

Agent-based and contact network modeling applications for *Escherichia coli* transmission in
commercial feedlot settings

by

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Department of Diagnostic Medicine and Pathobiology
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Abstract

Shiga toxin-producing *Escherichia coli* (STEC) are recognized as a major food-borne pathogens with outbreaks, human infections, and occasional deaths associated with the consumption of contaminated foods. Cattle are recognized as a primary reservoir for STEC, though the transmission dynamics of STEC in feedlot cattle are not fully understood. Our current understanding of the transmission dynamics is dependent on longitudinal studies of naturally occurring STEC infections in cattle. A certified nonpathogenic strain of *E. coli* was used for a series of transmission experiments of a known source and concentration to better understand the transmission dynamics of STEC in commercial feedlot settings. The contact networks of feedlot cattle both within and between pens were characterized and an agent-based model was created to examine the influence of direct and indirect transmission routes on the pen-level prevalence of *E. coli* colonization.

Nonpathogenic *E. coli* strains were used as a surrogate to assess the transmission dynamics for STEC in cattle. Initially, three verified nonpathogenic *E. coli* strains (O19:H+, O101:H10, and O28:H43) were orally inoculated into weaned Holstein calves. All inoculated strains were able to colonize in the gastrointestinal tract of the calves, shed in their feces, and spread to their pen environment. Pen-level area under the curve (AUC) for fecal shedding concentrations of each nonpathogenic strain were evaluated and the strain with the highest pen average fecal AUC was selected for use in two independent inoculation trials that each occurred in one independent pen of 70 feedlot cattle. *E. coli* strain (O28:H43) was selected for use in these two inoculation trials. Oral inoculation of five randomly selected steers from each pen occurred for five days at the start of the study period. The inoculated strain was able to colonize, shed in the feces, spread to the pen environment, and transmit between feedlot steers. These data provide

baseline data on the shedding and transmission of a nonpathogenic *E. coli* strain in dairy calves, feedlot steers, and its detection in the pen environment. These data can be furthered used as a surrogate to better understand enteric pathogen transmission, such as STEC, in commercial U.S. feedlot systems to explore effective interventions options in a real-world setting.

To better inform the construction of network-based disease transmission models for cattle housed within confined-spaced systems, contact network modeling was used to quantify the contacts defined with a spatial threshold (SpTh) of 0.71 m and a minimum contact duration (MCD) of either 10, 30, or 60 seconds within three pens of feedlot cattle across consecutive years. Static, undirected, weighted contact networks were created for the full study duration and at vary timescales (24-h, 6-h, and h) to assess network heterogeneity. The influence on contact networks in feedlot cattle due to the variation in Real-Time Location System (RTLS) average tag read rate observed between the three years though the same system was used was examined. When the networks were down-scaled from higher average tag read rates to match the lower average tag read rates, the overall networks maintained similar network density and clustering, though the average edge weight between pairs of steers decreased. The high-resolution spatial and temporal contact data provided estimates for contact networks within U.S. commercial feedlot pens that can be used to better inform pathogen transmission models.

Contact network analysis was used to quantify contacts and compare the resulting static, undirected, weighted contact networks created from two neighboring pens of feedlot cattle and from across the shared fenceline at varying timescales (24-h, 6-h, and h). Contacts within-pen were defined at 0.71 m SpTh with a MCD of 10, 30, or 60 seconds. “Fenceline” contacts were defined with the same SpTh and MCD durations as within-pen contacts but contained a steer from each pen and occurred within the defined fenceline area, any location reading occurring

within 1 m of either side of the shared fenceline. On a full study duration, the contact networks created within each feedlot pen were densely connected. On shorter timescales (24-h, 6-h, h) the within-pen contact networks showed greater network heterogeneity in density and clustering metrics. The contact network created across the shared fenceline yielded a total network density of 17%. These findings can be used to better inform the construction of network-based disease transmission models for cattle within confined-spaced systems and additionally accounting for the transmission that occurs over man-made barriers (e.g., fencelines).

Finally, an agent-based model was created in Netlogo 6.2 using empirical data to explore the influence of direct steer-to-steer contact and indirect steer-to-pen environmental contact on the pen-level prevalence of *E. coli* colonization. Agents in the model were defined as individual steers and three pen environment areas (front, middle, rear). Direct contact between pairs of steers in the model was defined as cumulative 10 s temporal sampling windows (TSW) aggregated on a daily (24-h) level. Indirect contact between individual steers and the three pen environment areas were defined by using a proportion of location readings for each steer in each defined pen area for a given study day. Proportions were then averaged for each individual steer by study week in the model. Colony forming units (CFU) of *E. coli* transmitted by direct contact were modeled as one, five, and ten percent of the average $\log_{10}\text{CFU}/400\text{cm}^2$ of collected hide samples, and CFUs of *E. coli* per pen area were determined as one, five, and ten percent of the average $\log_{10}\text{CFU}/\text{g}$ of those pen soil surface samples that fell within that pen area for that study week. The colonization period of individual cattle within the model was defined as a weighted average of the time in study days in which all individual steers in the empirical data were positive in the digitally collected fecal samples. Preliminary results are suggestive that direct contact between animals is more important for pen-level prevalence for *E. coli* colonization than

indirect contact with pen environment. Additional work is needed to determine the R-nought of the *E. coli* modeled to compare it to published values of R-nought of STEC.

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Approved by:

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Chapter 1 - Social Network Analysis and applications in the cattle industry: A review

Introduction

Social network analysis (SNA) is the study of the connections or interactions among individuals within a population and the effects of those connections (Dubé et al., 2011; Martinez-Lopez et al., 2009; Newman, 2010). Social network analysis is also referred to as “structural analysis”, as it is a strategy used to analyze social structures in a defined population using networks and graph theory (Wellman and Berkowitz, 1988; Otte and Rousseau, 2002). One of the most well-known examples of a social network is the World Wide Web (Raghavan, 2001). Social network analysis is a helpful tool in both human and animal systems, with a wide range of applications from identifying connections among individuals through the Internet and online data to modeling the spread of both human and animal diseases (Cuomo and Maiorano, 2018; Salathé and Jones, 2010; Drewe, 2010).

Social network analysis aims to conceptualize the social structure of the network of members and the connections between those members. These interconnections between individuals are represented as a graph, containing a set of nodes and links. Members of a network are referred to as nodes, and the connections between members are referred to as links. A glossary of network terminology can be found in Table 1-1. Nodes, or vertices, within a network are the individuals which could represent actors, people, animals, farms, communities, states, etc. The relationships between nodes are represented by links. The size of a network is represented by the number of links within a graph. A directed link, also referred to as an arc, is a pair of

nodes that is represented graphically as an arrow drawn between the two nodes (Bang-Jenson and Gutin, 2008). The direction of the arrow represents the course of the connection from one node to the next node. For example, in a network describing the flow of information a directed link is used to track the flow of information from one hub to another. An undirected link, also referred to as an edge, is bidirectional and is represented graphically as a line. An undirected link treats both nodes in the connection as equals regarding that connection (Bang-Jenson and Gutin, 2008). For example, a network created from social media connections, an undirected link is made if Smith is friends with Johnson, then Johnson is also friends with Smith. A weighted graph associates a label, or weight, with every edge in the graph representing the frequency of that interaction or association. This weight can represent the length of the route between two nodes, the number of resources or information that can be shared between the two nodes, the number of contacts between the two nodes, or the amount of time these two nodes were in contact.

