

Research Article

Identifying the Shedding Patterns of Wildlife *Escherichia coli* O157:H7 in Experimentally Inoculated Neonatal Jersey Calves

Xunde Li^{1,2}, Elizabeth M Antaki Zukoski^{1,2}, Tran HB Nguyen¹, Bruce R Hoar³, Michele T Jay Russell^{2,4}, and Edward R Atwill^{1,2*}

¹Department of Population Health and Reproduction, University of California, USA

²Western Institute for Food Safety and Security, University of California, USA

³College of Agriculture and Natural Resources, University of Wyoming, University, USA

⁴Western Center for Food Safety, University of California, USA

*Corresponding author

Edward R Atwill, Department of Population Health and Reproduction, University of California, Davis, One Shields Avenue, Davis, CA, USA 95616; Tel:5307542154; Email: ratwill@ucdavis.edu

Submitted: 06 February 2020

Accepted: 24 February 2020

Published: 28 February 2020

ISSN: 2379-948X

Copyright

© 2020 Li X, et al.

OPEN ACCESS

Keywords

- *Escherichia coli* O157:H7
- Wildlife
- Calves
- Neonatal
- Dose-dependent

Abstract

Cattle are considered to be a major reservoir host of Shiga-toxin producing enterohemorrhagic *Escherichia coli* O157:H7. Contaminated foods of bovine origin that have been exposed to bovine or wildlife *E. coli* O157:H7 isolates are important vehicles of human infections, which can lead to the deadly hemolytic uremic syndrome (HUS). The objective of this study was to determine possible dose-dependent pathogen shedding patterns of a wildlife *E. coli* O157:H7 strain in neonatal calves. We inoculated six calves, 1-2 day old calves, with either 10⁸ or 10⁹ CFU and collected fecal samples for 11 days post-inoculation to determine the shedding patterns of the inoculated *E. coli* O157:H7. Calves were then euthanized, with tissues collected for culture. One calf inoculated at 10⁸ CFU and both calves inoculated at 10⁹ CFU became infected. The calf inoculated at 10⁹ CFU, which shed the *E. coli* O157:H7 isolate for the entire 11 days post-inoculation, had a wider distribution of colonized tissues compared to the other 10⁹ CFU and 10⁸ CFU inoculated calves. We conclude that the wildlife strain has a dose-dependent shedding pattern with a calculated infectious dose (ID50) of 10⁸ CFU.

ABBREVIATIONS

EHEC: Enterohaemorrhagic *Escherichia coli*; *E. coli* O157:H7: *Escherichia coli* O157:H7; Stxs: Shiga toxins; HUS: Hemolytic Uremic Syndrome; A/E: Attaching/Effacing; ID50: 50% Infectious Dose; BHI: Brain Heart Infusion; PBS: Phosphate Buffered Saline; LB: Luria Base; eaeA: Intimin; HlyA: Hemolysin A; IACUC: Institutional Animal Care and Use Committee; TRACS: Teaching and Research Animal Care Services; RAMS: Rectoanal Mucosal Swabs; TSB: Tryptic Soy Broth; IMS: Immunomagnetic Separation; PFGE: Pulsed Field Gel Electrophoresis

INTRODUCTION

Escherichia coli are a Gram-negative, rod-shaped bacterium of the Enterobacteriaceae family. Most *E. coli* strains colonize the gastrointestinal tract of humans and mammals as part of the normal flora. However, some can evolve into pathogenic strains by acquiring virulence factors through plasmids, transposons, bacteriophages, and/or pathogenicity islands. These pathogenic *E. coli* are characterized by serogroups, pathogenicity mechanisms, clinical symptoms, or virulence factors [1,2]. One major pathogenic group of *E. coli* strains are the enterohaemorrhagic *Escherichia coli* (EHEC), which can produce Shiga toxins (Stxs) and cause non-specific diarrhea, hemorrhagic colitis, and

potentially life-threatening hemolytic uremic syndrome (HUS) in humans. The occurrence of massive outbreaks of Shiga toxin-producing *E. coli* infections, especially resulting from the serotype O157:H7 and the risk of developing HUS (the primary cause of acute kidney failure in children in many developed countries) make this pathogen a public health problem of serious concern [3]. Sources of human infection include contaminated foods, primarily ground beef, raw milk, lettuce, spinach, and fresh fruits, as well as waterborne transmission in rural areas through contact with feces from infected animals [4,5].

