Horizontal Transmission of Escherichia coli O157:H7 during Cattle Housing

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ABSTRACT

Ruminant livestock, particularly cattle, is considered the primary reservoir of Escherichia coli O157:H7. This study examines the transmission of E. coli O157:H7 within groups of cattle during winter housing. Holstein Friesian steers were grouped in six pens of five animals. An animal inoculated with and proven to be shedding a marked strain of E. coli O157: H7 was introduced into each pen. Fecal (rectal swabs) and hide samples (900 cm² from the right rump) were taken from the 36 animals throughout the study. Water, feed, and gate or partition samples from each pen were also examined. Within 24 h of introducing the inoculated animals into the pens, samples collected from the drinking water, pen barriers, and animal hides were positive for the pathogen. Within 48 h, the hides of 20 (66%) of 30 cohort animals from the six pens were contaminated with E. coli O157:H7. The first positive fecal samples from the noninoculated cohort animals were detected 3 days after the introduction of the inoculated steers. During the 23 days of the study, 15 of 30 cohort animals shed the marked E. coli O157: H7 strain in their feces on at least one occasion. Animal behavior in the pens was monitored during a 12-h period using closed circuit television cameras. The camera footage showed an average of 13 instances of animal grooming in each pen per hour. The study suggests that transmission of E. coli O157:H7 between animals may occur following ingestion of the pathogen at low levels and that animal hide may be an important source of transmission.

The foodborne pathogen Escherichia coli O157:H7 poses a significant health risk to consumers and, consequently, a considerable economic threat to the beef industry. Serious human illnesses, including bloody diarrhea, hemolytic uremic syndrome, and in some cases death, have been attributed to this organism since it was first reported in 1982 (17). Consumption of beef and beef products contaminated with E. coli O157:H7 is a major cause of human infection worldwide (15, 17). However, more recently, numerous outbreaks have been reported due to direct contact with animals and animal feces (11, 12, 19, 21, 23). Cattle are regarded as the primary reservoir of E. coli O157:H7, with fecal prevalence rates of 2 to 24% being reported (16, 29). Colonization of the gastrointestinal tract appears to be transient, with typical shedding patterns involving long periods of low-level shedding punctuated by short periods of high-level shedding (2, 28).

It is generally accepted that the greatest capacity for pathogen control is through the implementation of an integrated farm-to-fork approach. Several strategies that aid the reduction of E. coli O157:H7 carcass contamination have been introduced during the slaughter process, such as steam pasteurization and organic acid washes (15). In contrast, control strategies are not so advanced at the farm level, where the primary sources of E. coli O157:H7 are found. This is mainly due to an insufficient understanding of the ecology of the pathogen on the farm, despite more than 20 years of research. Although an abundance of studies have examined the prevalence of E. coli O157:H7 in the feces of cattle of different type (beef, dairy), age, and regions, few studies specifically examine the modes of transmission of the organism between cattle under natural conditions. Several studies have looked at the transmission of verotoxigenic E. coli among small groups of calves or sheep under artificially controlled conditions (3, 7, 30, 36); however, this may not adequately represent cattle-to-cattle transmission on the farm. The objective of this study was to define the principal means of transmission of E. coli O157:H7 among adult cattle and to elucidate the role of the environment in its spread.

MATERIALS AND METHODS

Organism. A bovine fecal E. coli O157:H7 (VC 047) strain, isolated during a survey of an Irish cattle feedlot, was used throughout the study (29). The strain was negative for both vt1 and vt2 genes but possessed the genes that encode enterohaemolysin A and eae, as determined by PCR. To aid detection, the strain was made resistant to streptomycin sulfate (1,000 mg l⁻¹) and nalidixic acid (50 µg ml⁻¹) as described by Park (31) and stored on cryoprotective beads (Technical Services Consultants, Lancashire, England) at −20°C.

