Bacterial microflora of normal and telangiectatic
livers in cattle

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Objective—To identify potential bacterial pathogens in normal and telangiectatic livers of mature cattle at slaughter and to identify consumer risk associated with hepatic telangiectasia.

Sample Population—50 normal livers and 50 severely telangiectatic livers.

Procedure—Normal and telangiectatic livers were collected at slaughter for aerobic and anaerobic bacterial culture. Isolates were identified, and patterns of isolation were analyzed. Histologic examination of all livers was performed.

Results—Human pathogens isolated from normal and telangiectatic livers included Escherichia coli O157:H7 and group-D streptococci. Most livers in both groups contained bacteria in low numbers; however, more normal livers yielded negative culture results. More group-D streptococci were isolated from the right lobes of telangiectatic livers than from the left lobes, and more gram-negative anaerobic bacteria were isolated from left lobes of telangiectatic livers than from right lobes. All telangiectatic lesions were free of fibrosis, active necrotizing processes, and inflammation.

Conclusions and Clinical Relevance—The USDA regulation condemning telangiectatic livers is justified insofar as these livers contain more bacteria than normal livers do; however, normal livers contain similar species of microflora. Development of telangiectasia could not be linked to an infectious process. The finding of E coli O157:H7 in bovine livers suggests that information regarding bacterial content of other offal and muscle may identify sources of this and other potential foodborne pathogens and assist in establishing critical control points for the meat industry. (J Am Vet Med Assoc 2001;219:36–39)

Intrinsic bacteria include those that are found in deep tissues of healthy animals but not pathogens recovered from diseased animals or carriers that do not have clinical signs. There is published evidence to support the hypotheses that normal tissues may either be sterile or commonly contain bacteria. The conflicting reports may be explained by problems with technique, inadequate controls, or sample population differences. Translocated bacteria arrive in deep tissues from other sites. There are a number of mechanisms by which bacteria contaminate deep tissues. Translocation may occur in disease situations, and bacteria translocate from the gastrointestinal tract under a variety of conditions involving diet change, flora overgrowth, immunosuppression, and damage to the gastrointestinal mucosa. In addition, bacteria are translocated to tissues via the use of contaminated captive bolt guns and knives used early in the slaughter process.

Bovine hepatic telangiectasia (BHT) accounts for slightly >10% of all bovine liver condemnations in federally inspected slaughter facilities in the United States. According to USDA data, BHT resulted in condemnation of nearly 23 tons of product in 1997. Current USDA policy concerning the condemnation of telangiectatic livers is based primarily on aesthetics. The lesion is easily diagnosed by inspectors, but its biological importance is undetermined. Although several investigators have studied the histologic features and potential causes of BHT, there is no confirmed toxic or bacterial cause of the condition. The purpose of the study reported here was to identify potential bacterial pathogens in normal and telangiectatic livers of mature cattle at slaughter and identify consumer risk associated with hepatic telangiectasia.

Materials and Methods

Liver specimens—The 100 livers used in this study were collected at a slaughter facility on 5 collection days. Cattle were of beef breeds or beef crosses and were from a variety of locations in Texas, Oklahoma, and Louisiana. Information regarding husbandry of the cattle was unknown. Livers were classified as either normal or telangiectatic by USDA inspectors. After inspection, livers were rinsed with cold tap water and placed in a stainless steel pan that had been wiped with a 70% ethanol-saturated towel. Only telan-
giecatic livers with numerous obvious lesions were collected for bacteriologic culture. For comparison, nontelangiectatic normal livers were collected as control specimens on the same collection day. An equal number of samples of normal and telangiectatic livers were examined on any collection day.

**Collection methods**—On the first 2 collection days, 2 techniques were compared in order to identify the procedure that resulted in the least environmental contamination. The first method was used on the first collection day. Normal livers and livers condemned for diffuse telangiectasia but grossly free of other defects at slaughter were removed from the inspection line. Care was taken to avoid bile contamination of sampled livers. The interval between liver collection and sampling for culture was maintained at ≤ 20 minutes in order to allow maximal recovery of anaerobic bacteria. The parietal capsular surface of the liver was seared directly with a propane torch on the right and left lobes, and a 1-cm² piece of liver tissue was removed aseptically from each site. Specimens were homogenized in a sterile tissue grinder or a sterile mortar and pestle with 2.3 to 3.0 mL of sterile phosphate-buffered saline solution (PBSS). Two 5% sheep blood agar plates (1 aerobic, 1 preduced anaerobic) and 1 MacConkey agar plate were streaked for primary isolation, using a sterile 10-μL plastic loop. After streaking, anaerobic plates were placed into an anaerobic jar with an anaerobic generator. In addition, approximately 0.75 mL of homogenized tissue was added to tryptose broth, brain heart infusion broth (BHI) broth with 5% oxysta, and malachite green mannitol (MM) selenite broth. Tryptose broth and BHI with oxysta are used as enrichment media for recovery of aerobic and anaerobic microorganisms, respectively. The MM selenite broth was used as a selective medium for isolation of Salmonella spp. Specimens from the right and left lobes of the liver were placed into neutral-buffered 10% formalin for histologic study. Environmental contamination was monitored by exposing blood agar plates to air for 30 minutes at intervals throughout the collection day. Pairs of plates were used simultaneously, with 1 placed on the workbench and another on the opposite side of the 20 X 20-ft room used for bacteriologic culture of the specimens. To minimize contamination, gloves were changed between samples.