There are two main forms of SNA: ego-centric analysis and global network analysis. In an ego-centric network analysis, the objective is to analyze the network of one actor within the network or analyze the network on a micro-level (Otte and Rousseau, 2002; Provan et al., 2007). This method can also be done when network boundaries or actors are not known a priori. Egocentric networks focus on the “embeddedness” of an individual in a network and on dyadic relationships (Provan et al., 2007), such as in Sembiring et al.’s description of an ego-centric network of fish farmers and cage collectors in Indonesia that showed the social network’s benefit of assisting the farmers in marking and selling their harvested fish (2019).

In global network analysis, or whole network analysis, one tries to describe all the relationships between all units within a network (Otte and Rousseau, 2002). Global network analysis looks at the network in a macro-level and is done when the boundaries of the network are known and when a complete list of all actors within a network can be collected. In a global network analysis, all communities within a network are detected as data is collected on all actors by tracking or survey about their relationships with all other actors within the network (Lancichinetti and Fortunato, 2009; Newman, 2004; Yang and Leskovec, 2013).

The aim of this literature review is to describe network metrics commonly used in describing, analyzing, and comparing social networks. Additionally, we aimed to describe applications of SNA in the cattle industry.

Network Metrics

There are two main groups of metrics to describe networks. The first being measures of centrality, used to determine the importance of nodes within the network. The second being measures of cohesiveness, which are used to evaluate the connectivity of the network. Measures of centrality within a network assign rankings or numbers to individual nodes within a network relative to their position within the network. These rankings are used to identify the most influential players within a social network. Measures of cohesiveness in a network measure the connectedness among individuals within a network.

Measures of Centrality

One of the simplest measures used for quantifying the centrality of an individual in a network is degree centrality (Lui et al., 2020). In a network, degree is the number of individual connections for each node. In a directed network, out-degree is the number of edges leaving a

node, and in-degree is the number of edges entering a node. In social networks, those based off social behaviors in a group, in-degree is a measure of popularity and out-degree is a measure of expansiveness (Borgatti et al., 2018). Average degree is the average number of links per node, and weighted degree, or strength, is the sum of weights of the edges of the nodes. Degree distribution is the probability of these degrees over the whole network. Degree centrality uses only local information to assign importance to a node based on the number of immediate neighbors that node has within a network (Lui et al., 2020). A node with a high degree centrality therefore has more direct connections with other nodes within the network (Golbeck, 2015).

Another measure of network centrality is betweenness centrality. This metric measures the number of times a node lies on the shortest path between other nodes and is a measure of the number of times a node needs a given node to reach another node in the network (Otte and Rousseau, 2002). Nodes with a greater betweenness centrality act as “bridges” between subgroups within a network. Betweenness gauges the extent to which a node facilitates the flow of transmission (i.e., disease, information, etc.) across the whole network (Newman, 2005).

A key measure of node centrality in a network is closeness centrality (Freeman, 1978; Opsahl et al., 2010; Wasserman and Faust, 1994). First proposed in 1959 by Dijkstra, the natural distance between all pairs of nodes in a connected graph is the length of the shortest path between all nodes in the graph. The farness of a node is the sum of the distances to all other nodes, and the closeness of a node is the reciprocal of the farness (Bavelas, 1950; Sabidussi, 1966). Closeness centrality assigns scores of each node based on their ‘closeness’ to all other nodes within the network and is calculated as the reciprocal of the sum of the length of the shortest paths between the node and all other nodes in the network. The more centrally located a

node is the lower its total distance to all other nodes in a network (Golbeck, 2015). A higher closeness centrality score may indicate nodes that are best placed within a network to influence the entire network in the shortest amount of time.

Eigenvector centrality or EigenCentrality is a metric that measures a node's influence on the network based on the number of links an individual has with other nodes in a network (Golbeck, 2015). All nodes in a network are assigned a relative score based on the concept that connections to high-scoring nodes contribute more to the score of the node in question than connections to lower-scoring nodes. Eigenvector centrality considers how well a node is connected and with which nodes that particular node has connections with. A node with a high eigenvector score indicates a node who is connected to other high-scoring nodes (Newman, 2006; Negre et al., 2018) This metric identifies nodes with influence over the whole network, not just a node's directly connected neighbors.

A variant of Eigenvector centrality is PageRank centrality. This metric also assigns nodes a score based on their connections and their connections' connections, but also considers link direction and weight. It was first designed for ranking web content which uses hyperlinks between pages as a measure of importance, but it can also be applied to other forms of networks (Brin and Page, 1998). PageRank centrality accounts for the direction of the links and assigns each node a score based on its incoming links (its in-degree), this score is weighted based on the relative score of the originating node for that link (Page et al., 1999). This measure, like Eigenvector centrality, identifies influential nodes in a network whose influence in the network extends beyond just their direct connections.

Measures of Cohesiveness

The simplest measure of network cohesiveness is network density, or the number of connections present in a network over the total number of connections possible. Network density can also be referred to as the gamma index (Pritchard, 1984). This measure assesses how fully connected a network is and does not consider network directionality or the weight of the links (Sosa et al., 2020). In a complete graph, every node is directly connected to every other node.

Another metric of network cohesion is fragmentation. Fragmentation is the number of pairs of nodes that are disconnected from each other (Borgatti, 2006). Maximum fragmentation occurs when all nodes are isolated, and in a complete graph where every node is connected to every other node, then there is no fragmentation (Borgatti, 2006).

Within a network, path length is defined as the distance between two nodes. Average path length is therefore the average number of steps, or edges, along the shortest paths for all possible pairs of nodes within a network (Mao and Zhang, 2013). This measures the efficiency of a network's ability to move information, resources, or disease across the network (Sosa et al., 2020). Diameter is the longest path of the shortest paths in the network and has been used to examine the rapidness of information or disease transmission on a network (Sosa et al., 2020).

Another measure of network cohesion is transitivity or clustering coefficient. This metric measures the tendency of nodes within the network to cluster together (Watts and Strogatz, 1998). This metric follows the social sciences analogy "the friends of my friends are my friends" (Ozkan-Canbolat and Beraha, 2016). In many real-world networks, nodes tend to prefer to form communities with a high density of ties at a greater than average probability than ties randomly established between two nodes (Holland and Leinhardt, 1971; Watts and Strogatz, 1998). There

are two levels of clustering coefficient: global and local. Both measures are based on quantifying triplets. A triplet in a network consists of three nodes that are connected by either two edges (open triplet) or three edges (closed triplet, Sosa et al., 2020).

