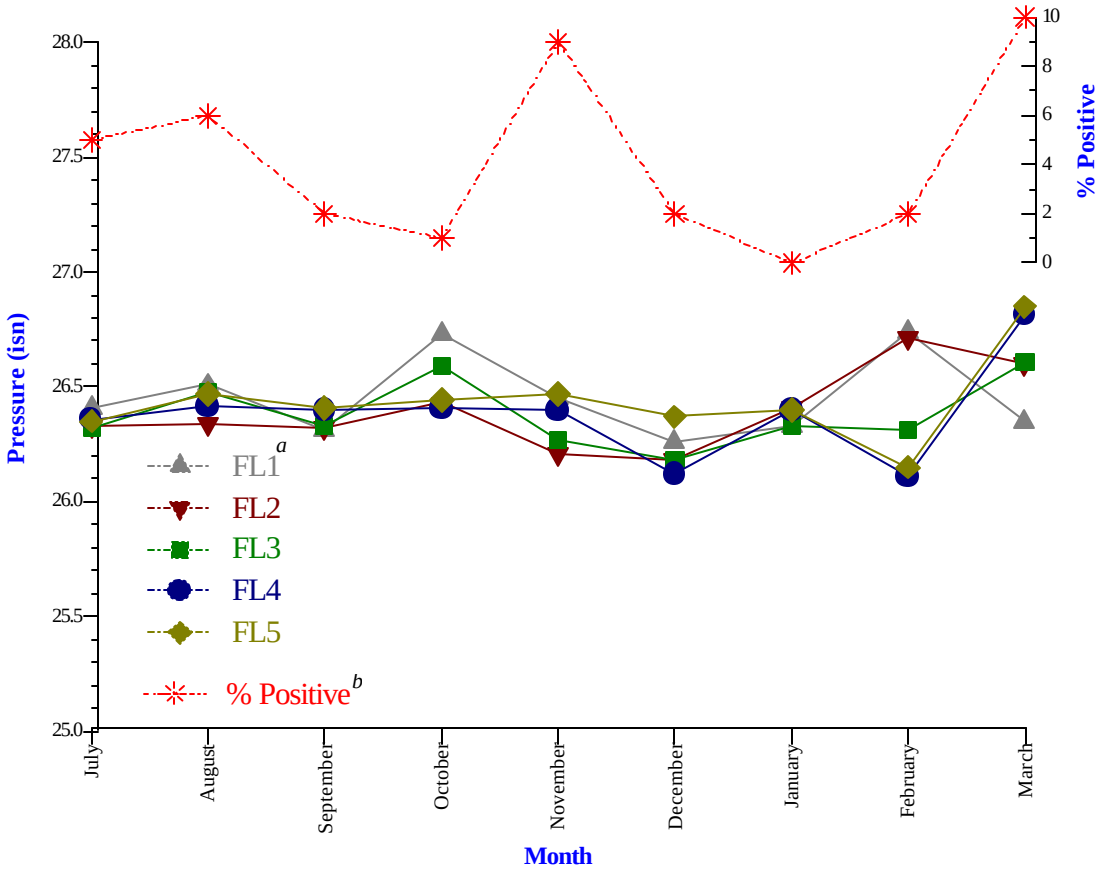

FIG. 10. Relationship between station pressures of the waste pond from July-March and prevalence of *E. coli* O157 in environmental samples.

[a] FL1-5: Feedlot 1-5.
[b] % total prevalence of *E. coli* O157.

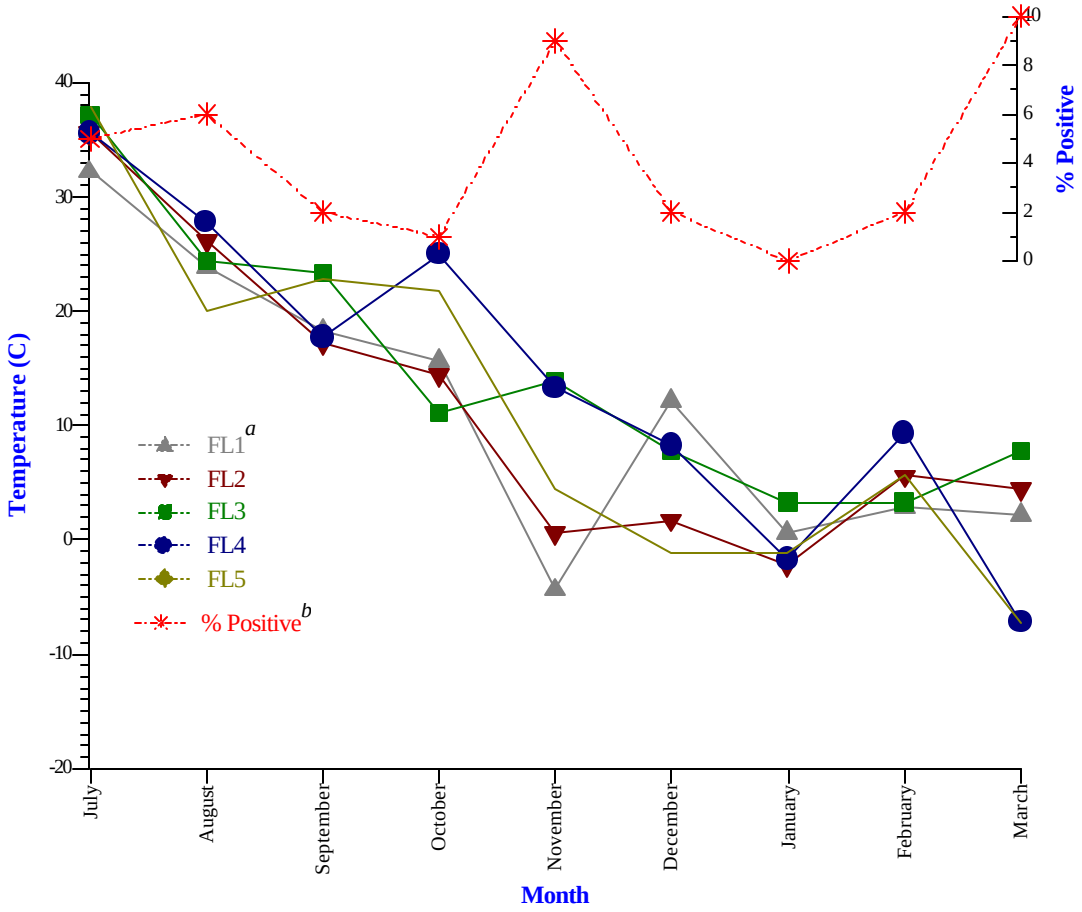

FIG. 11. Relationship between temperature dry bulb of the waste pond from July-March and prevalence of *E. coli* O157 in environmental samples.

[a] FL1-5: Feedlot 1-5.
[b] % total prevalence of *E. coli* O157.

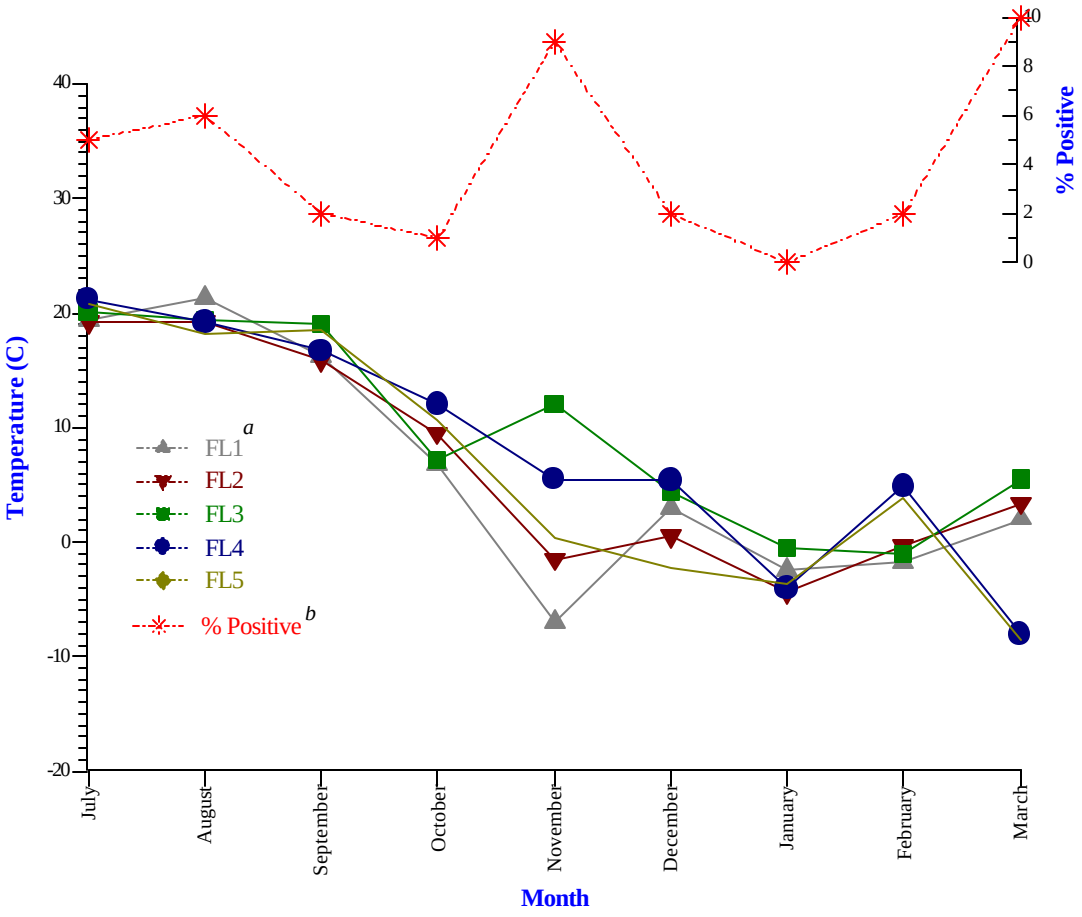

FIG. 12. Relationship between temperature wet bulb of the waste pond from July-March and prevalence of *E. coli* O157 in environmental samples.

[a] FL1-5: Feedlot 1-5.
[b] % total prevalence of *E. coli* O157.

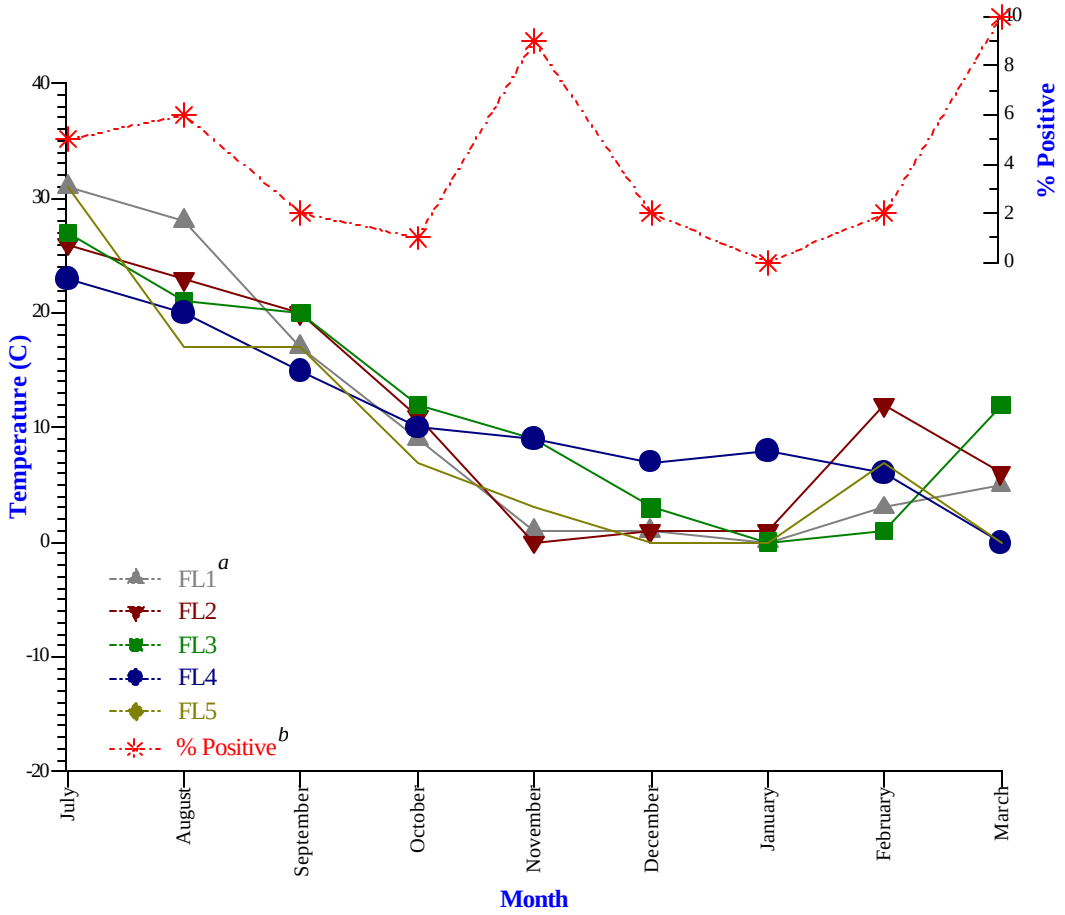

FIG. 13. Relationship between water temperature of the waste pond from July-March and prevalence of *E. coli* O157 in environmental samples.