Description of the animals and housing facilities. Thirty-six Holstein Friesian steers, aged 6 to 9 months, were selected by weight (289 ± 21 kg) and randomly assigned to one of two treatment groups (groups A and B). Group A was divided into three pens, each containing five animals, and one pen of three animals (to be used for inoculation). The animals were adapted to an ad lib barley-based concentrate diet for 3 weeks. Group B was di-
Preparation of inoculum. One bead that contained *E. coli* O157:H7 was aseptically transferred into 9 ml of brain heart infusion broth (Oxoid, Basingstoke, UK) and incubated at 37°C for 24 h. From this culture, 1 ml was transferred into 100 ml of brain heart infusion broth and incubated at 37°C for 18 h to achieve a stationary-phase culture. The culture was centrifuged at 3,000 × g for 10 min (Eppendorf Centrifuge 5403, Eppendorf, Hamburg, Germany) and washed three times in maximum recovery diluent (MRD; Oxoid), and the resultant pellet was resuspended in 10 ml of MRD. A 1-ml aliquot of this suspension, containing approximately 10^{10} CFU of *E. coli* O157:H7, was added to 50 ml of sterile distilled water.

Inoculation procedure. Six animals (three on the concentrate diet and three on the silage diet) were inoculated orally by syringe (Plastitek 60 ml, Becton Dickinson, Oxford, UK) with 50 ml of sterile distilled water that contained approximately 10^{10} CFU of *E. coli* O157:H7. Immediately after inoculation, each animal was dosed with 1 liter of sterile distilled water to wash down the culture. Fecal samples from the six inoculated animals were examined 24 h after inoculation for the presence of the marked strain, using the methods described below. All six animals were shedding the marked organism. One animal shedding *E. coli* O157:H7 was then placed into each of the six pens of five uninoculated steers consuming the same diet. The inoculated animal (876) from pen 2 in the concentrate group (Table 1) escaped from its pen on day 20 of the experiment. It was omitted from the experiment for the final 3 days.

Enumeration of *E. coli* O157:H7. Different collection and initial processing procedures were used for each sample type and are described in detail below. However, a common procedure for direct plating and enrichment of *E. coli* O157:H7 was used for all samples. To determine direct counts of *E. coli* O157:H7 present in each sample, aliquots were plated in duplicate onto sorbitol MacConkey agar (SMAC; Oxoid) that contained nalidixic acid (50 μg/ml) and streptomycin sulfate (1,000 μg/ml) (SMAC-nas) and incubated at 37°C for 48 h. Aliquots were also plated onto tryptone soy agar (Oxoid), incubated at 37°C for 2 h, overpoured with SMAC-nas, and reincubated for a further 48 h to allow for the recovery of injured cells. The direct counts reported throughout the paper were derived using the latter method. Samples for enrichment were incubated for 24 h at 37°C without agitation, then plated onto SMAC-nas, and reincubated at 37°C for 48 h. For all samples, identification of suspected positive colonies was confirmed by latex agglutination (Wellclex *E. coli* O157:H7, Murex, Dartford, UK). In addition, colonies from positive samples selected at random were confirmed by PCR, as previously described (32).

Sampling procedure. To avoid cross-contamination among pens during sampling, separate disposable overalls and shoe covers were worn in each pen. Latex gloves were worn and changed following collection of each sample. All samples were placed on ice immediately after collection and transported to the laboratory within 2 h.

### TABLE 1. Detection of Escherichia coli O157:H7 in fecal samples of inoculated cattle on two different diets during a 25-day period

<table>
<thead>
<tr>
<th>Pen no.</th>
<th>Animal identification no.</th>
<th>Concentrate diet</th>
<th>Grass silage diet</th>
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<tbody>
<tr>
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<td>-1^a</td>
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<td>1</td>
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<tr>
<td>1</td>
<td>608</td>
<td>5.3^b</td>
<td>3.7</td>
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<td>2</td>
<td>876</td>
<td>6.2</td>
<td>2.7</td>
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<tr>
<td>3</td>
<td>898</td>
<td>5.9</td>
<td>4.2</td>
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**Counts of *E. coli* on day:**

|    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
|    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |

^a Time −1 indicates 24 h after inoculation. Time 0 indicates 48 h after inoculation when each inoculated animal was placed in a pen of uninoculated cohorts.