Global clustering coefficient can be applied to both undirected and directed networks and gives an indication of the clustering within a network. This measure is based on triplets, or three connected nodes or closed triangles, over the possible number of triplets within a network (Luce and Perry, 1949) or the fraction of nodes connected to a common node, who are also connected (Newman, 2010). Local clustering coefficient gives an indication of the “embeddedness” of individual nodes within a network and is based on the number of triangles that nodes is a part of the degree of that particular node (Sosa et al., 2020) or the average probability that nodes connected to a particular node are also connected (Newman, 2010).

There are many network metrics that look at the division of nodes within the structure of a network. A subgraph of a network is a subset of the nodes in a network, and all the edges linking to those nodes. A clique is a subgraph of a network in which all nodes are connected, or a complete subgraph (Boyd and Ellison, 2007). A community, or cluster, is a group of densely inter-connected nodes that are sparsely connected to other nodes in the network (Zhou et al., 2009). In a directed network, the Giant Strongly Connected Components (GSCC) is the largest group of nodes that are mutually connected, and the Giant Weakly Connected Component (GWCC) is the GSCC plus all the nodes that have links into and out of the GSCC (Duncan et al., 2022). In models depicting disease outbreaks amongst nodes, assuming there are no changes or interventions applied to the network, the GSCC represents a reasonable lower bound on the maximum size of the final epidemic, whereas the GWCC provides a reasonable upper bound

(Kao and Kiss, 2010). The lower bound of the maximum size of an epidemic can be reduced by targeting nodes with the highest degree in a disassortative network, thereby reducing the size of the GSCC (Mewu et al., 2013).

Applications of SNA in Cattle

Cattle Movement Networks

Social network analysis is a valuable tool across multiple disciplines. The first studies to use SNA in veterinary science were used to explore the contact network among racehorses (Christley and French, 2003) and to predict tuberculosis (*Mycobacterium bovis*) transmission in wild brushtail possum (Corner et al., 2003). SNA specifically in the cattle industry has been used for many applications including the modeling of networks based on trade and cattle movements, modeling the transmission of infectious diseases between farms, and to better understand the social behavior of dairy cattle in groups.

One application of SNA that has been implemented in the cattle industry is the use of cattle movements to create networks. In 2014, the global cattle population was estimated to be 1.4 billion (Robinson et al., 2014) with the predicted global cattle population to increase to 2.6 billion by 2050 (Pica-Ciammarra et al., 2013). Globally, the movement of cattle is dependent on multiple factors related to the individual country, as well as factors of each individual producer. Animal movements have been found to be important facilitators of the spread of infectious diseases, particularly in long-distance transportation of exposed hosts (El Allaki et al., 2016; Enright and O'Hare, 2016). In the 2001 Foot-and-Mouth disease (FMD) outbreak in the United Kingdom, movements of infected animals (Gibbens et al., 2001; Haydon et al., 2002) and mixing of animals at livestock markets (Ferguson et al., 2001) contributed spread of the disease.

Understanding cattle movement patterns increases our understanding of the epidemiology of endemic and epidemic diseases, which results in more accurate risk assessments and more informed management decisions in the face of disease (Bajardi et al., 2012; Martínez-López et al., 2009).

Since the 2001 FMD epidemic in UK, SNA has been used to study the implications of livestock movements and evaluate the risk for disease transmission on an individual premise-level in multiple countries (Brennan et al., 2008; Kiss et al., 2006; Natale et al., 2009; Ortiz-Pelaez et al., 2006; Robinson and Christley, 2007; Volkova et al., 2010). The use of livestock movements in SNA has also been used to identify structural components of a network, such as the position of markets (Robinson and Christley, 2007), and exploring effective control measures (Dubé et al., 2009). The use of cattle movements and SNA have also been used to better develop risk-based surveillance and disease prevention programs (Aznar et al., 2011).

Networks for cattle movements have been created using a variety of methods. In the UK, the Cattle Tracing System is an online recording system for cattle births, deaths, and between-herd movements (BCMS, 2023). Movement records from this reporting system have been used to create networks based on British cattle movements (Duncan et al., 2022; Fielding et al., 2019; Fielding et al., 2020; Robinson and Christley, 2007; Woolhouse et al., 2005; Vernon and Keeling, 2009). Animal movements have also been used to create networks in multiple other countries including: Brazil (de Menezes et al., 2020), Canada (Dubé et al., 2008), Chile (Alocilla and Monti, 2022), Ecuador (Vinuela et al., 2022), France (Lal Dutta et al., 2014), Germany (Brzoska et al., 2020), Ireland (Brown et al., 2019), Italy (Bajardi et al., 2011; Natale et al.,

2009), Paraguay (Avalos et al., 2022), Sweden (Frössling et al., 2012; Lindström et al., 2009), Switzerland (Vindondo and Voelkl, 2018), and Uruguay (VanderWaal et al., 2016).

Whereas in the United States, there is currently no mandated cattle tracing system, livestock movements between states are mostly recorded by Interstate Certificates of Veterinary Inspection (ICVI). These certificates are issued by an accredited veterinarian that certifies that animals are visually healthy at the time of inspection prior to the movement and are the most accessible source of information in the country regarding animal movements (Forde et al., 1998; Portacci et al., 2013). However, there are some exceptions in which a shipment can occur without an ICVI including shipping directly to slaughter plants or movements that occur intrastate thus resulting in incomplete data on movements. Both Buhnerkempe et al. (2013) and Cabezas et al., (2021) used SNA to describe the patterns of livestock movements within the contiguous U.S. using ICVIs.

In developing countries there is often not an official form of recording animal movements but understanding the network of animal movements in these countries is important for disease surveillance and control. To generate networks in these countries, such as Cambodia, Thailand, and Madagascar, researchers have used questionnaires to identify players involved in the networks (Nicolas et al., 2013; Noopataya et al., 2015; Poolkhet et al., 2016). By design, these generated networks were limited to only partial networks with a small sample size and not a full network. However, they do provide a useful tool for better understanding the livestock movement in their respective countries and the control of infectious disease in these networks.

The application of SNA to cattle movement networks has allowed for the description of network structure and metrics and trade communities in multiple countries. Networks in

Argentina, Brazil, France, United Kingdom, United States, and Uruguay have demonstrated heterogeneity in the variation of movements incoming to a farm, county, state, or province compared to outgoing movements (Aznar et al., 2011; Buhnkempe et al., 2013; Cabezas et al., 2021; Lal Dutta et al., 2014; León et al., 2006; Volkova et al., 2010; VanderWaall et al., 2016). VanderWaal et al. proposed that these nodes within a contact network created from cattle movements in Uruguay likely represented premises of finishing cattle, as these farms have a higher turnover of animals, thus allowing them to accept multiple batches of animals (i.e., incoming movements) per year (2016). In contrast, nodes with a high out-degree, particularly in networks representing the United States, represented counties or states with a large number of cow-calf operations (Buhnkempe et al., 2013; Cabezas et al., 2021). These premises represent breeding and development operations that would be shipping more animals out of the premises than they would be receiving. The use of SNA has assisted in discovering the impact of influential “nodes” across complex networks (Albert et al., 2000; Robinson and Christley, 2007; Mweu et al., 2013; Palisson et al., 2016). Through better characterization and understanding of these potentially influential premises within livestock movement networks we can better target surveillance and response efforts in regard to disease events.