On the second collection day, the culturing technique was modified in a manner that shortened the interval between collection and culture and reduced manipulations and possible contamination. This second technique was identical to the first, except that homogenizing specimens was not performed. A 1-cm² piece of tissue was placed directly into the broth cultures for enrichment; the liver surface was seared and incised, and a sterile swab was stabbed directly into the liver and streaked onto primary agar plate cultures. Because results were similar to the first method used, and because identical results were obtained when both methods were used in tandem during the third collection, the second technique was used for the fourth and fifth collections. Data from all collection days were included in statistical analyses.

**Identification of aerobic bacteria**—Biochemical tests were conducted to identify all isolates. Initial tests for gram-positive bacteria included: triple sugar iron agar (TSIA), urea agar, blood agar with sodium azide, and salt mannitol agar. Results of these tests were verified by use of gram-positive identification cards. Isolates confirmed as Streptococcus spp underwent further testing for classification into Lancefield groups.

Initial biochemical tests for gram-negative isolates included TSIA, lysine iron agar (LIA), urea agar, motility test media, and tryptophane broth. Results of identification by these tests were confirmed, using analytic growth strips.

The E. coli isolates were subcultured on MacConkey agar with sorbitol, and those organisms that grew on the MacConkey with sorbitol agar and that did not ferment sorbitol underwent testing for the cellular antigen configuration of E. coli O157: H7. All broth cultures were incubated at 37°C for 72 hours. Plates also were held for 72 hours and checked at 24-hour intervals. For those plates with no bacterial growth but with growth in broth culture, another blood agar plate was streaked from the broth culture and monitored every 24 hours for 72 hours.

**Identification of anaerobic bacteria**—Upon achieving isolated colonies, an aerotolerance test was performed on isolates from anaerobic blood agar plates in order to distinguish obligate anaerobes from facultative anaerobes. Facultative anaerobes were characterized, using the same procedures as those used for the aerobically isolated microorganisms. Obligate anaerobes were gram-stained, and a tryptophane degradation test was performed. Subsequently, organisms were identified by use of an aerobe identification card.

**Salmonella spp enrichment and culture**—Bacteriologic cultures of liver specimens for Salmonella spp were performed, using an enrichment broth, MM selenite, and incubation for 18 to 24 hours. At this time, a subculture was made onto xylose lysine desoxycholate (XLD) medium and MacConkey agar. All potential Salmonella isolates underwent a Salmonella spp screen, using TSIA, LIA, and tryptophane.

**Histologic procedures**—After fixation in neutral-buffered 10% formalin, the central portions of individual specimens taken from the right and left lobes adjacent to culture sites were processed for histologic examination. Specimens were paraffin-embedded and stained with H&E.

**Statistical analyses**—Data were analyzed by use of Fisher exact test methodology. Significance was set at P < 0.05. Because many livers had multiple bacterial isolates, 1 to 3 isolates/genera were obtained, and most isolates were organisms with limited pathogenic potential, isolates were grouped for analysis as gram-negative aerobes, gram-negative anaerobes, group-D streptococci, nongroup-D streptococci, E. coli O157:H7, and E. coli non-O157:H7. Because of the public health importance of E. coli O157:H7 and group-D streptococci, data related to these isolates were analyzed separately.

**Results**

**Bacteria**—Specimens from 20 of 50 normal livers and 9 of 50 telangiectatic livers did not yield bacterial growth; this difference was significant (P = 0.02). Seventy-seven isolates were obtained from telangiectatic livers, and 49 isolates were obtained from normal livers. Most isolates were obtained from the enrichment broths. Thirteen of the 100 livers yielded an isolate on primary culture plates, and these consisted of only 3 organisms: E. coli (9 isolates), group-D streptococci (4 isolates), and Clostridium perfringens (1 isolate). Growth of these primary isolates on plates was considered moderate, because there were ≤ 30 colony-forming units.