^b Direct counts of *E. coli* O157:H7 in log g⁻¹ (detection limit, log 1.4 CFU g⁻¹).

^c Positive samples detected by enrichment only (detection limit, log 0.95 CFU g⁻¹).

^d *E. coli* O157:H7 not isolated from the sample.

^e NS, not sampled.
TABLE 2. Escherichia coli O157:H7 fecal shedding patterns of cohort animals (assigned individual tag numbers) during a 23-day period following natural infection from exposure to an experimentally inoculated pen mate.

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<th>Pen no.</th>
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<td>44&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Grass silage diet</td>
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<td>11</td>
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</table>

<sup>a</sup> Time 0 indicates when an inoculated animal was placed in a pen of unoinoculated cohorts.

<sup>b</sup> E. coli O157:H7 not isolated from any cohort animal.

<sup>c</sup> Identification of animal when positive samples were detected by enrichment (detection limit, log 0.95 CFU g<sup>-1</sup>.

**Fecal samples.** Fecal samples (approximately 0.1 g) were obtained from the rectum of each animal using two cotton-tipped swabs (Bibby Sterilin, Staffordshire, UK) inserted simultaneously while the animal was eating at the feed barrier. Following withdrawal, individual swabs were immediately placed into two sterile 30-ml tubes (Sterilin) that contained 5-ml volumes of modified tryptone soya broth (mTSB; Oxoid) plus nalidixic acid (50 µg/ml) and streptomycin sulfate (1,000 µg/ml) (mTSB-nas). On return to the laboratory, each tube was vortexed for 1 min. A dilution series in 9-ml volumes of MRD was performed from one sample before direct plating. The duplicate swab was enriched at 37°C for 24 h. The detection limit for direct counts and enriched fecal samples was log 1.4 CFU g<sup>-1</sup> and log 0.95 CFU g<sup>-1</sup>, respectively. This was calculated by spiking feces in the laboratory with known numbers of E. coli O157:H7 (VC 047). Fecal samples were taken on days 0, 1, 2, 3, 4, 7, 9, 10, 11, 14, 15, 16, 21, 22, and 23.

**Hide samples.** Hide samples, approximately 900 cm<sup>2</sup>, were taken from the right rump area of each animal, using individual sterile cellulose sponges (Sydney Heath & Son Ltd., Staffordshire, UK), moistened with 10 ml of MRD, and placed into sterile stomacher bags (Seward Laboratory, London, UK). On return to the laboratory, sponges were placed in 90 ml of mTSB-nas and stomached in a Colworth stomacher (model BA 6024, A. J. Steward & Co. Ltd., London, UK) for 30 s. Samples were direct plated on days 0, 1, 2, 3, 4, 7, 9, 10, 11, 14, 15, 16, 21, 22, and 23.

**Water troughs.** Sterile 150-ml containers (Sterilin) were used to remove 25-ml samples from the water trough of each pen. On return to the laboratory, samples were filtered through a 0.2-µm pore-size filter (Sartorius, Goettingen, Germany). The filter was placed in 20 ml of mTSB-nas and vortexed for 1 min. All samples were plated directly and enriched on days 1, 2, 3, 4, 7, 9, 14, and 23. The limit of detection for direct counts was -0.4 log ml<sup>-1</sup>. Sediment samples, approximately 10 g, were taken from each water trough on day 7 and added to 90 ml of mTSB-nas before enrichment.

**Feed samples.** Samples of feed, approximately 50 g of both silage and concentrates, were collected from each pen on day 3 and placed in sterile 150-ml containers (Sterilin). Samples were added to 200-ml volumes of mTSB-nas, stomached for 1 min, and enriched.