In both developing and developed countries, nodes representing middlemen, traders, dealers, and markets have been found to be influential within their respective networks (Christley et al., 2005; Bajardi et al., 2011; Natale et al., 2009; Noopataya et al., 2015; Poolkhet et al., 2016; Rautureau et al., 2011). Markets and traders act as hubs and represent nodes of high animal movement that connect a large number of farms and are important for disease control and preventions (Robinson and Christley, 2007). The mixing of animals at auction markets is

important for the dissemination of disease, through both the mingling of cattle at the market and the re-distribution of cattle to different farms after the sale (Ferguson et al., 2001; Gibbens et al., 2001; Robinson and Christley, 2007). In a SNA of the Danish cattle movements from 2000-2009, the removal of markets from the network resulted in the disappearance of GSCCs which demonstrates the usefulness of targeted application of control measures on highly connected premises in the face of a potential epidemic (Mweu et al., 2013).

Like markets, in some networks there are highly connected individual herds that have been found to have the potential to spread disease more effectively than averagely connected herds (Dutta et al., 2014; Grear et al., 2014; Hidano et al., 2016; Natale et al., 2009; Vidondo and Voekl, 2018). In VanderWaal et al.'s (2016) network based off the livestock movement in Uruguay, the top 10% of most highly connected farms in the network accounted for 72-83% of the animals moved annually. These highly connected farms are candidates for 'super-spreaders' (VanderWaal et al., 2016) and may be disproportionately influential in the spread of infectious diseases (Lloyd-Smith et al., 2005; Natale et al., 2009). These farms often have frequent incoming and outgoing movements which increase their potential to acquire an infected animal and to spread the infection further in the network (Natale et al., 2009). A node that is more highly central in a network, or has a higher closeness value, would allow for disease to spread quicker within the network. This is due to the ease at which through this node you can reach all other nodes in a network through a shorter number of steps. Natale et al (2009) examined the effect on outbreak size of removing outgoing movements from premises selected either randomly or based on centrality measures. Removing the top 1% of nodes based on centrality measures decreased outbreak size more than removing 20% of nodes randomly, with removal based on

degree most effective. By using centrality measures such as closeness centrality and eigenvector centrality, it was possible to characterize the premises based on their potential to cause large epidemics (Natale et al., 2009). Identification of these farms within a network allows for targeted restrictions to be implemented to reduce the potential spread of disease. Targeted intervention of these farms potentially fragments potential transmission chains, thus reducing the size and extent of a potential outbreak (VanderWaal et al., 2016). Additionally, these highly connected farms can be important sentinels for surveillance, as targeted sampling at these locations would increase sensitivity of active surveillance systems compared to random sampling of farms (VanderWaal, 2016). However, comparison of network metrics between countries can be difficult due to differences in population size, data recording, production types, inclusion of different types of movements such as to slaughter plants and markets, and the time frame of the analysis (VanderWaal et al., 2016).

Just as SNA has been used to describe the network structure and identify influential nodes in multiple cattle movement networks, it has also been used to describe the transmission risk and impact of disease within a network. Mohr et al. investigated the change in the dynamics of Foot-and-Mouth disease transmission in the livestock network of Scotland through the rewiring of movements between premises (2018). Manipulation of the observed contact network by movement restrictions while redirecting movements between holdings to avoid nonsensical activities such as avoiding movements to markets on days in which markets were inactive, significantly impacted disease control (Mohr et al., 2018). Brown et al., examined the risk of bovine tuberculosis transmission in the cattle movement network of Northern Ireland and concluded that the cattle movement network in this area is highly interconnected and this herd

connectivity may play an important role in the maintenance of bovine tuberculosis within the study population (2019).

Another finding of SNA applications on cattle movement networks is that many of the resulting networks had properties of scale-free and small-world networks (Bigras-Poulin et al., 2006; Christely et al., 2005; Natale et al., 2009; Robinson and Christley, 2007). Scale-free networks are characterized by having a highly heterogenous degree distribution that follows a highly right skewed “power-law” (Barabasi and Albert, 1999). Multiple networks show a farm-level connectivity that is extremely right skewed, with the majority of premises having low connections within the network and a few having a high number of connections (Natale et al., 2009; Volkova et al., 2010). Small-world networks are characterized by local clustering with ties that act as short-cuts reducing the path length between clusters (Watts and Strogatz, 1998). In many of the networks described above, there is the presence of “hubs” within the networks either in the form of livestock markets or individual farms. These “hubs” within networks contribute to the small-world and scale-free properties of the network (Natale et al., 2009). These highly connected “hubs” not only act as short-cuts between clusters in the networks; targeted intervention towards these highly connected nodes may limit the spread of infection of disease compared to the same intervention strategy applied to a random node in the network that is likely weakly connected (Albert et al., 2000; Natale et al., 2009).

Cattle Social Interaction Networks

Though SNA has predominantly been used to assess social behavior of wildlife and zoo animals, it can also be used to understand the social interactions of farm animals and has become increasingly popular for assessing the social behavior of dairy cattle (Foris et al, 2019; de Freslon

et al., 2019; Rocha et al., 2020). The applications include the use of social networks to identify social interactions and behaviors within groups of cattle to better understand group behavioral structures (Foris et al., 2019), disease transmission within groups (de Freslon et al., 2019), and increase animal welfare (George and Bolt, 2020) and productivity (Boyland, 2015). Cattle, like many species of animals, live in social groups with complex relationships and social structures (Wey et al., 2008; Sueur et al., 2011). These social structures are an important aspect of the animal population and influence behavior characteristics (Myers, 1983; Whitehead, 2009).

For decades researchers and producers have relied on visual observation to monitor the behavior of cattle, however visual monitoring is subjective, limited by the demands of training personnel, and requires extensive time and labor (Richeson et al., 2018). Due to advances in technology, cattle location and movement can be more easily quantified objectively and continuously, and from this data cattle behavior may be inferred. Some of these technologies to monitor individual cattle location and movement include the use of ear tags equipped with radio frequency identification (RFID) or remote, real-time location systems (RTLS) technology (Bikker et al., 2014; Shane et al., 2016; Stankovski et al., 2012), accelerometers (Arcidiacono et al., 2017; Robert et al., 2009), pedometers (Devant et al., 2012; Umemura, 2013), Global Positioning Systems (GPS) technologies (Godsk and Kjærgaard, 2011; Ungar et al., 2005), and motion collars (Meunier et al., 2017). RFID ear tags work by wirelessly transmitting the identity of an object in the form of a unique sequence of numbers and letters using radio waves (Grooms, 2007). Though RFID allows for batch-reading of animals and is efficient in traceability and behavior monitoring, ear tissue has been shown to be influential on the read range and received signal strength indicator of these tags (Adrion et al., 2017). RTLS is an automated sensor-based