The natural reservoir of *E. coli* O157:H7 is in the intestinal tracts of animals, particularly cattle and small domesticated ruminants, like sheep and possibly goats. It can also be carried by pigs, poultry, other domestic farm animals, birds, insects, and wildlife, such as rodents and white-tailed deer. Prevalence within wildlife carriers can be low, but can provide a resource for transmission within local areas and distant geographic locations [6-8]. Ferens and Hovde, 2011, found *E. coli* O157:H7 in 11.4% of cattle (n=1407), 1.2% of swine (n=1102), 3.6% of sheep and goats (n=364), and in 5.2% of 154 fly pools, where some isolates from cattle, swine, and flies shared indistinguishable subtypes of the bacteria. In another study, 1,071 wild rodents were trapped from October 2009 to August 2011 in the Salinas Valley region to

determine the fecal prevalence and risk factors associated with the occurrence of *E. coli* O157:H7, *Salmonella* spp., *Cryptosporidium* spp., and *Giardia* spp. within agricultural production systems. It was determined that 0.2% of the fecal samples collected from the wild rodents were positive for *E. coli* O157:H7 [9].

There has been extensive work using animal models to better understand the dynamic pathogenic mechanisms of EHEC, especially with regards to serotype O157:H7. It has been shown that neonatal animals, especially calves, are the susceptible age class for these types of diseases and symptoms, which include diarrhea, hyperemia, fibrinous exudates, enteritis, and the formation of attaching/effacing (A/E) lesions within the intestinal tissues. In contrast, older ruminants have transient watery diarrhea, but are not seriously affected by an *E. coli* O157:H7 infection. It is thought that *E. coli* O157:H7 primarily colonizes the rectoanal junction in cattle, which is then responsible for the persistent shedding pattern. The capacity for persistence with episodic shedding has been seen in studies, but there also can be a significant detection problem since the infected animals can be asymptomatic [10-13].

To reduce the risk of *E. coli* O157:H7 transmission from animals to humans, it's necessary to determine if the bacterium's pathogenesis differs among various wildlife strains. Here, we examined the shedding patterns among neonatal Jersey calves inoculated with a wildlife *E. coli* O157:H7 strain isolated from an agricultural production system. Our objective was to determine dose-dependent characteristics in the host clinical response correlated with isolation in daily fecal excretion and tissue-based cultures.

MATERIALS AND METHODS

Standard growth curve of bacterial strain

The wildlife *Escherichia coli* O157:H7 strain was isolated from a deer mouse (*Peromyscus maniculatus*) November 2009 in Monterey County, CA on a spinach produce ranch. Three growth curves were made by incubating a bead of stock inoculum, taken from -80°C frozen banks (Pro-lab, Richmond Hill, Ohio, USA), in 150mL of brain heart infusion, BHI (Sigma-Aldrich, St. Louis, Missouri, USA) broth. Each broth was incubated, plated, and original concentrations were calculated as previously described by Antaki-Zukoski et al., 2018 [14]. The *E. coli* O157:H7 strain was also evaluated for both Shiga toxins, intimin (*eaeA*), and hemolysin A (*HlyA*) virulence genes using an endpoint analysis (Eppendorf, Hauppauge, New York, USA) according to the PCR protocol [3] (data not shown).

Ethics statement

All animal experiments were conducted under the approval by the Institutional Animal Care and Use Committee (IACUC) at the University of California Davis Animal Care and Use program (AUP #15459). Experimental procedures followed the federal guidelines outlined in the "Animal Welfare Act" and "Health Research Extension Act", where personal protective equipment, standard operating protocols, daily cleaning/observation/animal enrichment, and sedation/euthanasia/necropsy methods were described in detail for the review and approval by the committee.