[a] FL1-5: Feedlot 1-5.
[b] % total prevalence of *E. coli* O157.

from an increase in the number of *E. coli* O157 in the feces, prolonged duration of shedding of cattle or an increase in the rate of new or repeated infections of cattle.

Kudva et al. (66) suggested that *E. coli* O157 in cattle and sheep manure may survive for months under wet environmental conditions, and recovery of these bacteria is less likely from dried layers of manure. In wet weather, during spring and summer months, cattle wading through mud could possibly bring to the surface organisms surviving in the moist soil. Lynn et al. (72) showed the ability of *E. coli* O157 to multiply prolifically in the environment if provided moisture and a nutrient source. In addition, Davies et al. (31) reported that marine sediments are able to provide an environment in which *E. coli* have sufficient nutrients to survive and multiply.

Prevalence of *E. coli* O157 on cattle hides

Results of the examination of hide swabs from commercially slaughtered cattle for the presence of *E. coli* O157 are shown in Table 6. Hide samples were collected from the ventral brisket areas of 100 cattle over two days using a sponge sampling technique.

Overall, 56% (56 of 100) of the hide samples were positive for *E. coli* O157. The presence of *E. coli* O157 on hides was different on each day with 14% (7 of 50) positive for the first and 98% (49 of 50) for the second collection date. These data are not surprising as cattle have been implicated as an *E. coli* O157 reservoir in cattle surveys and traceback studies (25, 68). *E. coli* O157 is carried in the intestinal tract of cattle and shed in the feces of the animals (23, 39, 124). The hide of cattle is known to be a primary source for *E. coli* O157 contamination of beef, and bacteria can be

TABLE 6. Prevalence of *E. coli* O157 on cattle hides[a] at a commercial slaughter plant

Day	No. Samples	No. of *E. coli* O157 positive	% Positive
1	50	7	14
2	50	49	98
Total	**100**	**56**	**56**

[a] 100 hide samples were collected by swabbing 450 cm^2 of brisket surface area using sponge immediately after the animal was stunned.

transferred onto the carcass from the hide during the slaughter and dressing processes (93). All cattle included in the current study were observed to be reasonably clean; however, hides were frequently contaminated with *E. coli* O157. These results support a number of previous studies which reported that a visibly clean hide may not necessarily be pathogen free (73, 84, 97).

No attempt was made in this study to find a precise explanation for the different *E. coli* O157 prevalence on each day; however, it may be related to varying contact among cattle. Similar findings were reported by Elder et al. (36) who found 11 of 29 lots (38%) to have at least one hide positive for *E. coli* O157, with 11% (38 of 355) overall *E. coli* O157 prevalence on hides, and prevalence ranging from 0% to 89% and a mean of 13%. Varied levels of the bacteria may be due to differences in fecal shedding of individual animals, or differences in survival rates of organism either on hide and/or animal-related environments (93). A number studies have revealed significant variation in the number of animals shedding *E. coli* O157 in feces (1-11%) (39, 55) which would directly translate to variation on hides.

The area of hide sampled in this study may have resulted in a high percentage of *E. coli* O157-positive samples. Reid et al. (93) reported that the brisket area of hide is the most heavily contaminated with *E. coli* O157. The brisket area may pose the greatest risk for contamination of carcass surfaces, as it is frequently contaminated, and the initial cut during dehiding passes centrally through the brisket (93). The high prevalence of *E. coli* O157 on the brisket-associated hide area may be due to animals lying down on contaminated ground either on the farm, during transportation, in lairage

(accommodation for farm animals), and/or by contact with the floor within the stunning box (93).

The overall prevalence of *E. coli* O157 on cattle hides is higher in the current study than previous reports have suggested (36, 93). In this study hide samples were collected in the summer months, in which the presence of *E. coli* O157 is expected to peak. As mentioned previously, a number of studies have found that peak *E. coli* O157 fecal shedding rates occur during summer and early fall, and vary from 0% to 61% on some farms (93). *E. coli* O157 excreted in the feces in cattle populations are spatially and temporally clustered, typically lasting 3-4 weeks (55, 76, 103). In addition, use of immunomagnetic separation and enrichment in the current study may have enhanced the isolation of this organism (60, 56, 68).

Differences in levels of *E. coli* O157 on hides may occur as a result of various factors, including fecal shedding, farming systems, transport, and lairage-related conditions (93). Prevalence of cattle hide contamination of slaughtered cattle with *E. coli* O157 in the current study differed by day of collection, 14% and 98% for day one and two, respectively. This variation may have been caused by multiple factors, including a) slaughtered cattle originating from different farms, b) hygienic conditions during farm-to-slaughterhouse transportation and duration of transport, c) differences between slaughter house design, practices and hygienic condition along unloading-to-stunning areas (93). Numerous surveys (7, 105) have identified several modes of transmission for *E. coli* O157 to cattle hides, including animal-to-animal, animal-to-lairage, and environment-to-animal.

Characterization of isolates

All isolates were serologically confirmed as O157:H7 and/or O157:NM isolates using Latex agglutination which the isolates react with monoclonal antibodies directed to O157 lipopolysaccharide and the H7 flagella. Of the 103 *E. coli* O157 isolates found, 47 originated from environmental sources, including 30 H7 positive and 17 H7-negative. Of the 56 isolates obtained from hide samples were 42 H7 positive and 14 were H7-negative (Table 7 and 8).

Serotypes of *E. coli* O157 isolates in feedlot. Table 7 summarizes serotype information regarding the 47 *E. coli* O157 isolates collected from environmental samples during this study. The majority (64%) of isolates were identified as H7 positive, and 36% were H7 negative (O157: NM). The distribution of the O157:H7 serotype was highest in July (n = 7), and lowest on January (n = 0) (Table 7). *E. coli* O157:H7 was isolated from at least one sample in 17 of 45 sample sets (38%). Most *E. coli* O157:H7 were isolated from pond sludge, water and chute samples. This study indicates that the overall prevalence of *E. coli* O157:H7 in the feedlot environment is much higher than that of *E. coli* O157: NM. Unfortunately, from the results of this study it is not possible to explain the reasons for the higher prevalence of *E. coli* O157:H7 over *E. coli* O157: NM. Overall, at least one *E. coli* O157:H7 was recovered per month except in January. *E. coli* O157:H7 isolates were found in each source, ranging from 12 isolates in sludge to 1 in the retention pond, and 1 in the drainage samples.

Serotypes of *E. coli* O157 isolates on cattle hide. Table 8 summarizes information regarding the 56 *E. coli* O157 isolates collected from cattle hide

TABLE 7. *E. coli* O157 isolates from samples by month and sources

Month of Isolation	**Sludge**			**Water**			**Chute**			**RT**[a]			**Drainage**			**Total**
	Total	**H7**[b]	**NM**[c]	**Total**	**H7**	**NM**	**Total**	**H7**	**NM**	**Total**	**H7**	**NM**	**Total**	**H7**	**NM**	
July	**3**	3	0	**1**	1	0	**3**	3	0	**0**	0	0	**0**	0	0	**7**
August	**2**	1	1	**0**	0	0	**5**	4	1	**1**	1	0	**0**	0	0	**8**
September	**2**	1	1	**0**	0	0	**0**	0	0	**0**	0	0	**1**	1	0	**3**
October	**0**	0	0	**0**	0	0	**1**	1	0	**0**	0	0	**0**	0	0	**1**
November	**8**	4	4	**0**	0	0	**3**	1	2	**0**	0	0	**0**	0	0	**11**
December	**1**	1	0	**1**	0	1	**0**	0	0	**0**	0	0	**0**	0	0	**2**
January	**0**	0	0	**0**	0	0	**0**	0	0	**0**	0	0	**0**	0	0	**0**
February	**2**	1	1	**1**	0	1	**0**	0	0	**0**	0	0	**0**	0	0	**3**
March	**4**	1	3	**6**	4	2	**2**	2	0	**0**	0	0	**0**	0	0	**12**
Total	**22**	**12**	**10**	**9**	**5**	**4**	**14**	**11**	**3**	**1**	**1**	**0**	**1**	**1**	**0**	**47**

[a] RT: retention pond
[b] H7: H7 flagella
[c] NM: Non-motile

TABLE 8. Serological identification of *E. coli* O157 on cattle hides at commercial slaughter plant

Collection day	No. of *E. coli* O157 positive	*E. coli* O157:H7	*E. coli* O157:NM
1	7	3	4
2	49	39	10
Total	**56**	**42**	**14**

during the course of this study. *E. coli* O157:H7 was isolated more frequently (75%) than O157:NM (25%). *E. coli* O157:H7 was isolated on both the first (43%) and second (80%) collecting date. No attempts to find precise explanations for the difference in *E. coli* O157:H7 prevalence were made in this study. However, it can be assumed that the high prevalence of *E. coli* O157:H7 over *E. coli* O157:NM is due to multiple factors, including the different origins of cattle from different farms, and the possibility that *E. coli* O157:H7 may attach more readily to the hide than *E. coli* O157:NM.

Antimicrobial resistance compared to isolation source

Antimicrobial resistance was investigated in 317 *E. coli* O157 isolates recovered from 103 positive samples during the course of this study (Table 9). The results of the *in vitro* susceptibility testing of *E. coli* O157 isolates are shown in Table 10. Seventy six (24%) of the isolates analyzed during this study were susceptible to all 23 antimicrobials. Of the 317 isolates characterized in the current study, approximately 56% (176/317) displayed resistance to sulfisoxazole, and 45% (143/317) were resistant to cephalothin. In addition, frequencies of 0-66% of sulfisoxazole, and 41-100% cephalothin resistance were found in the *E. coli* O157 isolates tested in this study (Table 10). Antimicrobial use in bovines may be a factor in the emergence of antimicrobial resistance in *E. coli* O157 recovered from feedlot environment and on cattle hides.