Differences between normal and telangiectatic livers for the species of bacteria that were isolated were not detected, except that telangiectatic livers yielded more gram-negative anaerobes from the right lobes and more group-D streptococci from the left lobes than did normal livers (Table 1). Aerobic gram-positive isolates consisted of 3 Corynebacterium group JK isolates and 49 isolates were obtained from normal
Discussion

Hepatic telangiectasia is a common lesion of cattle at slaughter, and pathologists consider it to be an incidental finding. Several causes of BHT have been proposed, including vitamin E-selenium deficiency, ischemia induced by bacterial emboli, increased hydrogen sulfide concentration in portal blood, and immune-mediated disease. Regardless of the cause, substantial amounts of bovine products are condemned because of BHT. A primary purpose of this study was to identify potential pathogens associated with BHT that would provide scientific justification for liver condemnation. Although gram stains have been used in numerous studies to detect bacteria in liver lesions, in only 1 previous study was bacteriologic culture of telangiectatic lesions performed. Four affected livers were examined by use of techniques similar to ours, and bacteria were not detected. In our study, 2 human pathogens were isolated. Group-D streptococci are opportunistic human pathogens occasionally associated with urinary tract infections, biliary tract disease, and endocarditis. * Escherichia coli O157:H7 is an important pathogen associated with hemolytic-uremic syndrome. Both pathogens were found in normal and telangiectatic livers, but they were isolated more commonly from livers with telangiectasis. These organisms could pose a threat to food handlers and the general public who come in contact with raw product or consume inadequately cooked product. With the exception of 4 of 37 isolates of group-D streptococci, these organisms were isolated only from enrichment cultures, indicating that they were in low numbers. Although there were more isolates from telangiectatic livers, the total number of isolates of E coli O157:H7 was low (6/50 telangiectatic livers, 1/50 normal livers). The risk presented by these organisms cannot be predicted completely from this study. Because only livers were examined by use of bacteriologic culture in these cattle, it cannot be stated whether liver constitutes a greater threat than other components of the offal or muscle.

Because portal blood flow is divided and directed differently in the liver of some species, bacteria isolated from right and left liver lobes were compared. In telangiectatic livers, gram-negative aerobes were isolated more commonly from the right lobe than from the left lobe, whereas group-D streptococci were isolated more frequently from the left lobe than from the right lobe. Presumably, these organisms originate from the gastrointestinal tract and arrive by translocation via the bloodstream.
The cattle were from a variety of sources, and specimens were collected at 5 time points during a 12-month period; thus, several variables were uncontrolled in this study. The distribution of isolates in normal livers differed from that of telangiectatic livers. Because there were few isolates from both groups of livers, caution in conclusions is warranted. Differences among isolates from normal and telangiectatic livers may merely reflect variation related to the uncontrolled variables in this study.

This study was not designed to address the cause of BHT. Histologic examination was performed to better evaluate the livers in this random population of cattle. Analysis of the histologic lesions supported the belief that BHT results from a necrotizing process that occurs sporadically and does not induce enough hepatocellular necrosis to trigger regeneration or scarring. The mild bile duct regeneration seen in some livers was interpreted to be the result of unrelated processes and was not consistently associated with telangiectasis. Results of this study provide no evidence for a relationship between local hepatitis (sawdust liver) and BHT.1 No instances of sawdust liver were seen grossly (more than 500 livers were observed grossly on each collection day) or histologically. If hepatitis preceded BHT, one would expect to see typical gross or microscopic necrotic lesions; however, telangiectatic lesions, whether large or small, lacked inflammation and immune-cell infiltration. It could be argued, however, that because all lesions observed were quiescent and, therefore, longstanding, the necrotic cells characteristic of sawdust liver had been removed. Lesions interpreted to be similar to telangiectasis have been induced experimentally, using portal vein inoculation of *Haemophilus somnus*; however, the culture techniques used in our study were capable of culturing *H somnus*, and that organism was never isolated. Also of interest is the low recovery of *Fusobacterium* spp (4 isolates from normal livers and 8 isolates from telangiectatic livers). It has been suggested that telangiectatic lesions may provide a microenvironment for *Fusobacterium* spp to grow, favoring formation of hepatic abscesses. The data do not strongly support the belief that hepatic abscesses result commonly from spontaneous growth of *Fusobacterium* organisms in foci of telangiectasia in bovine livers. Indeed, the pathogenesis of bovine liver abscesses involves showering of livers by organisms released through a damaged rumen mucosa, which is seen infrequently at slaughter in healthy range cattle.

Results of this study indicate that bovine livers collected at slaughter may harbor small numbers of intrinsic bacteria and potential human pathogens. Although only 100 livers were examined in this study, results indicate that contamination of telangiectatic livers may have some scientific basis, because these livers had bacterial contamination in excess of that seen in normal livers. An unexpected finding was the multiple isolations of *E coli* O157:H7. It is believed that *E coli* O157:H7 in meat at slaughter comes from external contamination. Results of experimental infection indicate that *E coli* O157:H7 in the gastrointestinal tract of cattle do not routinely translocate to deep tissues.11 Knowledge regarding bacterial contamination of tissues other than liver, possible influences of age and diet, and possible involvement of other pathogens are as yet unknown.

References