monitoring system that uses Bluetooth or WiFi capabilities to record the time stamped location of animals; this technology has been used to classify the location of animals in predefined pen areas and allow estimation of the probability of animals participating in behaviors such as eating or drinking (Chapa et al., 2021; Shane et al., 2016). Accelerometer technology measures the linear acceleration of an object along the X-, Y-, and Z-axis (Barwick, 2016) to provide movement data. This technology can be attached to animals and has been popular due to their small size and affordable price (Delagarde and Lamberton, 2015). Pedometers are electronic devices that transmit information about the number of steps an animal takes over a set time (Mazier et al., 2006). Pedometers have been successfully used to monitor the walking and grazing bite activity of cattle (Devant et al., 2012; Umemura, 2013) and have been used to show the increased of walking activity of cows in estrus (de Mol, 2000; Koelch, 1992; Lehrer et al., 1992; Scholfield, 1988). Neither accelerometers nor pedometers provide location data. GPS technology has also been used to track animals' positions continuously and automatically with accurate recognition of individual activities (Godsk and Kjærgaard, 2011; Ungar et al., 2005). GPS technologies are best used in outside and remote locations, however due to signal weakening, they are not easily applied for indoor data collection and analysis of behaviors (Arcidiacono et al., 2017). Motion collars combine accelerator and GPS technology to capture individual animal position and activity level while engaged in different behavioral activities (González et al., 2015). These advanced technologies allow researchers to quantify cattle behaviors objectively and continuously. However, work is still needed on the application of these technologies to ensure maximum sensitivity and specificity when detecting cattle behavior,

reliable and repeatable data, cost and benefit analysis, and integration into everyday practice for both researchers and producers (Richeson et al., 2018).

Cattle are hierarchical in their social interactions, and these hierarchies are subject to change depending on the addition or removal of individuals from the group (Sołtysiak and Nogalski, 2010). Understanding the regrouping of associations and interactions of the remaining individuals after the removal of animals or the introduction of new animals is important to understanding dynamics of a regrouped herd (George and Bolt, 2020) as changing animals within milking strings or resorting feedlot cattle. Social hierarchy in an animal group may be determined by observing the attitudes of the animals and assigning individuals to different ranks based on their dominance or subordination behaviors in the observed group (Kowalski, 2000; Rogalski, 1972). More dominant cows are those that displace others from feeding and resting areas and are never harassed by subordinates. Allowing hierarchies to establish is desirable in establishing a stable social structure and can lead to positive social behaviors, such as social grooming, which can reduce stress levels (Boyland et al., 2016).

Boyland et al. quantified the SNA of a dynamic group of 110 lactating dairy cows at two social scales: group and individual level (2016). At the group level, they found significant network centralization and no evidence of community sub-structure. This was similar to the findings in small herds of dairy cows and calves examined by Gyax et al. (2010) and de Freslon et al. (2019), who also found that each network was connected as one single component. Individuals in these networks were either directly or indirectly connected to all other individuals.

At the individual animal level, Boyland et al (2016) found evidence for differentiated social structures and relationship association strength between cows. These differences in

individually formed differentiated relationships were predicted by different individual cow attributes such as: lactation number, breed, gregariousness, and milk production (Boyland, 2015). This is consistent with previous findings that cows interact non-uniformly, often forming preferential relationships with some while avoiding others (Boyland et al., 2016; Gygax et al., 2010; Reinhardt and Reinhardt, 1981). This measure of tendency for individuals to associate with others that share similar characteristics is commonly observed in human groups with social preferences being impacted by similar race, ethnicity, age, religion, etc. (McPherson et al., 2001). The position of an individual cow within their social network has been linked to productivity, with evidence to suggest the more sociable or higher connected individuals have a higher milk yield (Boyland, 2015). Therefore, determining individual centrality measures may be important to take into consideration when sorting dairy cattle into new management groups. Individual attributes are important in shaping the contact structure of a group, add biological relevance to the observed social structures, and should be considered to avoid group disruptions thereby increasing animal welfare and to also increase the cost-effectiveness of prevention and control measures (de Freslon et al., 2019).

Another application of SNA and understanding the behavior structure of groups of cattle is an increased understanding of disease spread through individual groups. By better understanding the cattle contract structure we may be able to intervene in the transmission of pathogens more effectively on a farm (de Freslon et al., 2019). These applications of SNA have also been used to identify the level of social affiliation for individual animals, resulting in the identification of unhealthy animals with a lower level of social affiliation compared to healthy animals (Vimalajeewa et al., 2019). Like in the networks resulting from cattle movements, SNA

has been used to identify key individuals in a herd that may be particularly influential to the social structure (Boyland et al., 2016). These individuals at the center of the social network may have a disproportionate influence on the spread of pathogens in a group (Boyland, 2016; George and Bolt, 2020). It is important to take into consideration that the type of contact matches the transmission route of the pathogen of interest and that many pathogens have multiple routes of transmission (Craft, 2015). Infectious agents that spread through contact vary from indirect to direct transmission; contact between individuals increases in relative importance to pathogen transmission as one moves from environmental contamination, to vector-borne, to aerosol, to close-contact, and finally sexually transmitted diseases (Lembo et al., 2015).

Conclusion

Social network analysis is a multidisciplinary tool that aims to characterize the social structure of a network of individuals and their connections. SNA uses two groups of metrics to characterize network structure and individuals within a network. Measures of centrality aim to find influential individuals within a network. Examples of these measures include degree centrality, betweenness centrality, closeness centrality, Eigenvector and PageRank centrality. Measures of cohesiveness aim to quantify the connectedness of individuals within a network. These measures include network density, fragmentation, average path length, diameter, global and local cluster coefficient, and characterization of the network into clusters, cliques, and components.

Applications of SNA specific to the cattle industry include the modeling of networks based on trade and cattle movements, modeling the transmission of infectious diseases between farms, and modeling herd dynamics to better understand the social behavior of dairy cattle in

groups. Creation of networks based off of cattle movement records have identified influential “hubs” within the networks that can lead to better targeted application of surveillance and disease interventions. At a group level, it has been noted in multiple groups of dairy cattle that all individuals are connected to each other, either directly or indirectly. However, on an individual basis there is evidence of preferential relationships formed between dairy cows based on individual characteristics such as lactation number, breed, gregariousness, and milk production. These preferential social groupings form non-uniform interactions between dairy cows within a network. Social network analysis may be a useful future tool for veterinarians, policymakers, and researchers to predict the spread of disease within an individual herd, while also identifying the herds in a network that are at greatest risk of disease.

The lack of data describing the individual and group characteristics for beef cattle, specifically in feedlot settings, is a data gap barrier to better understanding of production-type differences in network structure and metrics. Also, patterns of contact of feedlot cattle need to be examined to provide more robust disease transmission models for within disease transmission within-pens and between-pens of feedlot cattle.