Animal experiments

Six male calves 1-2-days-old that had received colostrum were purchased from an approved dairy. Each calf was housed individually in the biosafety level-II animal facility at the Teaching and Research Animal Care Services (TRACS). The calves were fed commercial milk replacer without antibiotics twice a day and given free access to water. Bedding material was removed while pens were bleached and rinsed daily, to ensure fresh fecal collection. Upon arrival, each calf was deemed healthy by clinical examination and hematological analysis with a serum chemistry panel and complete blood count. Additionally, each calf was tested for the presence of *Cryptosporidium* spp. with an acid-fast stain performed on feces. In order to confirm that calves were not colonized with *E. coli* O157:H7, fresh rectal fecal samples and rectoanal mucosal swabs (RAMS) were collected for three consecutive days prior to inoculation with the wildlife *E. coli* O157:H7 [15]. Ten grams of each fecal sample was measured into 90mL of Tryptic Soy Broth (TSB) (Sigma-Aldrich, St. Louis, Missouri, USA), while the RAMS were placed in 50mL of TSB. The samples were incubated at 25°C for 2 hours, 42°C for 8 hours followed by immunomagnetic separation (IMS) using anti-O157 antibodies (Dynal Inc, Camarillo, California, USA) and cultured on MacConkey agar with sorbitol, cefixime, and potassium tellurite (Becton, Dickinson, Co, Sparks, Maryland, USA), and Rainbow agar (Biolog, Hayward, California, USA) for the presence of *E. coli* O157:H7 [14]. If any colonies were considered *E. coli* O157 suspects, traditional PCR was performed using a set of specific primers to detect O-antigen-encoding *rfb* regions of *E. coli* O157:H7 [3]. Once the calves were confirmed negative for *Cryptosporidium* spp. and *E. coli* O157:H7 prior infections, they were placed in individual pens, separated by one pen length. They were then assigned an *E. coli* O157:H7 inoculum dose.

Inoculation of animals

One micro bank bead for the wildlife strain, stored at -80°C, was placed in a 250mL flask containing 150mL of BHI. The flasks were incubated at 37°C for 6 hours, while shaking at 100 rpm. Once the determined optical density was reached, the flasks were placed on ice for 15 minutes. At this point the optical density was measured again and the concentration of the stock solution was calculated by using the growth curve from the *E. coli* O157:H7 strain. From the known stock solution concentration, the inoculums were prepared for the wildlife *E. coli* O157:H7 strain in 200mL of PBS.

In previous lab studies inoculating neonatal calves with wildlife *E. coli* O157:H7 strain isolated from a feral swine, at 10⁴ CFU and 10⁷ CFU showed that these inoculum doses were not high enough to cause an infection (data below). To ensure the feral swine and deer mouse strains were distinct from one another and from other *E. coli* O157:H7 isolates collected in California, a Pulsed Field Gel Electrophoresis (PFGE) was performed. The isolates were digested using the Center for Disease Control and Prevention PulseNet PFGE with *XbaI* restriction enzyme [16]. A dendrogram was constructed by analyzing each digest profile with band matching and phylogenetic clustering analysis methods in Gel ComparII (Applied Maths, Sint-Martens-Latem, Belgium) computer software. For this study, assuming that the two wildlife *E. coli* O157:H7 strains have similar pathogenicity patterns once

inoculated into a host, one calf served as a negative control, two calves were inoculated with 10^8 CFU (calf A and calf B), two calves were inoculated with 10^9 CFU (calf A and calf B), and one calf was inoculated with 10^{10} CFU (positive control) of the deer mouse wildlife *E. coli* O157:H7 strain. Serial dilutions from 10^{-1} to 10^{-10} were made in 9mL of PBS and plated in triplicate on LB for each stock solution and inoculum. After an overnight incubation at 37°C, the true concentration of each inoculum was calculated. The individually housed calves were intragastrically inoculated with their assigned inoculum dose in 200 ml PBS with a calf gastric feeder (VetOne, Boise, Idaho, USA) followed with another 200mL of PBS as a wash.

Daily sampling and fecal scoring

Fecal samples and RAMS were collected twice daily for the first two days post-inoculation (p.i.) and then collected once a day until the 11th day p.i. Fecal consistency was scored on a scale from 1-4, with 1=Normal, 2=Paste, 3=Liquid, and 4=Water (Table 1). All of the samples were collected in the afternoon and transported back to the laboratory in a refrigerated state. The samples were processed within 24 hours of sampling.