None of the 317 isolates were resistant to cefazolin, imipenem, gentamicin, and ciprofloxacin. Resistance profiles among isolates from sludge, water, and chute were largely similar to each other, whereas *E. coli* O157 isolates from hide showed a different pattern compared to the environmental isolates. *E. coli* O157 isolates showed the

TABLE 9. Sources and serotypes of *E. coli* O157 isolates

Source of isolation	Total number of isolates collected	Number of isolates by serotypes	
		O157:H7	O157:NM
Sludge	35	19	16
Water	19	8	11
Chutes	37	24	13
Retention pond	1	1	0
Drainage	4	2	2
Hide	221	69	152
Total	**317**	**123**	**194**

TABLE 10. The number of *E. coli* O157 isolates from various sources resistant to 23 antibiotics

Source of isolates	Resistance to																						
	FOX[a]	CXM	CTT	CAZ	K	TIC	S	IPM	G	NN	CZ	AMC	NA	CRO	TE	AN	C	CF	SXT	CIP	GM	AM	XNL
Sludge	7	5	5	4	1	6	6	0	23	0	0	7	6	2	10	1	7	16	1	0	0	10	8
Water	2	4	3	3	1	4	5	0	11	1	0	4	1	0	5	0	4	12	7	0	0	5	7
Chute	0	1	0	0	4	2	11	0	22	1	0	0	7	0	10	1	0	19	1	0	0	2	9
RT[b]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Drainage	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	1	0	4	0	0	0	0	0
Hide	0	0	0	0	0	0	0	0	120	0	0	0	18	0	3	1	0	91	1	0	0	0	14
Total	**9**	**10**	**8**	**7**	**6**	**12**	**26**	**0**	**176**	**2**	**0**	**11**	**32**	**2**	**28**	**4**	**11**	**143**	**10**	**0**	**0**	**17**	**38**

[a] FOX: Cefoxitin; CXM: Cefuroxime; CTT: Cefotetan;, CAZ: Ceftazidime; K: Kanamycin; TIC: Ticarcillin; S: Streptomycin; IPM: Imipenem; G: Sulfisoxazole; NN: Tobramycin; CZ: Cefazolin; AMC: Amoxicillin/clavulanic acid; NA: Nalidixic acid; CRO: Ceftriaxone; TE: Tetracycline; AN: Amikacin; C: Chloramphenicol; CF: Cephalothin; SXT: Trimethoprim-sulfamethoxazole; CIP: Ciprofloxacin; GM: Gentamicin; AM: Ampicillin; XNL: Ceftiofur.

[b] RT: retention pond.

highest level of resistance to sulfisoxazole and cephalothin. In addition, there was a high level of resistance to streptomycin, nalidixic acid, tetracycline, ampicillin, and ceftiofur. This finding supports previous studies that found *E. coli* O157 highly resistant to tetracycline, sulfamethoxazole, cephalothin, and ampicillin (41, 78, 129).

Similar levels of antimicrobial resistance occurred in *E. coli* O157 isolates from sludge and water samples. This similar pattern may be because both environmental samples originated from the same waste pond. In addition, *E. coli* O157 isolates from the hide were mainly resistant to sulfisoxazole (54%) and cephalothin (41%). All *E. coli* O157 isolates, regardless of the source of isolation, were susceptible to imipenem, cefazolin, ciprofloxacin, and gentamicin, similarly found in previous reports. Schroeder et al. (102) found all *E. coli* O157 isolates from different sources of isolation (human, cattle, swine, and food) were susceptible to cefoxitin, ceftriaxone, gentamicin, nalidixic acid, ciprofloxacin, ceftiofur, and trimethoprim-sulfamethozaxole.

The 35 *E. coli* O157 isolates recovered from sludge were most resistant to sulfisoxazole (66%), cephalothin (46%), ampicillin (29%), and to tetracycline (29%), and a small percentage (up to 23%) of isolates were resistant to other antimicrobials tested (Fig. 14). *E. coli* O157 isolates (n = 19) from water were widely resistant to all antimicrobials tested, except imipenem, cefazolin, ceftriaxone, amikacin, ciprofloxacin, gentamicin (Fig. 15). *E. coli* O157 isolates (n = 37) from chute were mainly resistant to streptomycin (30%), sulfisoxazole (59%), tetracycline (27%), cephalothin (51%), and ceftiofur (24%) (Fig. 16). One *E. coli* O157 isolate recovered from the retention pond

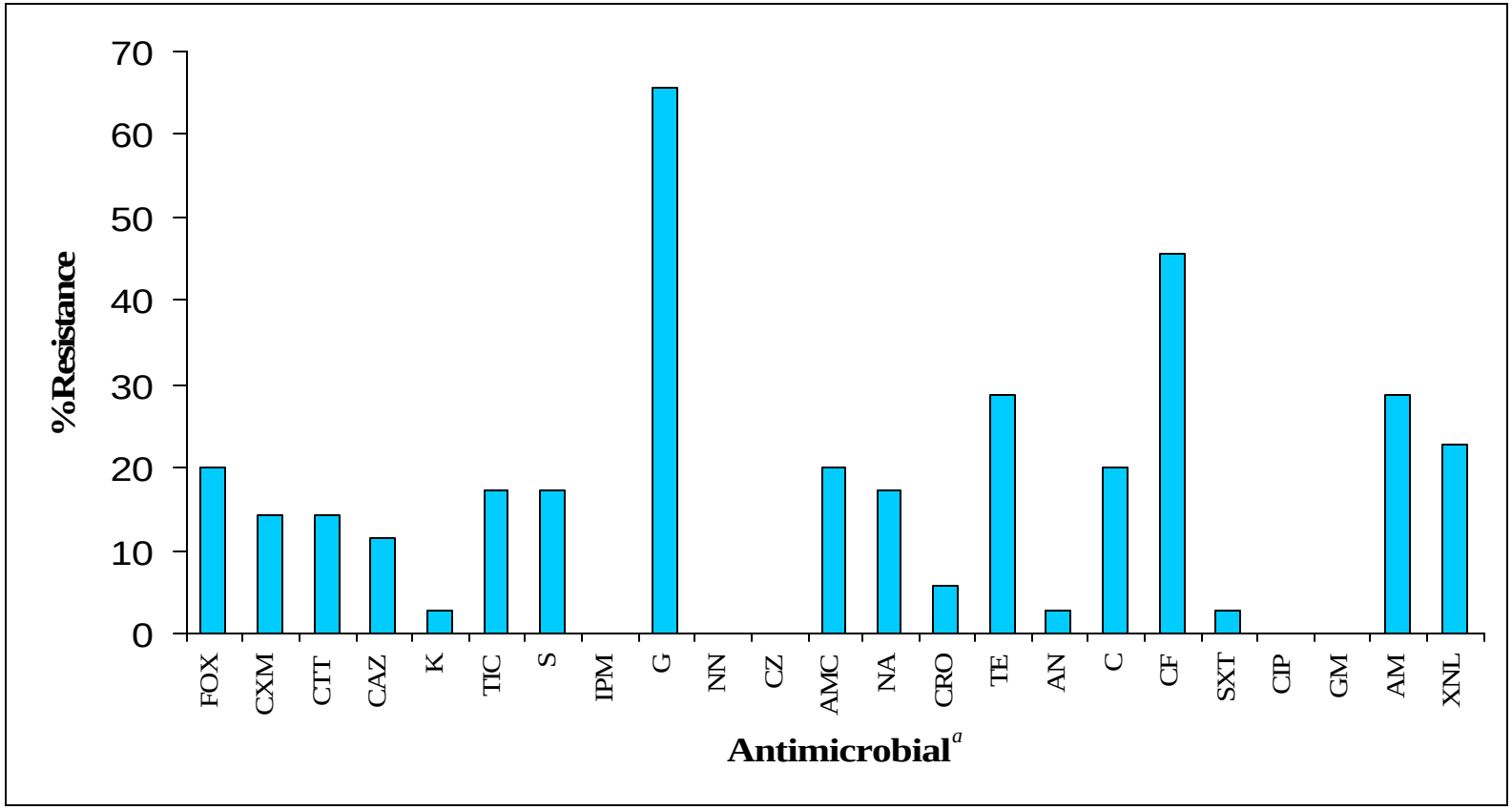

FIG. 14. Antimicrobial resistance among *E. coli* O157 isolates recovered from pond sludge (n = 35).

[a] FOX: Cefoxitin; CXM: Cefuroxime; CTT: Cefotetan;, CAZ: Ceftazidime; K: Kanamycin; TIC: Ticarcillin; S: Streptomycin; IPM: Imipenem; G: Sulfisoxazole; NN: Tobramycin; CZ: Cefazolin; AMC: Amoxicillin/clavulanic acid; NA: Nalidixic acid; CRO: Ceftriaxone; TE: Tetracycline; AN: Amikacin; C: Chloramphenicol; CF: Cephalothin; SXT: Trimethoprim-sulfamethoxazole; CIP: Ciprofloxacin; GM: Gentamicin; AM: Ampicillin; XNL: Ceftiofur.

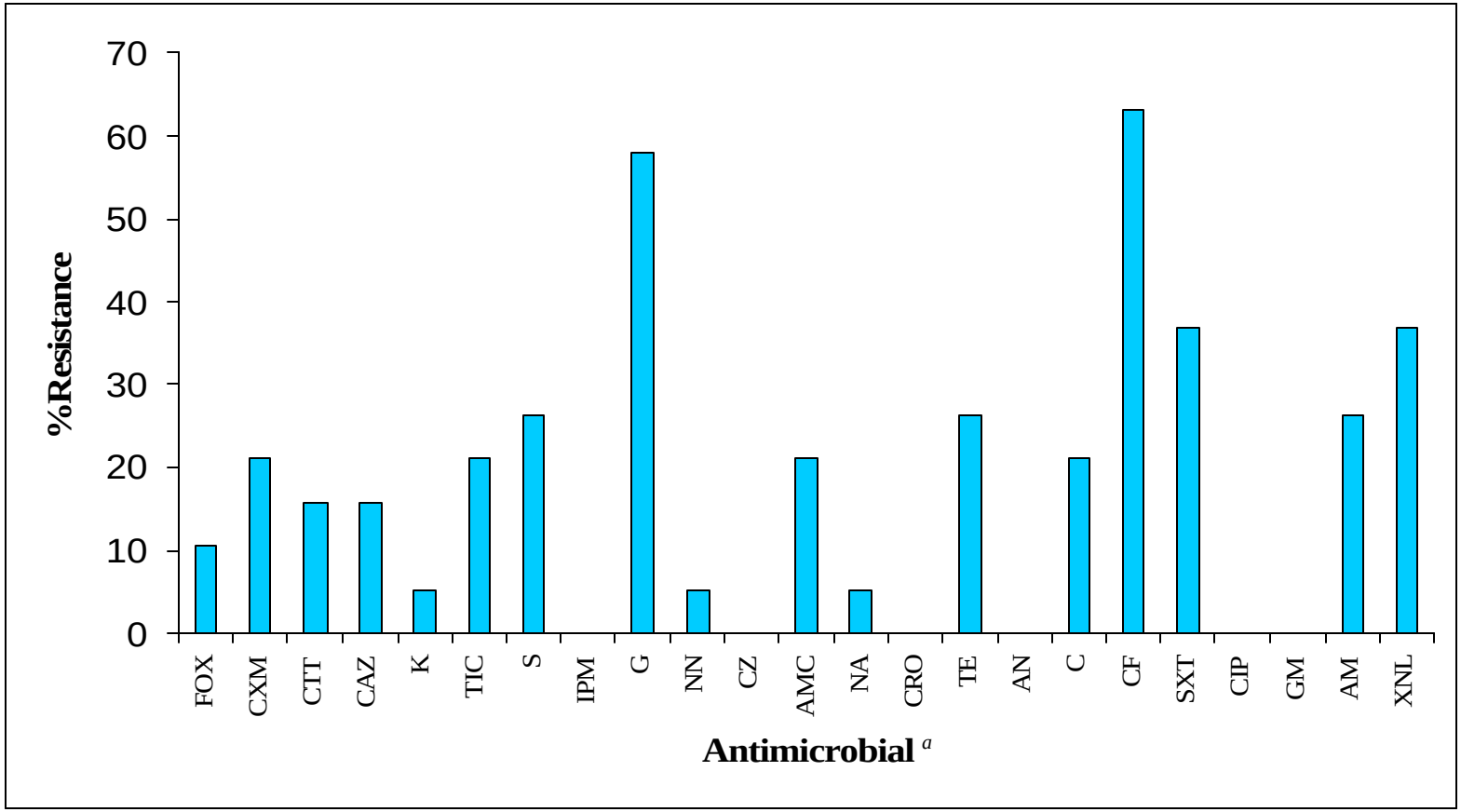

FIG. 15. Antimicrobial resistance among *E. coli* O157 isolates recovered from waste pond water (n = 19).