Literature Cited

1. Adrion, F., A. Kapun, E.-M. Holland, M. Staiger, P. Löb, and E. Gallmann. 2017. Novel approach to determine the influence of pig and cattle ears on the performance of passive UHF-RFID ear tags. *Comput. Electron. Agric.* 140: 168-179.
<https://doi.org/10.1016/j.compag.2017.06.004>.
2. Albert, R., H. Jeong, and A.L. Barabási. 2000. Error and attack tolerance of complex networks. *Nature*. 406(6819): 378-382. <https://doi.org/10.1038/35054111>.

3. Alocilla, O. and G. Monti. 2022. Network analysis of cattle movements in Chile: Implications for pathogen spread and control. *Prev. Vet. Med.* 204:105644.
<https://doi.org/10.1016/j.prevetmed.2022.105644>.
4. Arcidiacono, C., S.M.C. Porto, M. Mancino, and G. Cascone. 2017. Development of a threshold-based classifier for real-time recognition of cow feeding and standing behavioural activities from accelerometer data. *Comput. Electron. Agric.* 134: 124-134.
<https://doi.org/10.1016/j.compag.2017.01.021>.
5. Avalos, A., B. Durand, J. Naranjo, V. Maldonado, L. Canini, and G. Zanella. 2022. Analysis of cattle movement networks in Paraguay: Implications for the spread and control of infectious diseases. *PloS ONE*. 17(12): e0258999.
<https://doi.org/10.1371/journal.pone.0278999>.
6. Aznar, M.N., M.A. Stevenson, L. Zarich, and E.A. León. 2011. Analysis of cattle movements in Argentina, 2005. *Prev. Vet. Med.* 98:119-127.
<https://doi.org/10.1016/j.prevetmed.2010.11.004>.
7. Bajardi, P., A. Barrat, F. Natale, L. Savini, and V. Colizza. 2011. Dynamical patterns of cattle trade movements. *PloS ONE*. 6:e19869. <https://doi.org/10.1371/journal.pone.0019869>.
8. Bajardi, P., A. Barrat, L. Savini, and V. Colizza. 2012. Optimizing surveillance for livestock disease spreading through animal movements. *J. R. Soc. Interface*. 9: 2814-2825.
<https://doi.org/10.1098/rsif.2012.0289>.
9. Bang-Jensen, J. and G. Gutin. 2009. *Digraphs: Theory, algorithms, and applications*. Springer Monographs in Mathematics (second ed.), Springer-Verlag London Ltd., London.B

10. Barabasi, A.-L., and R. Albert. 1999. Emergence of scaling in random networks. *Science*. 286(5439): 509-512. <https://doi.org/10.1126/science.286.5439.509>.
11. Barwick, J. 2016. On-animal motion sensing using accelerometers as a tool for monitoring sheep behavior and health status. [Unpublished PhD Thesis]. University of New England.
12. Bayelas, A. 1950. Communication patterns in task-oriented groups. *J. Acoust. Soc. Am.* 22(6): 725-730. <https://doi.org/10.1121/1.1906679>.
13. BCMS. 2023. CTS Online. (Last accessed 1 January 2023). <https://secure.services.defra.gov.uk/wps/portal/ctso>
14. Bikker, J.P., H. van Laar, P. Rump, J. Doorenbos, K. van Meurs, G.M. Griffioen, and J. Dijkstra. 2014. Technical note: Evaluation of an ear-attached movement sensor to record cow feeding behavior and activity. *J. Dairy. Sci.* 97(5): 2974-2979. <https://doi.org/10.3168/jds.2013-7569>.
15. Bigras-Poulin, M., R.A. Thompson, M. Chriél, S. Mortensen, and M. Greiner. 2006. Network analysis of Danish cattle industry trade patterns as an evaluation of risk potential for disease spread. *Prev. Vet. Med.* 76: 11-39. <https://doi.org/10.1016/j.prevetmed.2006.04.004>.
16. Borgatti, S.P. 2006. Identifying sets of key players in a social network. *Comput. Math. Organ. Theory*. 12: 21-34.
17. Borgatti, S.P., M.G. Everett, and J.C. Johnson. 2018. *Analyzing social networks*. Sage. Los, Angeles, CA.
18. Boyd, D.M., and N.B. Ellison. 2007. Social network sites: definition, history, and scholarship. *J. Comput. Mediat. Commun.* 13(1): 210-230.

19. Boyland, N.K. 2015. The influence of social networks on welfare and productivity in dairy cattle. [Unpublished PhD Thesis]. University of Exeter.
20. Boyland, N.K., D.T. Mlynski, R. James, L.J.N Brent, and D.P. Croft. 2016. The social network structure of a dynamic group of dairy cows: from individual to group level patterns. *Appl. Ani. Behav. Sci.* 174: 1-10.
<https://doi.org/10.1016/j.applanim.2015.11.016>.
21. Brennan, M.L., R. Kemp, and R.M. Christley. 2008. Direct and indirect contacts between cattle farms in north-west England. *Prev. Vet. Med.* 84(3): 242-260.
<https://doi.org/10.1016/j.prevetmed.2007.12.009>.
22. Brin, S., and L. Page. 1998. The anatomy of a large-scale hypertextual web search engine. *International Conference on World Wide Web*. Brisbane, Australia: Elsevier Science Publishers B. 30: 107-117.
23. Brown, E. A.H. Marshall, H.J. Mitchell, and A.W. Byrne. 2019. Cattle movements in Northern Ireland form a robust network: implications for disease management. *Prev. Vet. Med.* 170: 104740. <https://doi.org/10.1016/j.prevetmed.2019.104740>.
24. Brzoska, L., M. Fischer, and H.H.K. Lentz. 2020. Hierarchical structures in livestock trade networks- A stochastic block model of the German cattle trade network. *Front. Vet. Sci.* 7:281. <https://doi.org/10.2289/fvets.2020.00281>.
25. Buhnerkempe, M.G., D.A. Grar, K. Portacci, R.S. Miller, J.E. Lombard, and C.T. Webb. 2013. A national-scale picture of U.S. cattle movements obtained from interstate certificate of veterinary inspection data. *Prev. Vet. Med.* 112(3-4): 317-329.
<https://doi.org/10.1016/j.prevetmed.2013.08.002>.