Analytical assays for detecting *E. coli* O157:H7

Each 10g fecal sample and RAMS were placed in separate whirl packs (eNasco, Fort Atkinson, Wisconsin, USA) containing either 90mL TSB for fecals and 50mL TSB for RAMS, and then incubated for 2 hours at 25°C, 8 hours at 42°C, and held overnight at 6°C. *E. coli* O157:H7 was recovered using IMS and the Dynal Bead Retriever, with 50 µL of washed beads streaked for isolation on Rainbow agar and another 50 µL streaked for isolation on Sorbitol MacConkey Agar [14]. As performed in our laboratory, this IMS method has been shown to be able to detect as few as 1CFU/10g of calf feces (Data not shown). Two suspect colonies per positive plate were confirmed as *E. coli* O157:H7 using PCR as described above [17]. Confirmed colonies were stored at -80°C in Microbank vials for further analysis.

Euthanasia and necropsy

All calves were euthanized with pentobarbital sodium (Euthasol solution, Virbac, Fort Worth, Texas, USA) intravenously and necropsied on the 11th day p.i. Intestinal sections of the duodenum, jejunum, ileum, cecum, spiral colon, distal colon, and rectum were collected for bacterial culture (approximately 5cm in length). Sections of pancreas, liver, mesenteric lymph node, gall bladder, lung and kidney were also taken for culture (approximately 5g). Each segment of intestine was tied with string by both ends so that the contents and the mucosa could be cultured for the presence of *E. coli* O157:H7. Once transported to the laboratory, one end of the tissue segment was cut and the contents were collected in a whirl pack bag containing 90mL of TSB. The intestinal tissue was then cut longitudinally to expose the mucosa and placed in a separate whirl pack bag with 90mL of TSB. The non-intestinal tissues were each placed in a bag containing 50mL of TSB. All TSB bags were then incubated for 2h at 25°C, 8h at 42°C, and then held overnight at 6°C. *E. coli* O157:H7 was recovered using the same method as described above.

Table 1: Explanation of daily fecal scoring for each of the six experimental calves.

Score	Status	Description
1	Normal	Forms a pile on bedding
2	Paste	Pile has a flatter surface area
3	Liquid	Fecal pile runs through bedding
4	Water	Fecal contains flecks of tissue and material

Data analysis

The ID50 was determined for the wildlife *E. coli* O157:H7 strain with binary logistic regression using StataIC 12 (StataCorp LP, College Station, Texas, USA) computer software by calculating the number of calves that became infected over the total number of calves used for each inoculum dose. With these percentages, a curve was generated to determine the dose at which 50% of the calves became infected with the inoculated *E. coli* O157:H7 strain.

RESULTS AND DISCUSSION

Our definition of an infected calf was one that shed the wildlife *E. coli* O157:H7 inoculum for more than 48 hours p.i. and was culture positive in at least one of its tissues at necropsy on day 11 p.i. This was to account for passage of the inoculum through the gastrointestinal tract without attaching/colonizing the actual tissues. The wildlife *E. coli* O157:H7 strain used for this study was PCR-confirmed positive for both Shiga toxins, the attaching/effacing (*eaeA*), and the hemolysin A (*HlyA*) genes. By PFGE analysis, the deer mouse *E. coli* O157:H7 strain was different from the feral swine strain as well as three other *E. coli* O157:H7 strains collected from bovine feces in various parts of California (Figure 1).

Higher average fecal scores were observed in the 10^9 CFU inoculum group compared to the calves inoculated with 10^8 CFU. The positive control calf stayed consistent at a score of 1, while the negative control calf fluctuated between a score of 1-3 (Figure 2). IMS and culture was performed on daily fecal and RAMS collections for the detection of the inoculated *E. coli* O157:H7 strain. Calf B from the low inoculum dose shed for six days, while Calf A stopped shedding after four days post-inoculation. Both calves inoculated at 10^9 CFU shed longer than the lower dose inoculum group, with Calf B shedding for 7 days and the Calf A shedding for the entire duration of the trial. The positive control also shed the entire experimental time of eleven days. The *E. coli* O157:H7 inoculum was never recovered from the negative control. Table 2 represents the IMS daily collection results.