[a] FOX: Cefoxitin; CXM: Cefuroxime; CTT: Cefotetan;, CAZ: Ceftazidime; K: Kanamycin; TIC: Ticarcillin; S: Streptomycin; IPM: Imipenem; G: Sulfisoxazole; NN: Tobramycin; CZ: Cefazolin; AMC: Amoxicillin/clavulanic acid; NA: Nalidixic acid; CRO: Ceftriaxone; TE: Tetracycline; AN: Amikacin; C: Chloramphenicol; CF: Cephalothin; SXT: Trimethoprim -sulfamethoxazole; CIP: Ciprofloxacin; GM: Gentamicin; AM: Ampicillin; XNL: Ceftiofur.

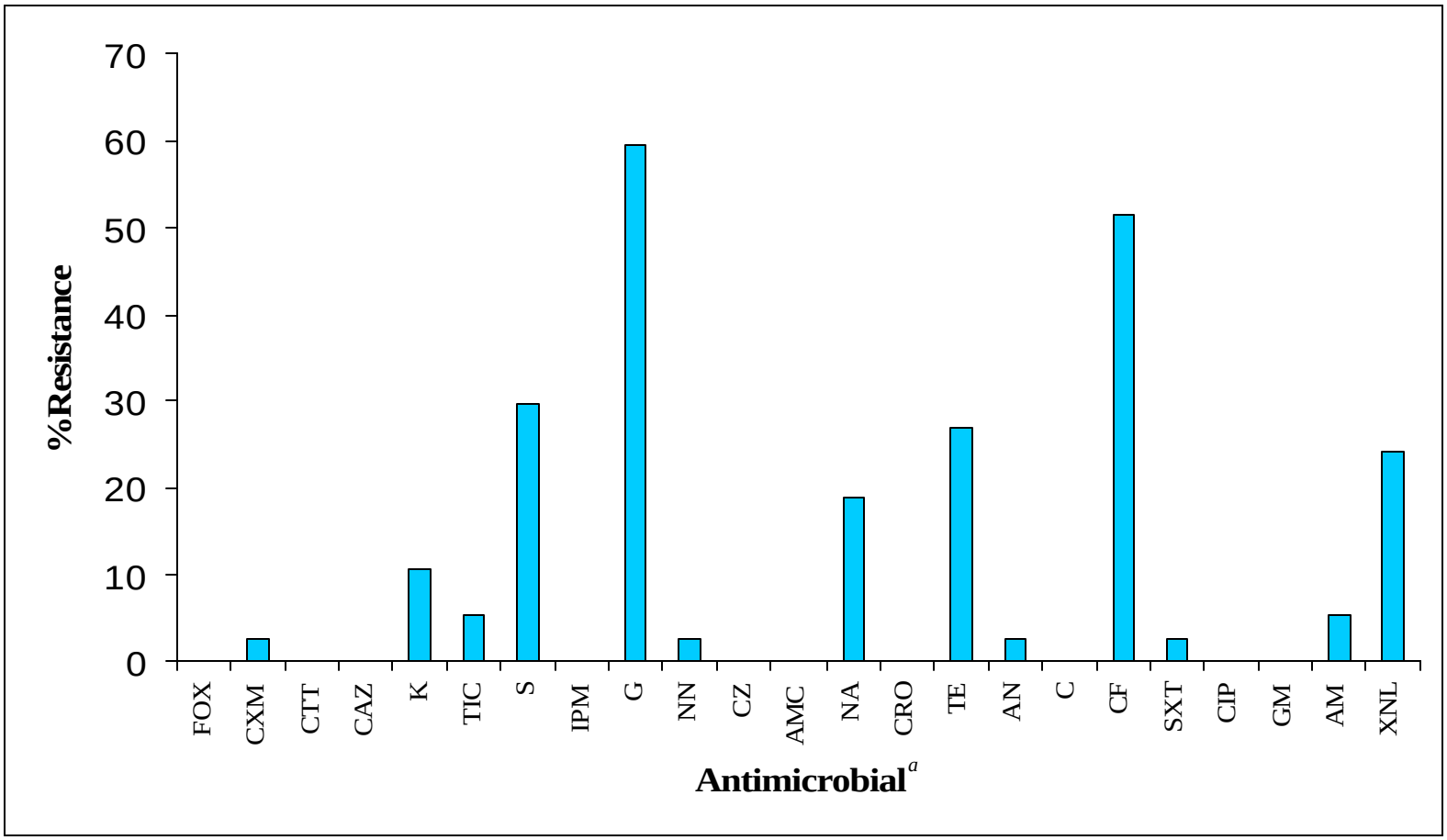

FIG. 16. Antimicrobial resistance among *E. coli* O157 isolates recovered from chute (n = 37).

[a] FOX: Cefoxitin; CXM: Cefuroxime; CTT: Cefotetan;, CAZ: Ceftazidime; K: Kanamycin; TIC: Ticarcillin; S: Streptomycin; IPM: Imipenem; G: Sulfisoxazole; NN: Tobramycin; CZ: Cefazolin; AMC: Amoxicillin/clavulanic acid; NA: Nalidixic acid; CRO: Ceftriaxone; TE: Tetracycline; AN: Amikacin; C: Chloramphenicol; CF: Cephalothin; SXT: Trimethoprim-sulfamethoxazole; CIP: Ciprofloxacin; GM: Gentamicin; AM: Ampicillin; XNL: Ceftiofur.

was only resistant to cephalothin and susceptible to all other antimicrobials tested (Fig. 17). Virtually, all 4 *E. coli* O157 isolates from drainage were susceptible to most of the antimicrobials tested. It was noted that 25-100% of *E. coli* O157 isolates from drainage were resistant to streptomycin (100%), cephalothin (100%), and amikacin (25%), (Fig. 18). *E. coli* O157 isolates from hide were most 54% resisted to sulfisoxazole, 41% to cephalothin, 8% to nalidixic acid, 1% to tetracycline, and 6% to ceftiofur; however, less than 1% of 221 hide isolates was resistant to amikacin, and to trimethoprim-sulfamethoxazole (Fig. 19).

A high level of resistance to sulfamethoxazole and tetracycline among *E. coli* O157:H7 isolates recovered from humans and cattle has been previously reported (102). In the current study, it is not surprising that tetracycline-resistance among *E. coli* O157 isolates from the feedlot environment was discovered since sulfa drugs and tetracycline are approved for use in cattle production (Food and Drug Administration, The FDEA Approved Animal Drugist, the Green Book) (40). In the current study, approximately 9% of *E. coli* O157 isolates recovered were resistant to tetracycline. This finding supports the Schroeder et al. (102) study which found approximately 10% of Shiga Toxin-producing *E. coli* isolated from humans, cattle, swine, and food were tetracycline resistant. Parallel to Schroeder et al. (102), our study showed low overall resistance to amoxicillin-clavulanic acid (3%) among 317 *E. coli* O157 isolates. Only 20 and 21% of *E. coli* O157 isolates recovered from sludge and water sample, respectively, were discovered to exhibit amoxicillin-clavulanic acid resistance. However, Galland et al. (41) has shown among *E. coli* O157:H7 isolated from 47% cattle (27 of 57) were resistant to

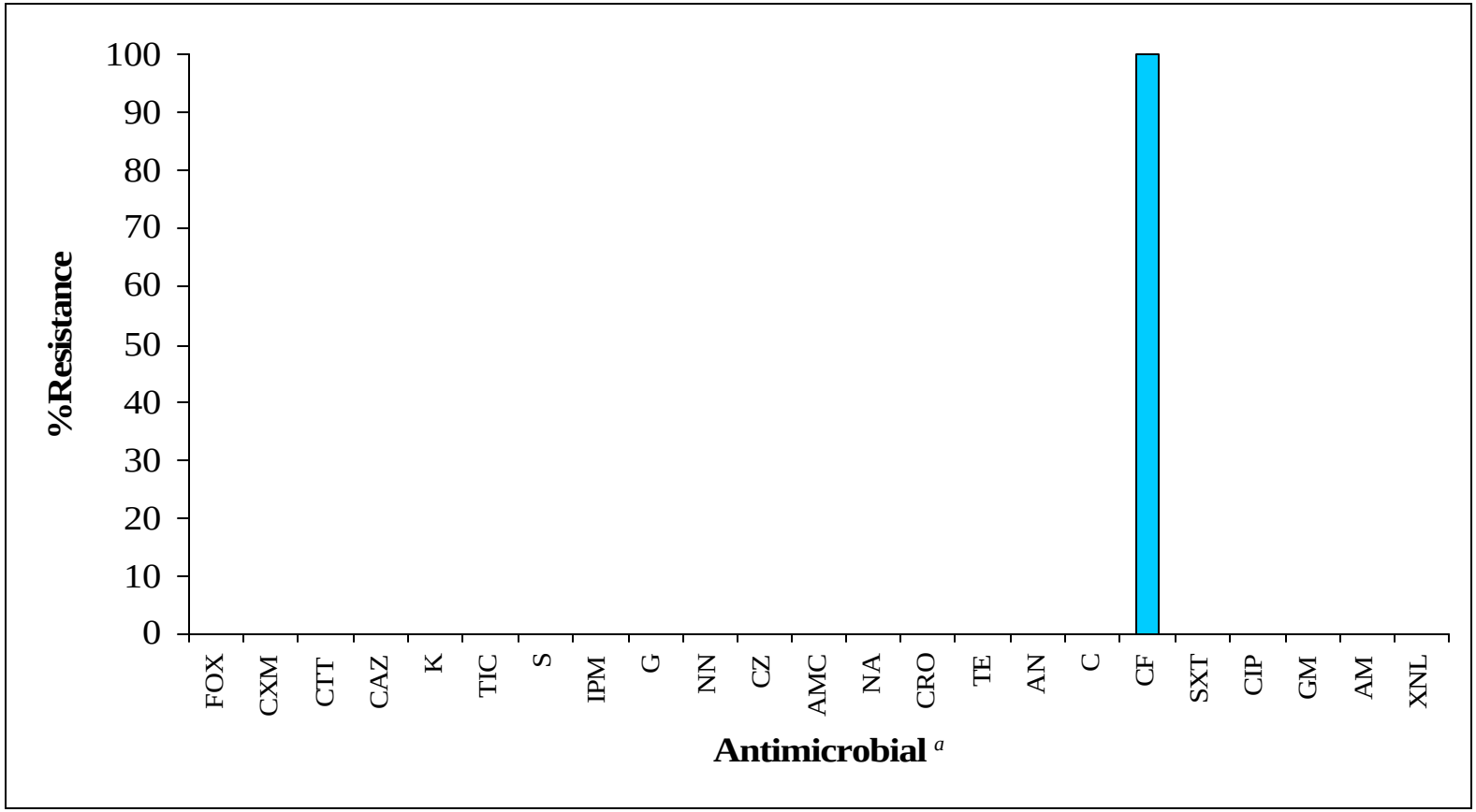

FIG. 17. Antimicrobial resistance among *E. coli* O157 isolates recovered from retention pond (n = 1).

[a] FOX: Cefoxitin; CXM: Cefuroxime; CTT: Cefotetan;, CAZ: Ceftazidime; K: Kanamycin; TIC: Ticarcillin; S: Streptomycin; IPM: Imipenem; G: Sulfisoxazole; NN: Tobramycin; CZ: Cefazolin; AMC: Amoxicillin/clavulanic acid; NA: Nalidixic acid; CRO: Ceftriaxone; TE: Tetracycline; AN: Amikacin; C: Chloramphenicol; CF: Cephalothin; SXT: Trimethoprim-sulfamethoxazole; CIP: Ciprofloxacin; GM: Gentamicin; AM: Ampicillin; XNL: Ceftiofur.