26. Cabezas, A.H., M.W. Sanderson, C.Y. Lockhart, K.A. Riley, and C.J. Hanthorn. 2021. Spatial and network analysis of U.S. livestock movements based on interstate certificates of veterinary inspection. *Prev. Vet. Med.* 193: 105391. <https://doi.org/10.1016/j.prevetmed.2021.105391>.
27. Chapa, J.M., L. Lidauer, A. Steininger, M. Öhlschuster, T. Potrusil, M. Sigler, W. Auer, M. Azizzadeh, M. Dillich. And M. Iwersen. 2021. Use of real-time location system to detect cows in distinct functional areas within a barn. *J. Dairy. Sci. Com.* 2(4): 217-222. <https://doi.org/10.3168/jdsc.2020-0050>.
28. Christley, R.M., and N.P. French. 2003. Small-world topology of UK racing: the potential for rapid spread of infectious agents. *Equine. Vet. J.* 35: 586-589. <https://doi.org/10.2746/042516403775467298>.
29. Christley, R., S. Robinson, R. Lysons, and N. French. 2005. Network analysis of cattle movement in Great Britain. *Proc. Soc. Vet. Med. Epidemiol. Prev. Med.* 234-243.
30. Corner, L.A.L., D.U. Pfeiffer, and R.S. Morris. 2003. Social-network analysis of *Mycobacterium bovis* transmission among captive brushtail possums (*Trichosurus vulpecula*). *Prev. Vet. Med.* 59: 147-167. [https://doi.org/10.1016/S0167-5877\(03\)00075-8](https://doi.org/10.1016/S0167-5877(03)00075-8).
31. Craft, M.E. 2015. Infectious disease transmission and contact networks in wildlife and livestock. *Phil. Trans. R. Soc. B.* 370: 20140107. <https://doi.org/10.1098/rstb.2014.0107>.
32. Cuomo, S., and F. Maiorano. 2018. Social network data analysis and mining applications for the internet of data. *Concurr. Comput. Pract. Exp.* 30(15): e4527. <https://doi.org/10.1002/cpe.4527>.

33. de Freslon, I., B. Martínez-López, J. Belkhiria, A. Strappini, and G. Monti. 2019. Use of social network analysis to improve the understanding of social behavior in dairy cattle and its impact on disease transmission. *Appl. Anim. Behav. Sci.* 213:47-54. <https://doi.org/10.1016/j.applanim.2019.01.006>.
34. de Menezes, T.C., I. Luna, and S.H.G. de Miranda. 2020. Network analysis of cattle movement in Mato Grosso Do Sul (Brazil) and implications for Foot-and-Mouth Disease. *Front. Vet. Sci.* 7:219. <https://doi.org/10.3389/fvets.2020.00219>.
35. de Mol, R.M. 2000. Automated detection of estrus and mastitis in dairy cows. [Unpublished PhD Thesis]. Wageningen University.
36. Delagard, R., and P. Lamberton. 2015. Daily grazing time of dairy cows is recorded accurately using the Lifecorder Plus device. *Appl. Anim. Behav. Sci.* 165: 25-32. <https://doi.org/10.1016/j.applanim.2015.01.014>.
37. Devant, M., S. Marti, and A. Bach. 2012. Effects of castration on eating patterns and physical activity of Holstein bulls fed high-concentrate rations under commercial conditions. *J. Anim. Sci.* 90(12): 4505-4513. <https://doi.org/10.2527/jas.2011-4929>.
38. Dijkstra, E.W. 1959. A note on two problems in connexion with graphs. *Numerische Mathematik.* 1: 269-271.
39. Drewe, J.A. 2010. Who infects whom? Social networks and tuberculosis transmission in wild meerkats. *Proc. R. Soc. B.* 277: 633-642. <https://doi.org/10.1098/rspb.2009.1775>.
40. Dubé, C., C. Riddle, D. Kelton, and B. McNab. 2008. Comparing network analysis measures to determine potential epidemic size of highly contagious exotic diseases in fragmented

- monthly networks of dairy cattle movements in Ontario, Canada. *Transb. Emerg. Dis.* 55: 382-392. <https://doi.org/10.1111/j.1865-1682.2008.01053.x>.
41. Dubé, C., C. Riddle, D. Kelton, and B. McNab. 2009. A review of spatio analysis terminology and its application to food-and-mouth disease modelling and policy development. *Transb. Emerg. Dis.* 56: 73-85. <https://doi.org/10.1111/j.1865-1682.2008.01064.x>.
42. Dubé, C., C. Ribble, D. Kelton, and B. McNab. 2011. Introduction to network analysis and its implications for animal disease modelling. *Rev. sci. tech. Off. int. Epiz.* 30(2):425-436.
43. Duncan, A.J., A. Reeves, G.J. Gunn, and R.W. Humphry. 2022. Quantifying changes in the British cattle movement network. *Prev. Vet. Med.* 198:105524. <https://doi.org/10.1016/j.prevetmed.2021.105524>.
44. Dutta, B.L., P. Ezanno, and E. Vergu. 2014. Characteristics of the spatio-temporal network of cattle movements in France over a 5-year period. *Prev. Vet. Med.* 177: 79-94. <https://doi.org/10.1016/j.prevetmed.2014.09.005>.
45. El Allaki, F., N. Harrington, and K. Howden. 2016. Assessing the sensitivity of bovine tuberculosis surveillance in Canada's cattle population, 2009-2013. *Prev. Vet. Med.* 134: 145-152. <https://doi.org/10.1016/j.prevetmed.2016.10.012>.
46. Enright, J.A., and A. O'Hare. 2016. Reconstructing disease transmission dynamics from animal movements and test data. *Stoch. Enviorn. Res. Risk. Assess.* 31: 369-377. <https://doi.org/10.1007/s00477-016-1354-z>.

47. Ferguson, N.M., C.A. Donnelly, and R.M. Anderson. 2001. The foot-and-mouth epidemic in Great Britain: pattern of spread and impact of interventions. *Science*. 292(5519): 1155-1160. <https://doi.org/10.1126/science.1061020>.
48. Fielding, H.R., T.J. McKinley, M.J. Silk, R.J. Delahay, and R.A. McDonald. 2019. Contact chains of cattle farms in Great Britain. *R. Soc. Open. Sci.* 6. <https://doi.org/10.1098/rsos.180719>.
49. Fielding, H.R., T.J. McKinley, R.J. Delahay, M.J. Silk, and R.A. McDonald. 2020. Effects of trading networks on the risk of bovine tuberculosis incidents on cattle farms in Great Britain. *R. Soc. Open. Sci.* 7. <https://doi.org/10.1098/rsos.191806>.
50. Forde, K., A. Hillberg-Seitzinger, D. Dargatz, and N. Wineland. 1998. The availability of state-level data on interstate cattle movements in the United States. *Prev. Vet. Med.* 37: 209-217.
51. Foris, B., M. Zebunke, J. Langbein, and N. Melzar. 2019. Comprehensive analysis of affiliative and agonistic social networks in lactating dairy cattle groups. *Appl. Ani. Behav. Sci.* 210: 60-67. <https://doi.org/10.1016/j.applanim.2018.10.016>.
52. Freeman, L. C., 1978. Centrality in social networks: Conceptual clarification. *Social Networks* 1, 215-239.
53. Frössling, J., A. Ohlson, C. Björkman, N. Håkansson, and M. Nöremark. 2012. Application of network analysis parameters in risk-based surveillance- examples based on cattle trade data and bovine infections in Sweden. *Prev. Vet. Med.* 105: 202-208. <https://doi.org/10.1016/j.prevetmed.2011.12.011>.