**Pen barrier.** Unlimited areas of wooden partition and metal feed barriers from each pen were swabbed using the sponge method described above. The sponge was added to 90 ml of mTSB-nas and stomached for 1 min before enrichment. Samples were taken on days 1, 2, 4, 7, and 23.

**Overshoes.** On exiting the pens after sampling, disposable overshoes were removed and placed in a sterile stomacher bag. On return to the laboratory, 90 ml of mTSB-nas was added to each bag, stomached for 1 min, and then enriched. Overshoes were sampled on day 9.

**RESULTS**

All animals remained healthy for the duration of the experiment. The fecal shedding patterns of the six inoculated animals are presented in Table 1. At 24 h after inoculation, the three inoculated animals consuming the concentrate and silage diets shed the marked E. coli O157:H7 at concentrations of 5.3 to 6.2 log g<sup>-1</sup> and 4.5 to 5.1 log g<sup>-1</sup>, respectively. When these inoculated animals were introduced to the six pens (48 h after inoculation), each containing five uninoculated cohorts, E. coli O157:H7 was detected in the inoculated animals’ feces at concentrations of 2.7 to 4.2 and 3.8 to 4.0 log g<sup>-1</sup> for the concentrate- and silage-fed animals, respectively. During the following 23 days, positive E. coli O157:H7 fecal samples were detected sporadically from the six inoculated animals (Table 1).

Table 2 presents the fecal shedding pattern of the cohort animals from the six pens. Three days after the introduction of the inoculated animals, two of five cohort animals from one of the concentrate pens had fecal samples positive for the marked organism. Within 4 days at least one cohort animal tested positive in each of the six pens. Over the 23 days of the study, 9 of 15 cohort animals from the concentrate treatments and 6 of 15 from the silage treatments tested positive at least once for the E. coli O157:H7 isolate. Generally, the cohorts did not shed the organism on consecutive sampling days. Three of nine cohort animals on the concentrate diet tested positive on two or more sampling occasions. One cohort animal tested positive on days 3, 16, 21, and 22. Two of six cohort animals on the silage diet tested positive on two occasions.
TABLE 3. Positive Escherichia coli O157:H7 hide and environmental samples recovered during a 23-day period following the introduction of 1 experimental inoculated animal into six pens of five uninoculated cohort animals

<table>
<thead>
<tr>
<th>Pen no.</th>
<th>Concentrate diet</th>
<th>Grass silage diet</th>
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<tr>
<td></td>
<td>Positive samples recovered on day:</td>
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<td>1</td>
<td>H (6/6)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>H (4/6)</td>
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<td>1</td>
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<td>H (5/6)</td>
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<td>2</td>
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<td>H (2/6)</td>
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</tbody>
</table>

<sup>a</sup> H, hide; W, water; P, pen.
<sup>b</sup> Time 1, 24 h after an inoculated animal was placed in a pen of uninoculated cohorts.
<sup>c</sup> Number of hide samples positive for E. coli O157:H7. Hides sampled on days 1, 2, 4, 7, 10, 15, and 23.
<sup>d</sup> No positive samples on this day.
<sup>e</sup> E. coli O157:H7 detected on pen barrier. Samples taken on days 1, 2, 4, 7, and 23.
<sup>f</sup> E. coli O157:H7 detected in water. Samples taken on days 1, 2, 3, 4, 7, 9, 14, and 23.

E. coli O157:H7 was detected only by enrichment (0.95 to 1.4 log g<sup>–1</sup>) in 13 of the 15 positive cohort animals. One cohort animal in the concentrate treatment shed the marked organism at 4.2 and 3.8 log g<sup>–1</sup> on days 21 and 22, respectively, having previously tested positive by enrichment on days 3 and 16. Another cohort animal in a separate pen but on the same diet shed the organism at 3.0 log g<sup>–1</sup> on one occasion.