Since the negative control calf received fecal scores between 1-3, it was determined that there could be another pathogen causing these clinical signs. A possible conclusion was that the calf contracted *Cryptosporidium* spp. before the experiment and was pre-screened negative, but the infection caused clinical signs while in the experiment. It has been reported that neonatal calves infected with the protozoan *Cryptosporidium* spp. are usually asymptomatic, and mainly stressed animals show clinical manifestations of the disease associated with various degrees of diarrhea [18]. The negative control calf might have been exposed to the *Cryptosporidium* spp. protozoa prior to or during transportation, and then stressed due to the experiment,

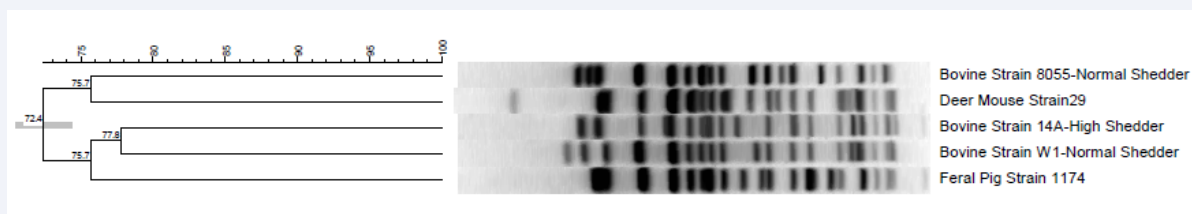


Figure 1 PFGE dendrogram of both wildlife and bovine *Escherichia coli* O157:H7 isolated from various parts of California, USA. Bovine strain 8055 isolated from Yolo County, Deer mouse inoculum strain isolated from Monterey County, Bovine strain 14A isolated from Imperial County, Bovine strain W1 isolated from Colusa County, and Feral pig strain 1174 isolated from San Benito County.