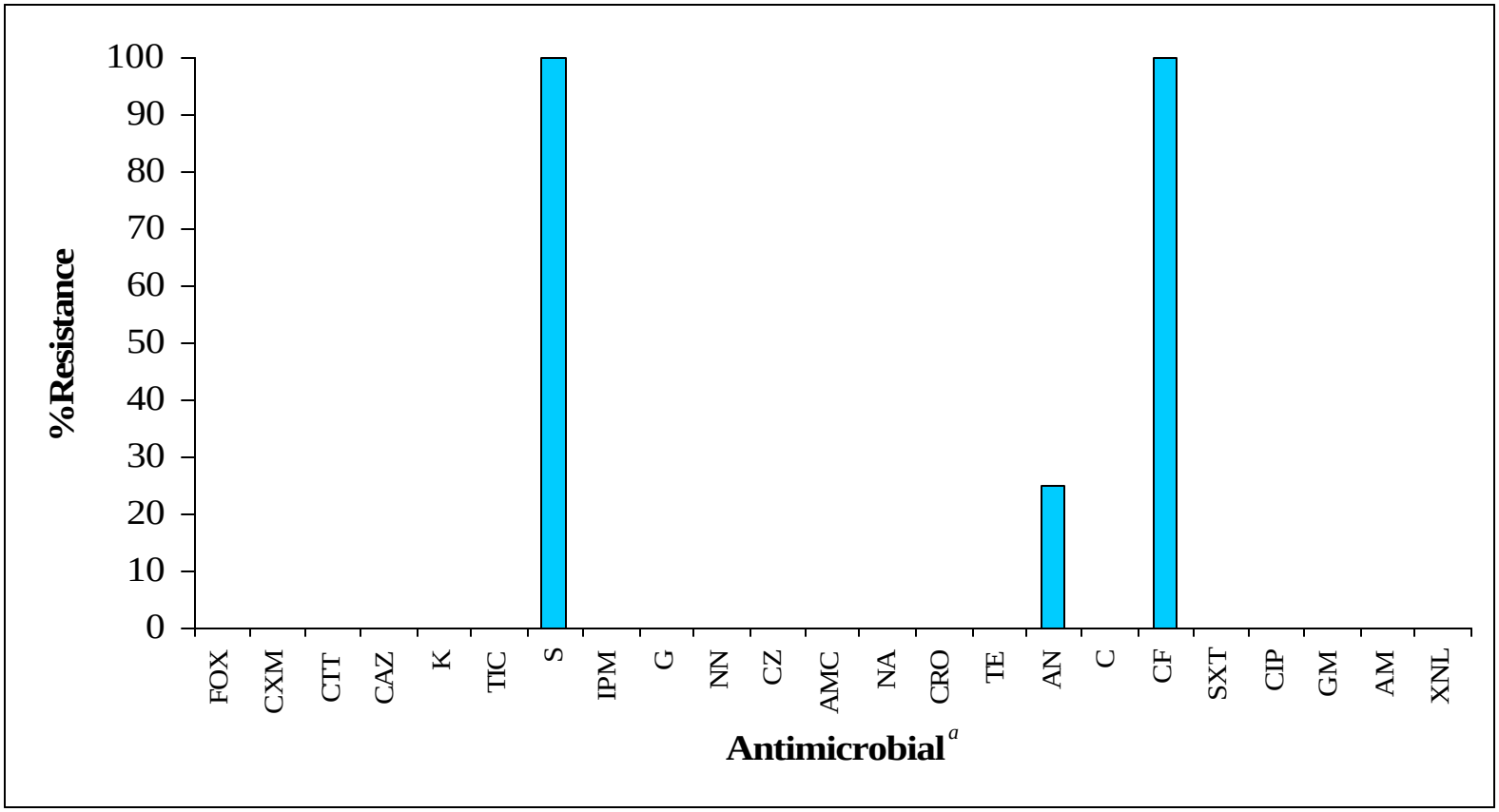

FIG. 18. Antimicrobial resistance among *E. coli* O157 isolates recovered from drainage (n = 4).

[a] FOX: Cefoxitin; CXM: Cefuroxime; CTT: Cefotetan;, CAZ: Ceftazidime; K: Kanamycin; TIC: Ticarcillin; S: Streptomycin; IPM: Imipenem; G: Sulfisoxazole; NN: Tobramycin ; CZ: Cefazolin; AMC: Amoxicillin/clavulanic acid; NA: Nalidixic acid; CRO: Ceftriaxone; TE: Tetracycline; AN: Amikacin; C: Chloramphenicol; CF: Cephalothin; SXT: Trimethoprim -sulfamethoxazole; CIP: Ciprofloxacin; GM: Gentamicin; AM: Ampicillin; XNL: Ceftiofur.

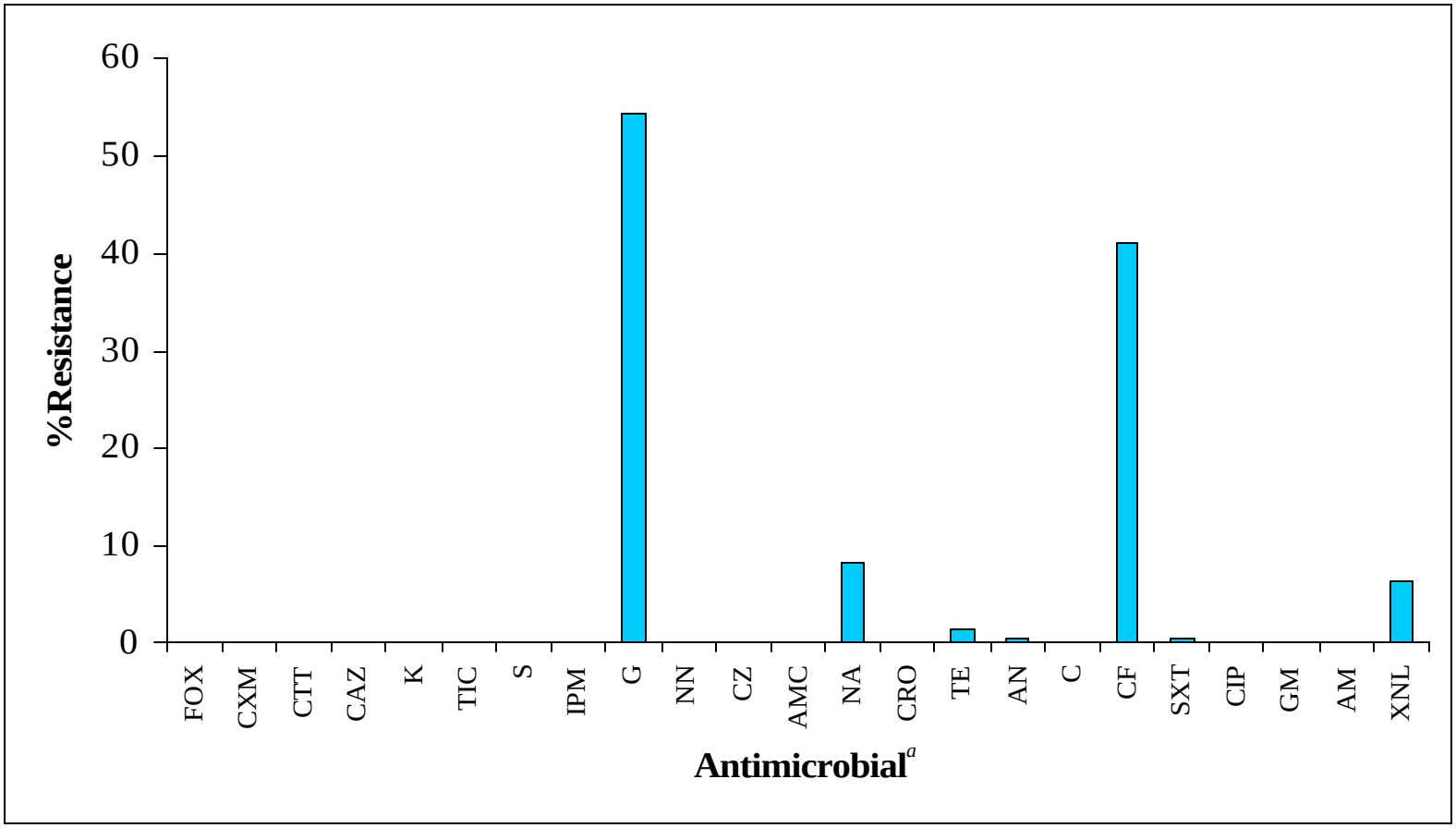

FIG. 19. Antimicrobial resistance among *E. coli* O157 isolates recovered from hide (n = 221).

[a] FOX: Cefoxitin; CXM: Cefuroxime; CTT: Cefotetan;, CAZ: Ceftazidime; K: Kanamycin; TIC: Ticarcillin; S: Streptomycin; IPM: Imipenem; G: Sulfisoxazole; NN: Tobramycin; CZ: Cefazolin; AMC: Amoxicillin/clavulanic acid; NA: Nalidixic acid; CRO: Ceftriaxone; TE: Tetracycline; AN: Amikacin; C: Chloramphenicol; CF: Cephalothin; SXT: Trimethoprim-sulfamethoxazole; CIP: Ciprofloxacin; GM: Gentamicin; AM: Ampicillin; XNL: Ceftiofur.

amoxicillin-clavulanic acid. The difference between the study of Galland et al. (41) and ours may be due to different methodologies (dilution susceptibility test) used to determine resistance as well as a difference in resistance breakpoint (>4/2 µg/ml versus 20/10 µg/ml). In addition, antibiotic resistance may be due to temporal and geographical differences of the studies, as the Galland et al. (41) study was conducted in the southwestern regions of Kansas over an 11-month time frame.

A number of previous studies have demonstrated that antibiotic-resistant microorganisms recovered from food animals may colonize the human population via the food chain through occupational exposure or waste runoff from animal production facilities (118, 127). Therefore, the possibility exists that the resistance of *E. coli* O157 to certain antibiotics may be transferred from cattle to the environment.

Cattle are implicated as reservoirs of *E. coli* O157 (129); therefore, the continued use of sulfa drugs and tetracycline derivatives in cattle may increase resistance selection among these organisms (127, 129). It is a possible that these resistant bacteria may be transferred from cattle to environment. This possibility may affect the findings of resistance patterns of *E. coli* O157 isolates recovered from feedlot environment and cattle hides. Interestingly, only a small percentage of *E. coli* O157 isolates in this study were resistant to cefoxitin (3%), chloramphenicol (3%), and nalidixic acid (1%), and none of these antibiotics are approved for use in cattle in the U.S. A similar finding was observed by Schroeder et al. (102).

In this study, it was observed that there was a connection among the microbial ecosystems of cattle, and hide and feedlot environment; however, it may be difficult to identify the origin of the antimicrobial resistance observed in this study.

Multiple antimicrobial resistances

Only 9 (3%) of the isolates analyzed were resistant to more than 10 antimicrobials, however, 126 (40%) isolates were resistant to one or more antimicrobial (Table 11). Multiple antibiotic resistance frequencies were lowest for isolates from the retention pond and drainage, while isolates from sludge and water had the highest resistance frequencies.

Multiple antimicrobial resistance in *E. coli* O157 may be partially due to the spread of genetic elements such as plasmids, transposons, and integrons which can carry resistance to numerous antimicrobials (129). Schroeder et al. (102) demonstrated that the multiple antimicrobial-resistant phenotypes observed resulted from the spread of mobile genetic elements. In that study, ampicillin-resistant *E. coli* O157 isolates were also resistant to streptomycin and tetracycline, suggesting resistance genes for these drugs are linked on plasmids. A similar observation was found in the current study with multiresistance observed more frequently than in the Schroeder et al. study (102).

Genomic relatedness among *E. coli* O157 using rep-PCR DNA fingerprinting

A total of 101 representative *E. coli* O157 isolates were submitted for analysis by rep-PCR DNA fingerprinting to Bacterial BarCodes, Inc., Houston, Tex.). These 101 isolates consisted of 47 *E. coli* O157 isolates from feedlot environments and 54 hide isolates. Two primers were assessed for their ability to discriminate between the

TABLE 11. Multiple antimicrobial resistances among the 317 *E. coli* O157 isolates

No. of antimicrobials to which resistance was shown	Number of isolates	
	Hide (n=221)	Environmental (n=96)
0	66	10
1	99	27
2	41	21
3	12	11
4	1	6
5	1	4
6	0	3
7	0	1
8	0	2
9	0	2
>10	0	9

isolates: Uprime-B1 with the DiversiLab fingerprinting kit and Uprime-RI with the repPRO fingerprinting kit.