Table 3 summarizes the results for hide, water, and pen barrier samples. On introduction to the pens (48 h after inoculation), the hide of five of six inoculated animals was positive for the organism, at levels ranging from 1.2 to 4.0 log cm<sup>2</sup>. Within 24 h in pen 1 of the concentrate group, all five cohort animals had positive hide samples. After 48 h, 20 of 30 hides of cohort animals were positive for the marked E. coli O157:H7. On that day, direct counts of the organism were calculated for the 36 animals, with low levels ranging from −0.82 to −0.20 log cm<sup>2</sup> detected. Throughout the experiment, at least one positive hide sample was detected from 14 of 15 and 12 of 15 cohorts on the concentrate and silage treatments, respectively.

Two of three water troughs in pens that housed the animals fed concentrates were positive 24 h after the introduction of the shedding animals to the pens. All three water troughs from animals fed silage diets had at least one positive sample by day 4. Water samples were positive following enrichment (≤−0.4 log ml<sup>−1</sup>), with the exception of pen 3 from the concentrate treatment in which concentrations of 1.4 log ml<sup>−1</sup> were detected on day 1.

On exiting each pen on day 9, overshoes were examined for E. coli O157:H7. Two of three and one of three samples were positive from the concentrate and silage diet pens, respectively, following enrichment. Feed samples taken from each pen on day 3 were negative. Data from the video recording of cattle behavior were collected during a 12-h period, because the video quality was too poor to analyze during the night. The average number of times per hour that an animal groomed another in any pen was 12.5 (range, 0 to 27 times an hour). The average ambient temperature recorded in the shed throughout the study period was 5.4°C (range, 0 to 12.4°C).

DISCUSSION

E. coli O157:H7 has been isolated from multiple sources on the farm (16, 18, 33), with some studies citing the surface of pens, animal feed, and water in particular as important sources for transmission of the pathogen (7, 16, 33, 35). In the present study, the introduction of the animals inoculated with E. coli O157:H7 caused rapid contamination of the environment, with the first positive pen barrier and water samples detected within 24 h. However, although contamination occurred rapidly, the number of positive samples from these sites decreased as the study progressed. Feed from each pen was sampled on one occasion and was negative, although multiple sampling may have detected a positive sample (13). To investigate the potential for animal handlers to act as vectors of E. coli O157:H7 among groups of cattle, overshoes were sampled on exiting the pens, with three positive samples detected. Other studies have implicated animal handlers in the transmission of verotoxigenic E. coli among groups of animals (7). In the present study, this mode of transmission was prevented, because access to the pens was prohibited except on sampling days, when disposable clothing and overshoes were worn.
Although extensive environmental sampling has been performed in many other studies, sampling of the animal hide is often not done or only examined at time of slaughter (1, 15, 37). The results of the current study showed that in some pens all hides were positive for E. coli O157:H7 24 h after the introduction of the inoculated animal, proving that one animal shedding E. coli O157:H7 has the potential to contaminate the hides of several cohorts (25). It was also observed that on seven different sampling days E. coli O157:H7 was detected on the hide of at least one animal in a pen even though all fecal cultures from that pen were negative on that day. This is in agreement with findings of surveys in which E. coli O157:H7 was present in 5.9% of cattle fecal samples and 60.6% of hide samples (1). These findings suggest that E. coli O157:H7 contamination of the hide cannot be accurately interpreted from fecal sample results. Because animal hide is regarded as a major source of pathogens on beef carcasses (1, 15, 34), it would seem prudent to determine the prevalence of E. coli O157:H7 on hides in addition to feces to estimate the potential risk of carcass contamination.

The fact that some cohort animals began to shed the pathogen in the latter days of the study, when water and pen barrier samples were negative, suggested that the hide may be an important source of the pathogen for the animals. The results from the camera footage suggested that the time spent on social grooming (average of 13 instances an hour through all pens) was sufficient to ingest E. coli O157:H7 present in small amounts of fecal matter on the hide, thus causing colonization of the cohort animals. Therefore, it is reasonable to assume, particularly in housed animals where a greater level of animal grooming would be expected, that the hide may be one of the most significant sources of animal-to-animal transmission.