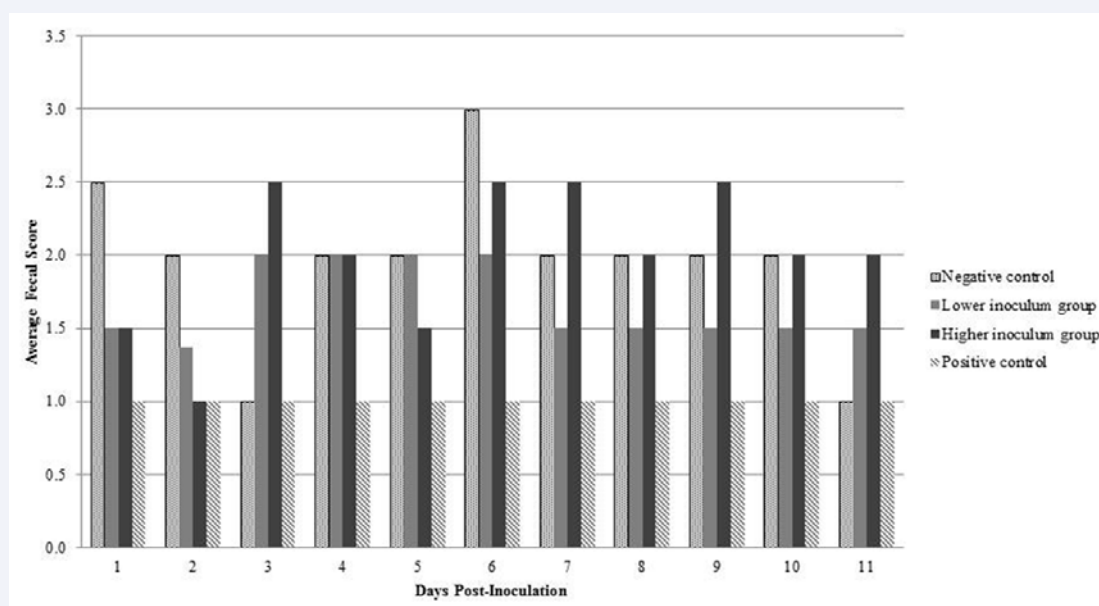


Figure 2 Average daily fecal scores for each inoculum group of experimental calves. Negative control: negative control calf; Lower inoculum group: average fecal scores from the calves inoculated with 10^8 CFU of the wildlife *Escherichia coli* O157:H7; Higher inoculum group: average fecal scores from the calves inoculated with 10^9 CFU of the wildlife *Escherichia coli* O157:H7; Positive control: positive control calf inoculated with 10^{10} CFU of the wildlife *Escherichia coli* O157:H7.

Table 2: Daily collection IMS results for shedding of the wildlife *Escherichia coli* O157:H7 in each calf following oral inoculation.

Days p.i.	Wildlife <i>E. coli</i> O157:H7 isolated from a Feral Swine						Wildlife <i>E. coli</i> O157:H7 isolated from a Deer Mouse					
	Negative	Calf A 10^4 CFU	Calf B 10^4 CFU	Calf C 10^4 CFU	Calf D 10^4 CFU	Calf A 10^7 CFU	Negative	Calf A 10^8 CFU	Calf B 10^8 CFU	Calf A 10^9 CFU	Calf B 10^9 CFU	Positive (10^{10} CFU)
1	-	-	-	-	-	-	-	+	+	+	+	+
2	-	-	-	-	-	-	-	+	+	+	+	+
3	-	-	-	-	-	-	-	+	+	+	+	+
4	-	-	-	-	-	-	-	+	-	+	-	+
5	-	-	-	-	-	-	-	-	-	+	+	+
6	-	-	-	-	-	-	-	-	+	+	+	+
7	-	-	-	-	-	-	-	-	-	+	+	+
8	-	-	-	-	-	-	-	-	-	+	-	+
9	-	-	-	-	-	-	-	-	-	+	-	+
10	-	-	-	-	-	-	-	-	-	+	-	+
11	N/A	N/A	N/A	N/A	N/A	N/A	-	-	-	+	-	+

(+) indicates presence of the *Escherichia coli* O157:H7 inoculum tested by Immunomagnetic Separation and PCR-confirmed on the day of collection from each calf; (-) indicates the *Escherichia coli* O157:H7 inoculum was not detected on the day of collection by Immunomagnetic Separation from each calf; (N/A) calves were euthanized the day prior.

Table 3: IMS detection of the wildlife *Escherichia coli* O157:H7 in intestinal tissue 11 days post-inoculation.

Tissue Segment	Negative	Calf A 10 ⁸ CFU	Calf B 10 ⁸ CFU	Calf A 10 ⁹ CFU	Calf B 10 ⁹ CFU	Positive 10 ¹⁰ CFU
Duodenum	-	-	-	+	-	+
Jejunum	-	-	-	+	-	+
Ileum	-	-	-	+	+	+
Cecum	-	-	-	+	+	+
Spiral Colon	-	-	+	-	-	+
Distal Colon	-	-	-	+	-	+
Rectum	-	-	-	+	-	+

(+) indicates that the wildlife *Escherichia coli* O157:H7 was detected in the tissue with Immunomagnetic Separation and PCR-confirmed, (-) indicates the wildlife *Escherichia coli* O157:H7 was not detected in the tissue.

causing clinical signs of diarrhea. This is in comparison to the positive control that had normal feces throughout the entire experiment. Consequently, clinical signs of diarrhea and fecal score differences between inoculum doses could not be correlated to the *E. coli* O157:H7 inoculum.

Due to animal welfare, a low dose inoculum experiment was not performed with this wildlife *E. coli* O157:H7 strain, but an *E. coli* O157:H7 strain isolated from feral swine was experimentally inoculated in neonatal calves in previous studies using doses of 10⁴ CFU and 10⁷ CFU (Table 2). Since no animals were considered to be infected with the feral swine strain, higher inoculum doses were used instead for the current study. This is a limitation, but assuming the two strains behave the same in neonatal calves, the infectious dose at 50% was calculated to show that 10⁸ CFU is the ID50 value for the deer mouse wildlife *E. coli* O157:H7 strain.