In the current study, the Uprime-B1 primer resulted in greater discrimination between the isolates than Uprime-RI primer and was, therefore, used for the interpretation (Fig. 20). Similarly, Dombek et al. (34) determined that the DNA fingerprints obtained with the BOX (Uprime-B1) primer were more effective for grouping bacteria strains than the DNA fingerprints obtained with REP (Uprime-RI) primers.

To reveal the relatedness of *E. coli* O157:H7 isolates, a dendrogram based on Uprime-B1 fingerprint data was constructed (Fig. 21). All isolates in this study were grouped into six clusters, separated at a relative genetic similarity of 53%; however, most isolates obtained from this study had rep-PCR banding patterns identical or closely similar to each other with an average similarity coefficient of 92%. Based on these results, the majority of the environmental (29/47) and hide (51/54) isolates fell into the first cluster.

As shown in Fig. 21, cluster 1 consisted of 80 isolates (29 *E. coli* O157 isolates from environmental feedlot and 51 *E. coli* O157 isolates from cattle hides) which appeared indistinguishable with similarity coefficients greater than 97%. Cluster 2 consisted of 9 isolates, all from environmental sources (mostly from feedlot 3), which appeared indistinguishable with similarity coefficients greater than 98%. These isolates were highly similar to the isolates in cluster 1 with an average similarity of 96%. Cluster 3 consisted of 2 isolates, 1 environment and 1 hide isolate. These isolates were highly

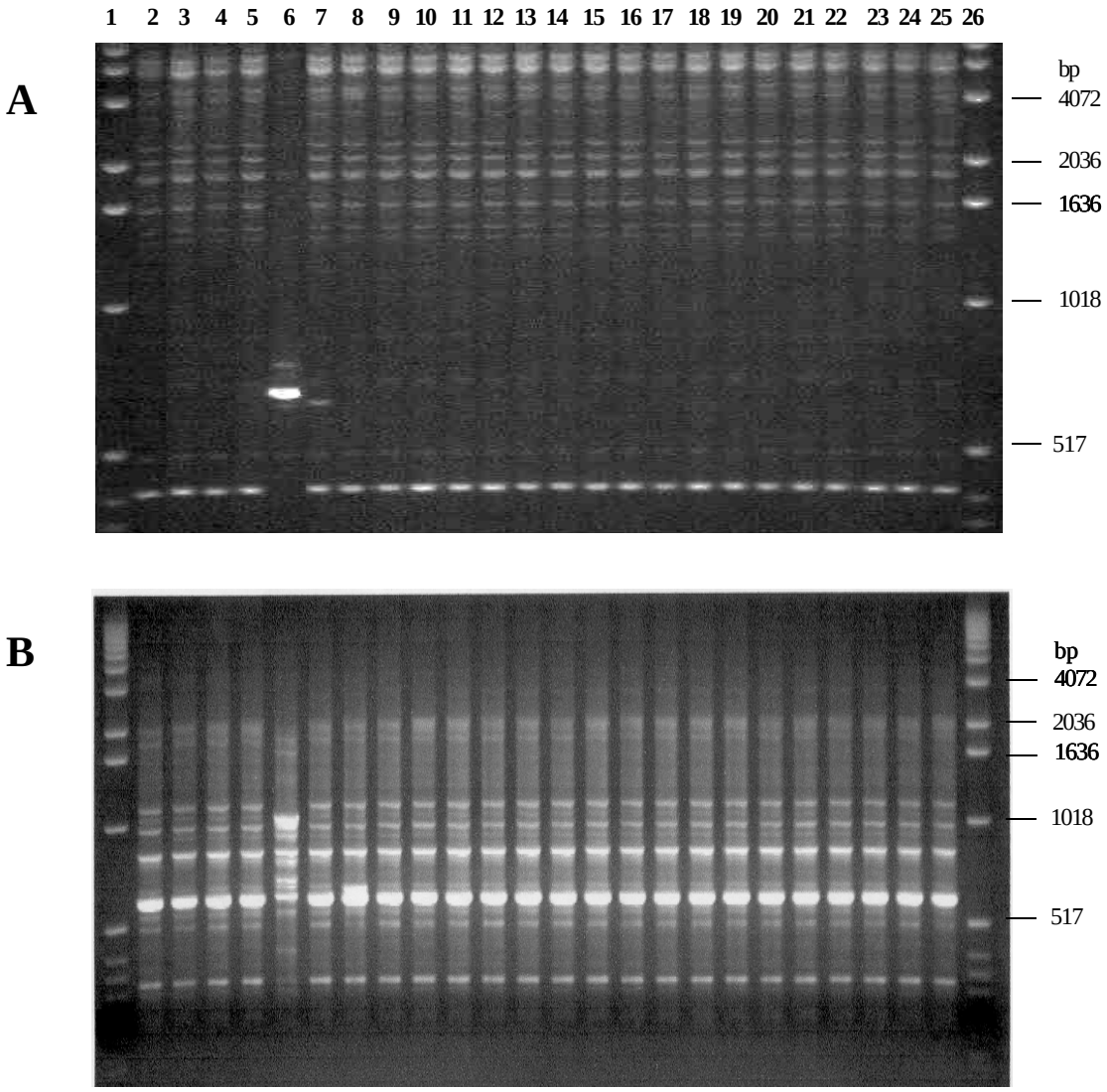

FIG. 20. rep-PCR DNA fingerprint patterns of *E. coli* O157 strains obtained from feedlot environment and cattle hides. (A) PCR DNA fingerprint patterns generated with Uprime-B1 primer with the DiversiLab fingerprinting kit. (B) PCR DNA fingerprint patterns generated with Uprime-RI primers with the repPRO fingerprinting kit. The *E. coli* O157 strains used for the fingerprint analysis shown in panel B are identical to the strains used for the analysis shown in panel A.

Lanes 1 and 26 contained an external standard, a 1-kb molecular weight ladder.
Lane 2, 8: *E. coli* O157:NM, from cattle hides, collecting day 1; Lanes3-7 *E. coli* O157:H7 from cattle hide, collecting day 1; Lane s 9, 10, 14, 16, 18, 19: *E. coli* O157:NM, from cattle hide, collecting day 2; Lane 11-13, 15, 17, 20-25: *E. coli* O157:H7 from cattle hide, collecting day 2.

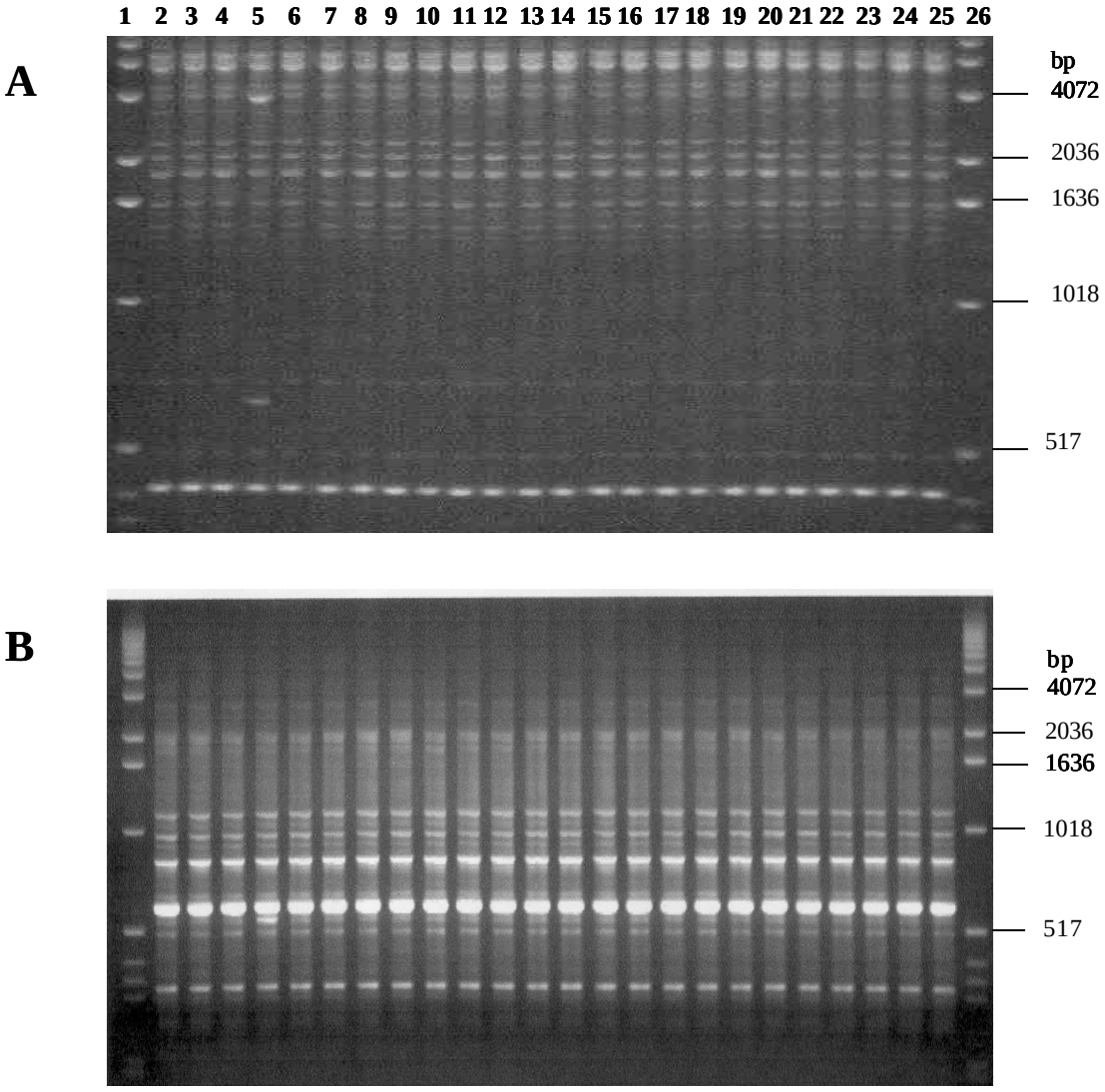

FIG. 20. Continued.

Lanes 1 and 26 contained an external standard, a 1-kb molecular weight ladder.
Lanes 8-9, 17: *E. coli* O157:NM, from cattle hide, collecting day 2; Lanes 2-7, 10-16, 18-25: *E. coli* O157:H7 from cattle hide, collecting day 2.