The infective dose of E. coli O157:H7 for cattle has been the subject of many experiments. Generally, in animal inoculation experiments cattle are inoculated with high levels of E. coli O157:H7 (10 log CFU) to achieve colonization, and it has been assumed that these levels of laboratory-grown strains of E. coli O157:H7 are the infective dose for cattle on the farm. It is reported that when experimental inoculation doses are reduced below this level, variable success rates in animal colonization are achieved. Cray et al. (9) showed that inoculation with doses of 7 log CFU of E. coli O157:H7 was insufficient to infect all cattle, whereas no shedding was recorded from five cattle inoculated with 4 log CFU. Shere et al. (36) inoculated calves at 6 log CFU but did not cause shedding in all calves despite repeated doses. In this study, the cohort cattle began to shed the organism when fecal shedding from inoculated steers was detectable only by enrichment or when E. coli O157:H7 was found solely on the hide. Given the low numbers of the organism present on the hides, the inoculation dose of E. coli O157:H7 ingested by the cohort animals was low, probably in the region of 2 to 3 log CFU. Horizontal transmission of E. coli O157:H7 has been previously reported in sheep when the animals were shedding less than 4 log CFU/g of feces or at levels only detectable by enrichment (8, 26). Elsewhere, in poultry the colonization potential of a laboratory Campylobacter isolate was increased 10,000-fold following a single passage in vivo (6). The results from these studies suggest that passage of a pathogen through the digestive tract may enhance its ability to colonize the species concerned when compared with a laboratory-grown strain. Consequently, significantly lower numbers of E. coli O157:H7 may be sufficient to colonize animals under natural conditions on the farm. This would not be unexpected given that the ingestion of 10 log CFU E. coli O157:H7 from the farm environment would be virtually impossible. Therefore, the form of the inoculum used in experimental inoculation studies (i.e., laboratory-grown or naturally occurring bacteria) may warrant further investigation.

The effects of diet on the fecal shedding patterns of animals colonized with E. coli O157:H7 have been the subject of much debate. Some studies have suggested that hay-fed cattle shed E. coli longer and in higher numbers than if fed on grain (20, 27), whereas others have suggested the opposite is true (14, 24). In this study, six and nine cohorts on the silage and concentrate diets, respectively, shed the organism in their feces on at least one occasion. It was also observed that one inoculated animal fed the silage diet ceased shedding after day 6, whereas a cohort animal on the concentrate diet commenced shedding E. coli O157:H7 in high numbers toward the end of the study. A definitive conclusion regarding the effect of diet on fecal shedding patterns cannot be established due to the small number of animals involved in this study. Large sample sizes would be required to allow for variations in animal shedding patterns, which are influenced by factors such as microbial flora, age, and immune status (10, 22, 38).

Feed withdrawal commonly occurs in beef cattle as a result of time spent during transport and in lairage before slaughter. The present study found no clear effect of fasting on E. coli O157:H7 shedding, which is similar to previous studies (4, 9, 26, 29). This is in contrast to other bacteria such as Salmonella spp., where a fasting period leads to a marked increase in fecal shedding of this organism (5). It is therefore unclear whether fasting of cattle will lead to an increased incidence of E. coli O157:H7 in the feces and subsequent carcass contamination.

This study has provided further insight into the ecology of E. coli O157:H7 on the farm. It was observed that cohort animals began to shed E. coli O157:H7 in their feces when the pathogen was being shed at very low levels by their inoculated pen mate or was present solely in the pen environment. The study suggests that the contamination of animal hide, in conjunction with animal grooming, may be an important source of E. coli O157:H7 for cattle, particularly if the infectious dose is lower than previously thought. Further work is necessary to establish the dose required to infect cattle with E. coli O157:H7 having passed through the animal as opposed to with laboratory-grown cultures.

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