On the 11th day post-inoculation, the calves were euthanized and necropsied. IMS and culture was performed on all tissues and intestinal contents that were collected from each calf. The wildlife *E. coli* O157:H7 inoculum was never recovered from the pancreas, liver, mesenteric lymph node, gall bladder, lung, or kidney from any of the calves. Recovery of *E. coli* O157:H7 wildlife strain from tissues is shown in Table 3. The *E. coli* O157:H7 inoculum was recovered from at least one intestinal tissue in all calves except for the negative control and Calf A inoculated at 10⁸ CFU. Due to its negative fecal and RAMS cultures after four days post-inoculation and tissue culture at necropsy, by our case definition Calf A at 10⁸ CFU was considered not infected.

The infectious ID50 for the wildlife *E. coli* O157:H7 strain was calculated to be 10⁸ CFU. One of two calves inoculated at 10⁸ CFU was considered infected. Both calves inoculated at 10⁹ CFU were deemed infected due to their long shedding period of *E. coli* O157:H7 [2,19]. Although our case numbers are limited, there was also a dose-dependent trend seen in the cultured tissues that were taken from each calf. It appeared that if a calf was inoculated with the higher dose of the wildlife *E. coli* O157:H7, it would shed for a longer period of time and have culture-positive tissues when tested at the end of the experiment.

CONCLUSION

Wild animals can potentially carry *E. coli* O157:H7, causing transmission to humans when they are in contact with farm animals or produce fields [20]. Only a limited number of experimental studies have evaluated the ability of wildlife species carrying and transmitting the *E. coli* O157:H7 pathogen through the environment or to other animals. Due to this limitation, we don't fully understand the potential virulence of these wildlife isolates. We have shown in this study that a wildlife *E. coli* O157:H7 isolate, from a deer mouse, has the capability to experimentally infect neonatal calves at a high inoculum dose of 10⁸CFU, with a wide range of intestinal tissue colonization if infection continues for a week or longer. Future studies could further examine the host-pathogen interaction between these wildlife isolates and their reservoirs by determining the infectious dose/clinical signs in deer mouse models.

ACKNOWLEDGEMENT

The authors are grateful to the Teaching and Research Animal Care services for their assistance on the purchasing, care, and transportation of the animals used for this experiment. The authors would also like to thank the California Animal Health and Food Safety, University of California, Davis, for their necropsy floor and their overall assistance. Support for this research was provided to the Western Center for Food Safety contract U01-003-572 from the U.S. Food and Drug Administration. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the U.S. Food and Drug Administration.

REFERENCES

- Lim JY, Yoon JW, Hovde CJ. A brief overview of *Escherichia coli* O157:H7 and its plasmid O157. J Microbiol Biotechnol. 2010; 20: 1-10.
- Naylor SW, Roe AJ, Nart P, Spears K, Smith DGE, Low CJ, et al. *Escherichia coli* O157:H7 forms attaching and effacing lesions at the terminal rectum of cattle and colonization requires the LEE4 operon. Microbiol. 2005; 151: 2773-2781.
- Philpott D, Ebel F. Methods in Molecular Medicine: *E. coli*-Shiga toxin methods and protocols; Humana Press Inc.: Totowa, New Jersey, USA, 2003; 978-59259-316-3.
- Dean-Nystrom E, Bosworth BT, Moon HW, O'Brien A. *Escherichia coli* O157:H7 requires intimin for enteropathogenicity in calves. Infect Immun. 1998; 66, 4560-4563.
- Jeamsripong S, Chase JA, Jay-Russell MT, Buchanan RL, Atwill ER. Experimental in-field transfer and survival of *Escherichia coli* from animal feces to romaine lettuce in Salinas Valley, California. Microorganisms. 2019; 7: 408.
- Nielsen EM, Skov MN, Madsen JJ, Lodal J, Jespersen JB, Baggesen DL. Verocytotoxin-producing *Escherichia coli* in wild birds and rodents in close proximity to farms. Appl Environ Microbiol. 2004; 70: 6944-6947.
- Fernens WA, Hovde CJ. *Escherichia coli* O157:H7: Animal reservoir and sources of human infection. Foodborne Pathog Dis. 2011; 8: 465-487.
- Singh P, Sha Q, Lacher DW, Del Valle J, Mosci RE, Moore JA, et al. Characterization of enteropathogenic and Shiga toxin-producing *Escherichia coli* in cattle and deer in a shared agroecosystem. Front Cell Infect Microbiol. 2015; 5: 29.
- Kilonzo C, Li X, Vivas EJ, Jay-Russell MT, Fernandez KL, Atwill ER. Fecal shedding of zoonotic food-borne pathogens by wild rodent in a