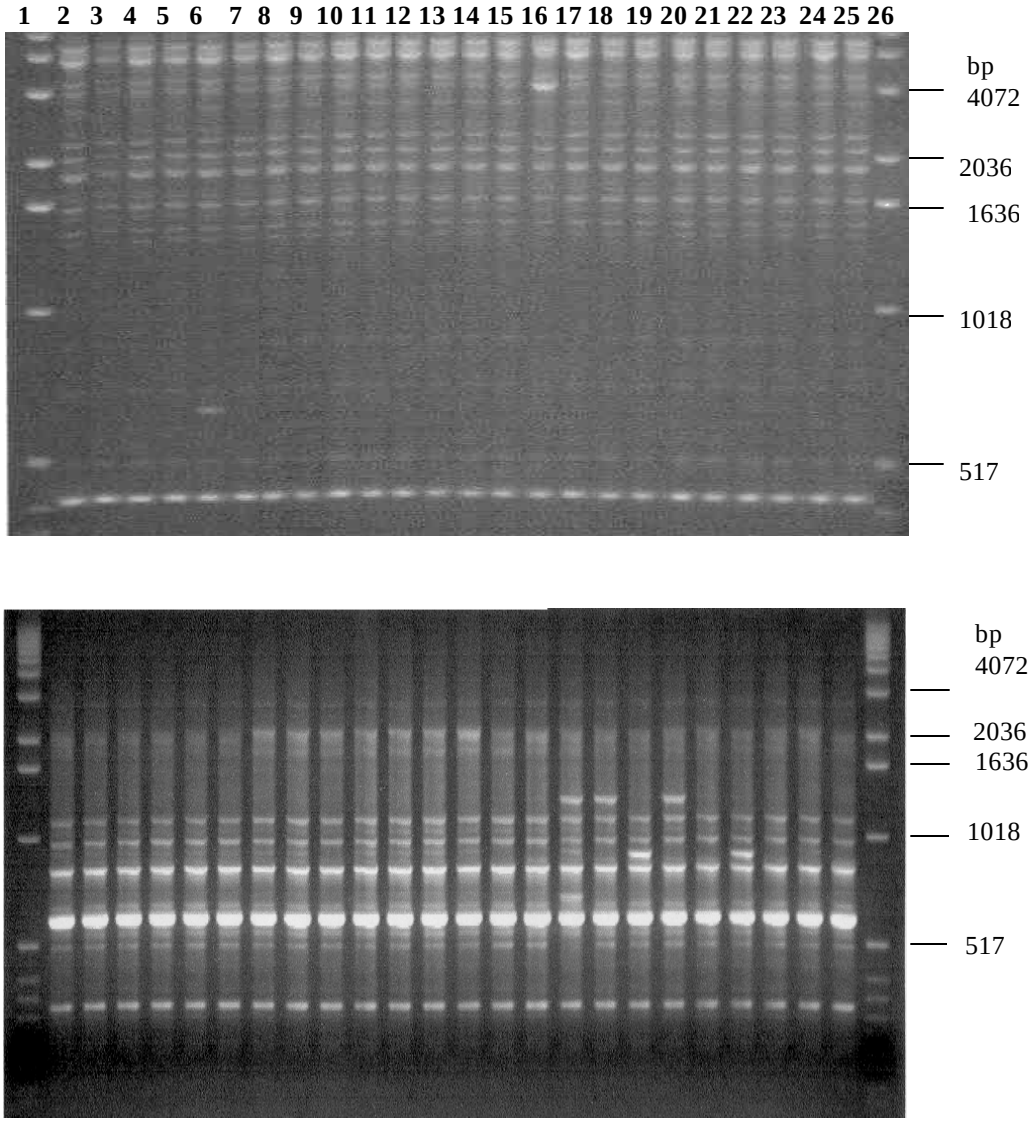

FIG. 20. Continued.

Lanes 1 and 26 contained an external standard, a 1-kb molecular weight ladder.
Lanes 2-7: *E. coli* O157:H7, from cattle hide, collecting day 2; Lanes 8-9, 11-13: *E. coli* O157:H7 from F4 on Jul; Lane 10: *E. coli* O157:H7 from F1 on Jul, Lane 14-15: *E. coli* O157:H7 from F5 on Jul; Lanes 16-17: *E. coli* O157:H7 from F2 on Aug; Lane 18: *E. coli* O157:NM from F2 on Aug; Lanes 19: *E. coli* O157:H7 from F3 on Aug; Lanes 21: *E. coli* O157:NM from F5 on Sept; Lanes 20, 22-23: *E. coli* O157:H7 from F5 on Sept; Lane 24: *E. coli* O157:H7 from F2 on Sept; Lane 25: *E. coli* O157:NM from F3 on Sept

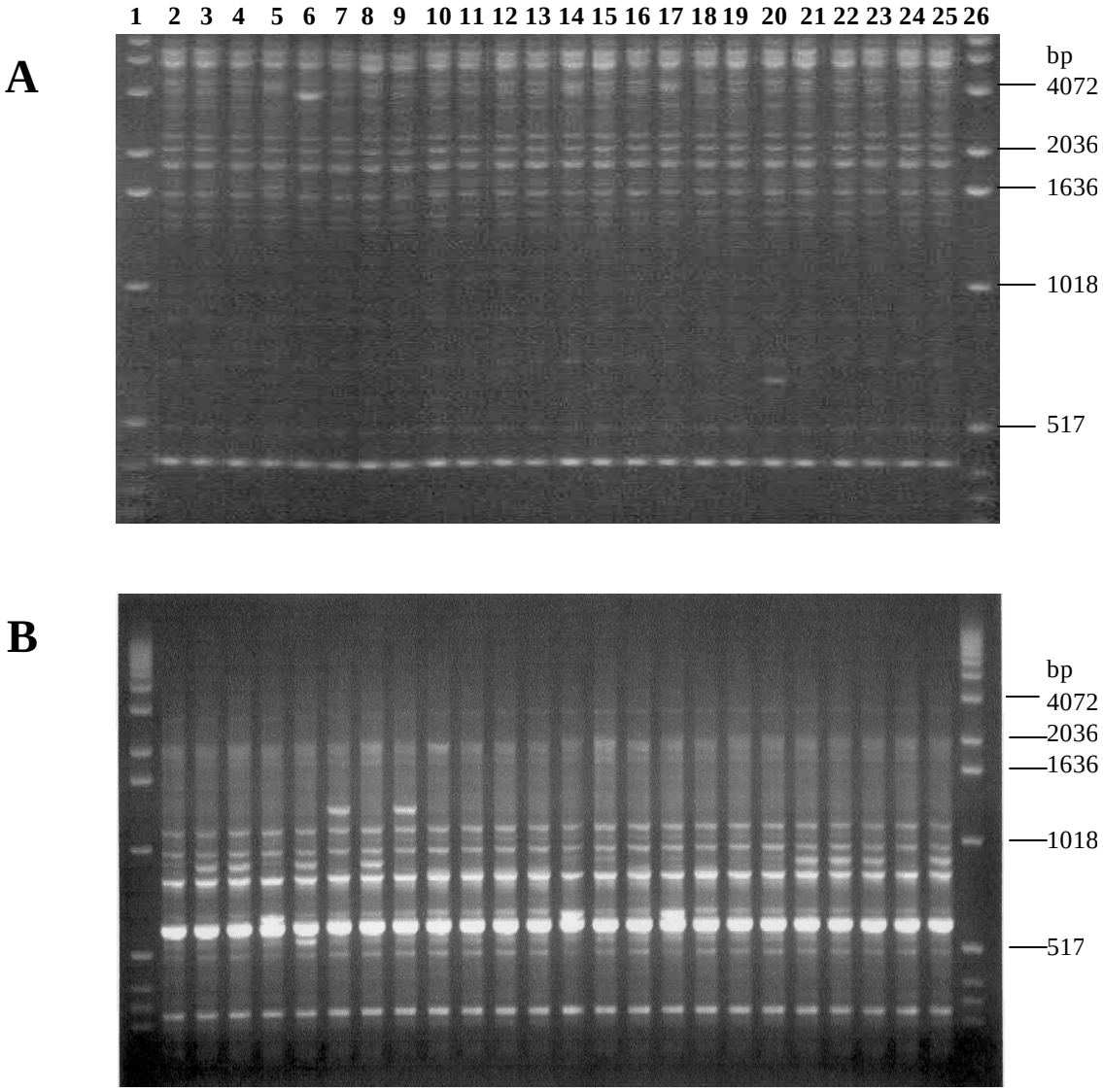

FIG. 20. Continued.

Lanes 1 and 26 contained an external standard, a 1-kb molecular weight ladder.
Lanes 2: *E. coli* O157:H7, from F5 on Oct; Lane 3-4: *E. coli* O157:H7 from F1 on Nov; Lane 5: *E. coli* O157:NM from F2 on Nov; Lanes 6, 8: *E. coli* O157:H7 from F2 on Nov; Lanes 7, 9: *E. coli* O157:NM from F2 on Nov; Lane 10: *E. coli* O157:NM from F4 on Dec; Lane 11: *E. coli* O157:H7 from F4 on Dec; Lane 12: *E. coli* O157:NM from F5 on Dec; Lane 13: *E. coli* O157:H7 from F5 on Dec; Lane 14: *E. coli* O157:NM from F1 on Dec; Lane 15: *E. coli* O157:H7 from F1 on Dec; Lane 16: *E. coli* O157:H7 from F1 on Feb; Lane 17: *E. coli* O157:NM from F2 on Feb; ; Lane 18: *E. coli* O157:NM from F5 on Feb; Lane 19: *E. coli* O157:NM from F2 on Mar; Lane 20: *E. coli* O157:H7 from F2 on Mar; Lane 21: *E. coli* O157:H7 from F3 on Mar; Lanes 22-25: *E. coli* O157:NM from F3 on Mar.

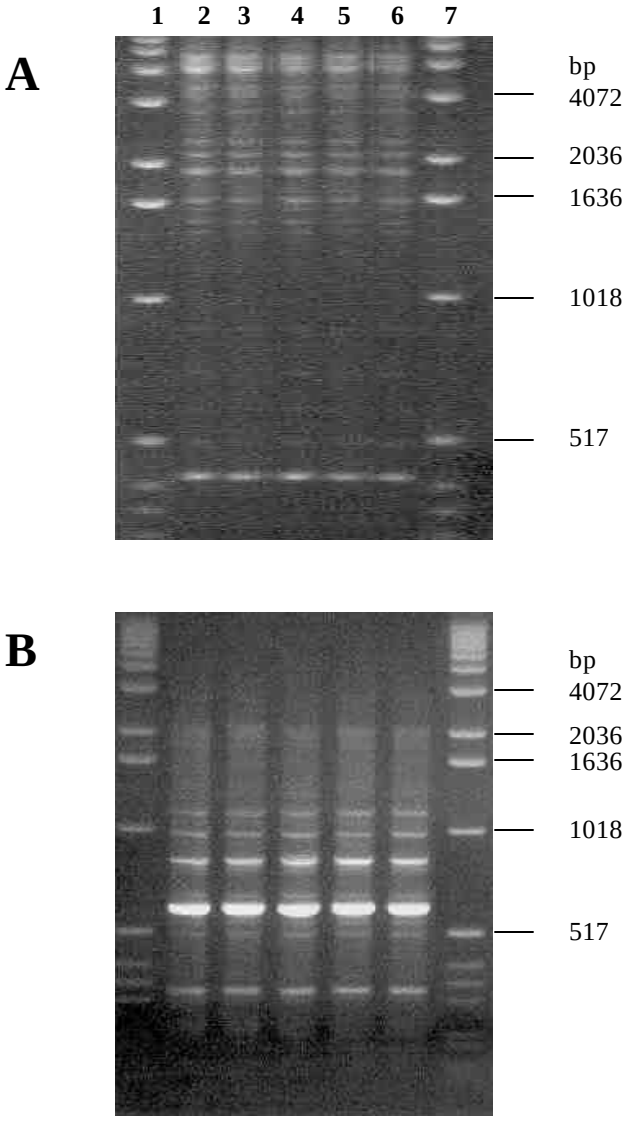

FIG. 20. Continued.

Lanes 1 and 7 contained an external standard, a 1-kb molecular weight ladder.
Lanes 2-5: *E. coli* O157:H7 from F3 on Mar; Lane 6: *E. coli* O157:NM from F3 on Mar.