- major agricultural region of the central California coast. *Appl Environ Microbiol.* 2013; 79: 6337-6344.
10. Naylor SW, Low CJ, Besser TE, Mahajan A, Gunn GJ, Pearce MC, et al. Lymphoid follicle-dense mucosa at the terminal rectum is the principal site of colonization of enterohemorrhagic *Escherichia coli* O157:H7 in the bovine host. *Infect Immun.* 2003; 71: 1505-1512.
 11. Cornick NA, Helgersson AF. Transmission and infectious dose of *Escherichia coli* O157:H7 in swine. *Appl. Environ. Microbiol.* 2004; 70: 5331-5335.
 12. Lim J, Li J, Sheng H, Besser T, Potter K, Hovde C. *Escherichia coli* O157:H7 colonization at the rectoanal junction of long-duration culture positive cattle. *Appl Environ Microbiol.* 2007; 73: 1380-1382.
 13. Baines D, Lee B, McAllister T. Heterogeneity in enterohemorrhagic *Escherichia coli* O157:H7 fecal shedding in cattle is related to *Escherichia coli* O157:H7 colonization of the small and large intestine. *Can J Microbiol.* 2008; 54: 984-995.
 14. Antaki-Zukoski EM, Li X, Pesavento PA, Nguyen THB, Hoar BR, Atwill ER. Comparative pathogenicity of wildlife and bovine *Escherichia coli* O157:H7 strains in experimentally inoculated neonatal Jersey calves. *Vet Sci.* 2018; 5: 1-11.
 15. Rice DH, Sheng HQ, Wynia SA, Hovde CJ. Rectoanal mucosal swab culture is more sensitive than fecal culture and distinguishes *Escherichia coli* O157:H7-colonized cattle and those transiently shedding the same organism. *J Clin Microbiol.* 2003; 41: 4924-4929.
 16. Ribot EM, Fair MA, Gautom R, Cameron DN, Hunter SB, Swaminathan B, et al. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for PulseNet. *Foodborne Pathog Dis.* 2006; 3: 59-67.
 17. Paton JC, Paton AW. Methods for detection of STEC in humans. In: Philpott, D., Ebel, F. (eds) *E. coli. Methods in Molecular Medicine™*, volume 73; Humana Press Inc.: Totowa, New Jersey, USA, 2003; 9-26.
 18. Díaz-Lee A, Mercado R, Onuoha EO, Ozaki LS, Muñoz P, Muñoz V, et al. *Cryptosporidium parvum* in diarrhetic calves detected by microscopy and identified by immunochromatographic and molecular methods. *Vet Parasitol.* 2010; 176: 139-144.
 19. Besser TE, Richards BL, Rice DH, Hancock DD. *Escherichia coli* O157:H7 infection of calves: infectious dose and direct contact transmission. *Epidemiol Infect.* 2001; 127: 555-560.
 20. Brando RF, Miliwebsky E, Bentancor L, Deza N, Baschkier A, Ramos MV, et al. Renal damage and death in weaned mice after oral infection with shiga toxin 2-producing *Escherichia coli* strains. *Clin Exp Immunol.* 2008; 153: 297-306.

Cite this article

Li X, Antaki Zukoski EM, Nguyen THB, Hoar BR, Jay Russell MT, et al. (2020) Identifying the Shedding Patterns of Wildlife *Escherichia coli* O157:H7 in Experimentally Inoculated Neonatal Jersey Calves. *J Vet Med Res* 7(2): 1183.