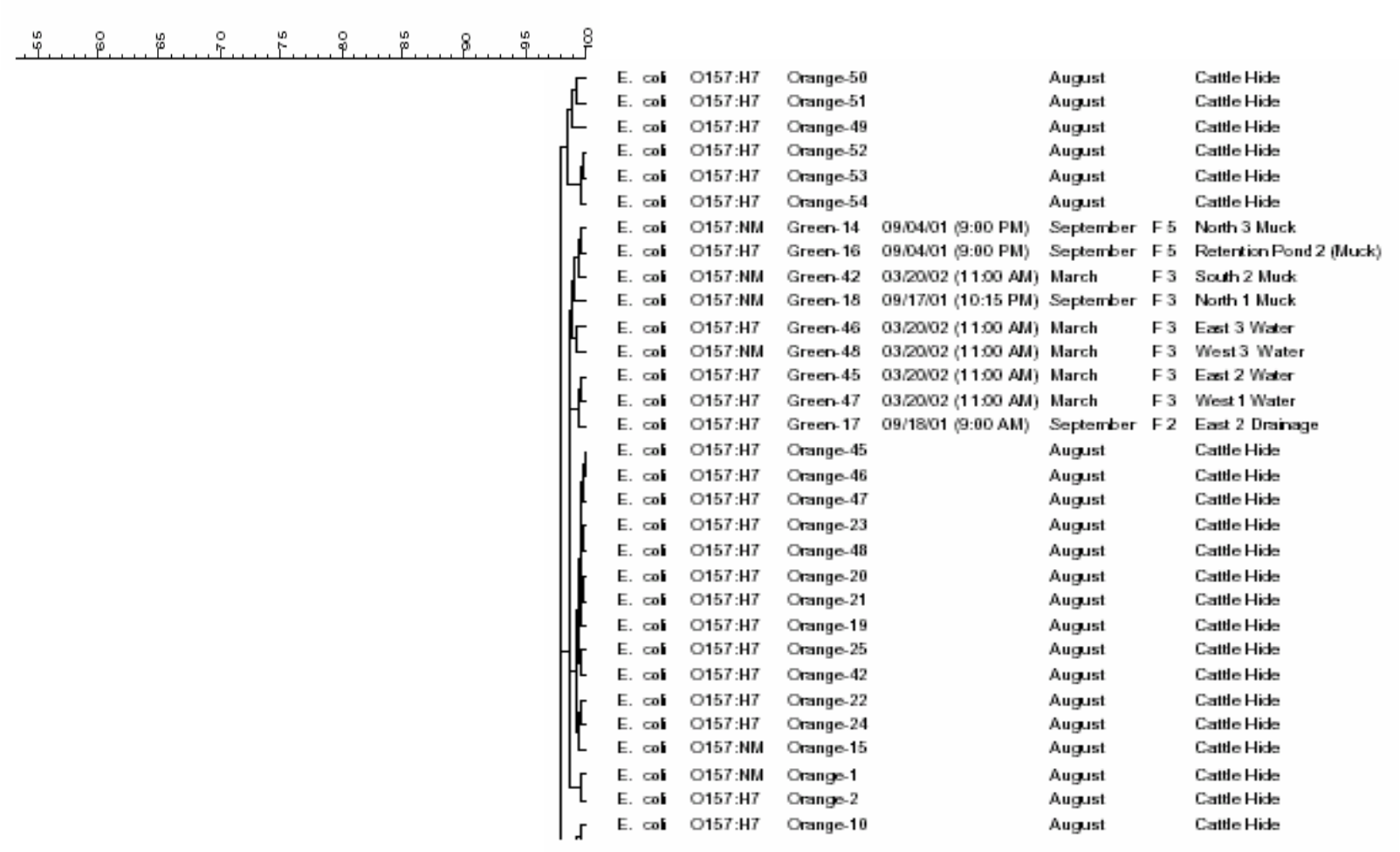

FIG. 21. Uprime-B1 dendrogram representing genetic relationships between *E. coli* O157 isolates based on rep-PCR fingerprints.
* Muck: pond sludge

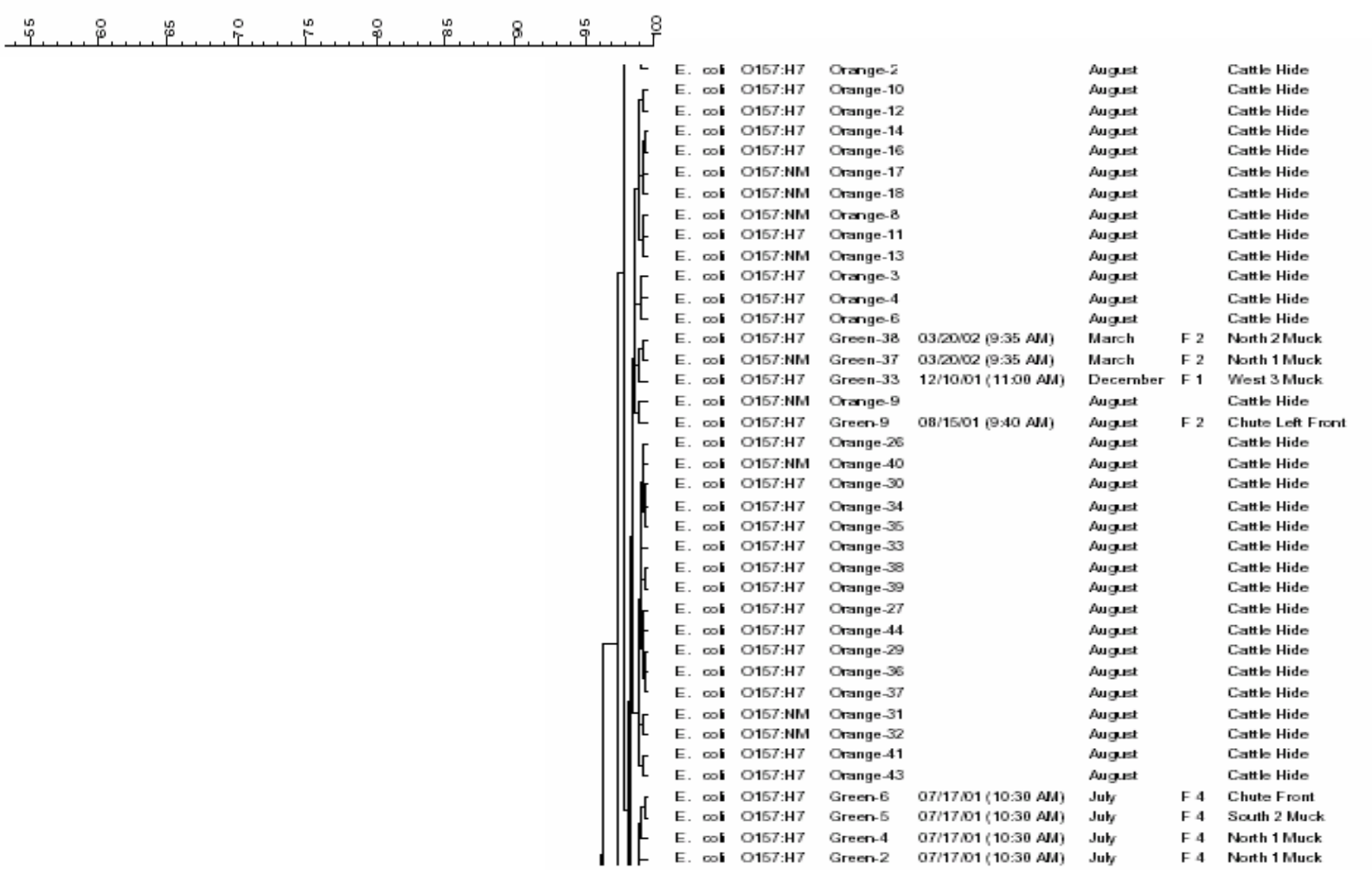

FIG. 21. Continued.

similar, if not indistinguishable, and had a similarity coefficient of 96%. These two isolates were also very closely related to the isolates in clusters 1 and 2 with an average similarity of 93%. Cluster 4 consisted of 5 environmental isolates. In this cluster, isolates from feedlot 2 (n = 3) and feedlot 5 (n = 1) appeared indistinguishable with a similarity coefficient greater than 99%. The *E. coli* O157:H7 isolate from the chute of feedlot 2 was highly similar to the other isolates in this cluster with a similarity coefficient of 95%. The isolates in cluster 4 were highly similar to the isolates in clusters 1 to 3 with an average similarity coefficient of 93%. Cluster 5 consisted of 4 isolates, 3 environmental and 1 hide isolate. These isolates appeared indistinguishable with a similarity coefficient of 98%. Again, these isolates were highly similar to the isolates in clusters 1 to 4, with an average similarity coefficient of 92%. Finally, cluster 6 contains only one *E. coli* O157:H7 isolated from a cattle hide. This isolate appears different from all other isolates in this sample set, with an average similarity of 53%.

Most of the isolates (100/101) obtained from this study had rep-PCR banding patterns closely related to each other. Due to the low potential of detecting identical strains from epidemiologically unrelated sources by chance alone, it is extremely likely that *E. coli* O157 isolates from the cattle production environment, with identical subtypes, are related in the terms of ecology and epidemiology (96, 94). The results of the current study revealed possible point source feedlot contamination and within feedlot transmission and the possibility of more than one source of *E. coli* O157:H7 in each feedlot. As most hide isolates were identified as the same type, it may be considered that rep-PCR may not be sufficiently discriminatory for *E. coli* O157 strains, and a use

of a more discriminatory method for the subtyping of *E. coli* O157 isolates may be advised. Akiba et al. (3) previously described the emergence of closely related PFGE clonal types during bovine colonization. Similarly, Mechie et al. (76) found all strains of *E. coli* O157 isolated throughout 15 month study of *E. coli* O157:H7 in a dairy herd be indistinguishable. However, Akiba et al. (3) and Faith et al. (37) studies found more than one type of *E. coli* O157 strains discovered in the experiment which determined genomic DNA by PFGE. This technique has been used in number of investigations of *E. coli* O157 in cattle environments (37, 51, 70, 91, 96, 103), and is regarded as the "gold standard" for subtyping of *E. coli* O157 in epidemiological studies (34).

Bacterial turnover associated with cattle and the cattle environment may create a specific condition for selected *E. coli* O157. Due to complexity, two previous studies (57, 131) suggested that bacterial turnover associated with rumen development and interactions with other gastrointestinal flora may result in the creation of a niche suitable for the colonization and proliferation of *E. coli* O157 strain acquired from the drinking water microcosm. The current results may suggest clonal spread of the *E. coli* O157 within the feedlots; however, there is the possibility of variation between strains that was not detected by rep-PCR. Similar strains isolated in feedlots indicate a common source. In addition, the current results indicated that *E. coli* O157 isolates may be able to maintain, transmit, and persist within feedlot environments. A similar finding was reported by Renter et al. (94) who determined isolation frequency and persistence of *E. coli* O157 strains from range cattle production environments over an 11-month study. Rice et al. (96), and Shere et al. (103) reported that *E. coli* O157 strains were persistently

isolated from a bovine production environment for up to 2 years. Similarly, Laegreid et al. (68) indicated the maintenance, transmission, and distribution characteristics of *E. coli* O157 subtypes isolated from production environments.

CONCLUSIONS

During the course of this study, *E. coli* O157 was isolated from hide and environmental samples. *E. coli* O157 was most often isolated with peak prevalence during November and March. The most commonly contaminated sites in the feedlot environment were the chute area and sludge from a waste water pond. When environmental factors, such as ambient, water, and pond sludge temperature were low temperature, a decrease in the prevalence of *E. coli* O157 in the feedlot environment was observed. No correlation with other environmental factors, such as liquid levels in the pond and electrical conductivity was observed. Cattle hide has been implicated as one of the major sources of *E. coli* O157. The number of positive hide samples varied widely between days which may reflect different animal husbandry practices between farms. A similar pattern in antibiotic resistance frequencies was detected in *E. coli* O157 isolates, from pond sludge and water samples, while hide isolates had unique antimicrobial resistance. This difference could reflect the intensive use of antibiotics in the cattle for therapeutic and prophylactic purposes, and in some cases as growth promoters to improve cattle production. Most *E. coli* O157 isolates from the feedlot environment and hide had a high prevalence of cephalothin and sulfisoxazole resistance. In addition, multiple antimicrobial resistance to more than 10 antibiotics was observed in *E. coli* O157 isolates from feedlot environment. The increasing prevalence of multiple antimicrobial resistance in the isolates of environmental and hide origins may have occurred due to intensive use of antimicrobial agents. A correlation may exist between

intensive use of antibiotics and increasing antimicrobial resistance in *E. coli* O157; however, there was no prior documentation in this study to support this observation. All strains of *E. coli* O157 isolated throughout this study were closely related with regard to rep-PCR DNA fingerprinting. There is the possibility that variation between *E. coli* O157 strains in this study was not detected by *rep*-PCR, and the use of a more discriminatory method may be advised. However, this finding may demonstrate the possible persistence of this specific strain on the cattle hide, since the majority of hide isolates were closely related and grouped into one cluster. This finding has been supported by previous research showing closely related strains. In addition, recovery of this strain from cattle hide, and from the feedlot environment on multiple visits, may indicate that the maintenance, transmission, and persistence of this strain are enhanced by the cattle production environment. Results obtained from this study may serve to assist in developing strategies for adjusting management practices at feedlots to minimize the potential for contamination of animals with *E. coli* O157:H7.

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