54. George, A.J., and S.L. Bolt. 2020. Understanding the social behavior of dairy cattle can benefit welfare and productivity. *Livestock*. 25(5).
55. Gibbens, J.C., J.W. Wilesmith, C.E. Sharpe, L.M. Mansley, E. Michalopoulou, J.B.M. Ryan, and M. Hudson. 2001. Descriptive epidemiology of the 2001 foot-and-mouth disease epidemic in Great Britain: the first five months. *Vet. Record*. 149(24): 729-743.
<https://doi.org/10.1136/vr.149.24.729>.
56. Godsk, T., and M. Kjærgaard. 2011. High classification rates for continuous cow activity recognition using low-cost GPS positioning sensors and standard machine learning techniques. *Lect. Note Comput. Sci.* 6870: 174-188.
57. Golbeck, J. 2015. *Introduction to Social Media Investigation: A Hands-on Approach* (1st. ed.). Syngress Publishing.
58. González, I.A., G.J. Bishop-Hurley, R.N. Handcock, and C. Crossman. 2015. Behavioral classification of data from collars containing motion sensors in grazing cattle. *Comput. Electron. Agric.* 110: 91-102. <https://doi.org/10.1016/j.compag.2014.10.018>.
59. Grear, D.A., J.B. Kaneena, J.J. Averill, and C.T. Webb. 2014. Local cattle movements in response to ongoing bovine tuberculosis zonation and regulations in Michigan, USA. *Prev. Vet. Med.* 114:201-212. <https://doi.org/10.1016/j.prevetmed.2014.03.008>.
60. Grooms, D. 2007. Radio frequency identification (RFID) technology for cattle. Extension Bulletin E-2970. Michigan RFID Education Task Force, Michigan State University Extension.
61. Gygas, L., G. Neisen, and B. Wechsler. 2010. Socio-spatial relationships in dairy cows. *Ethology*. 116(1): 10-23. <https://doi.org/10.1111/j.1439-0310.2009.01708.x>.

62. Haydon, D.T., M. Chase-Topping, D.J. Shaw, L. Matthews, J.K. Friar, J. Wilesmith, and M.E.J. Woolhouse. 2002. The construction and analysis of epidemic trees with reference to the 2001 UK foot-and-mouth outbreak. *Proc. R. Soc. Lond. B.* 270: 121-127.
<https://doi.org/10.1098/rspb.2002.2191>.
63. Hidano, A., T.E. Carpenter, M.A. Stevenson, M.C. Gates. 2016. Evaluating the efficacy of regionalization in limiting high-risk livestock trade movements. *Prev. Vet. Med.* 133: 31-41. <https://doi.org/10.1016/j.prevetmed.2016.09.015>.
64. Holland, P.W., and S. Leinhardt. 1971. Transitivity in structural models of small groups. *Comp. Group. Stud.* 2(2): 107-124.
65. Kao, R., and I. Kiss. 2010. Network concepts and epidemiological models. In: Stumpf, M., Wiuf, C. (Eds.), *Statistical and Evolutionary Analysis of Biological Networks*. Imperial College Press, pp. 85-112 (Chapter 5).
66. Kiss, I.Z., D.M. Green, and R.R. Kao. 2006. Infectious disease control using contact tracing in random and scale-free networks. *J. R. Soc. Interface.* 3: 55-62.
<https://doi.org/10.1098/rsif/2005/0079>.
67. Koelch, R.K. 1992. Application of activity, milk progesterone and heart rate sensors for dairy reproduction management. [Unpublished PhD Thesis]. Cornell University.
68. Kowalski, A. 2000. The phenomenon of dominance and its physiological implications in animals. *Med. Weter.* 56(9): 543-546.
69. Lal Dutta, B., P. Ezanno, and E. Vergu. 2014. Characteristics of the spatio-temporal network of cattle movements in France over a 5-year period. *Prev. Vet. Med.* 117: 79-94.
<https://doi.org/10.1016/j.prevetmed.2014.09.005>.

70. Lancichinetti, A., and S. Fortunato. 2009. Community detection algorithms: a comparative analysis. *Physical review E*, 80(5):056117.
71. Lehrer, A.R., G.S. Lewis, and E. Aizenbud. 1992. Estrus detection in cattle: A recent development. *Anim. Reprod. Sci.* 28: 355-361.
72. Lembo, T., H. Auty, K. Hampson, M.E. Craft, A. Dobson, R. Fyumagwa, E. Ernest, D. Haydon, R. Hoare, M. Kaare, F. Lankester, T. Mlengeya, D. Travis, and S. Cleaveland. 2015. Infectious Diseases in the Serengeti: What We Know and How We Know It. In *Serengeti IV: Sustaining biodiversity in a coupled human-natural system*. University of Chicago Press, pp: 532-581 (Chapter 19).
<https://doi.org/10.7208/chicago/9780226196336.003.0019>.
73. León, E.A., M.A. Stevenson, S.J. Duffy, M. Ledesma, and R.S. Morris. 2006. A description of the cattle movements in two departments of Buenos Aires province, Argentina. *Prev. Vet. Med.* 76: 109-120. <https://doi.org/10.1016/j.prevetmed.2006.04.010>.
74. Lindström, T., S. Sisson, M. Nöremark, A. Jonsson, and U. Wennergren. 2009. Estimation of distance related probability of animal movements between holdings and implications for disease spread modeling. *Prev. Vet. Med.* 91: 85-94.
<https://doi.org/10.1016/j.prevetmed.2009.05.022>.
75. Lloyd-Smith, J.O., S.J. Schreiber, P.E. Kopp, and W.M. Getz. 2005. Superspreading and the effect of individual variation on disease emergence. *Nature*. 438: 355-359.
<https://doi.org/10.1038/nature04153>.
76. Luce, R.D., and A.D. Perry. 1949. A method of matrix analysis of group structure. *Psychometrika*. 14(2): 95-116.

77. Lui, W.-C., L.-C. Huang, C.W.-J. Liu, and F. Jordán. 2020. A simple approach for quantifying node centrality in signed and directed social networks. *App. Netw. Sci.* 5(46). <https://doi.org/10.1016/j.appnet.2020.100288>.
78. Mao, G., and N. Zhang. 2013. Analysis of average shortest-path length of scale-free network. *J. Appl. Math.* 2013: 865643. <https://doi.org/10.1155/2013/865643>
79. Martínez-López, B., A. M. Perez, and J. M. Sánchez-Vizcaíno. 2009. Social network analysis. Review of general concepts and use in preventative veterinary medicine. *Transb. Emerg. Dis.* 56: 109-120. <https://doi.org/10.1111/j.1865-1682.2009.01073.x>.
80. Mazrier, H., S. Tal, E. Aizinbud, and U. Bargai. 2006. A field investigation of the use of the pedometer for early detection of lameness in cattle. *Can. Vet. J.* 47: 883-886.
81. McPherson, M., L. Smith-Lovin, and J.M. Cook. 2001. Birds of a feather: homophily in social networks. *Annu. Rev. Sociol.* 27(2001): 415-444.
82. Meunier, B., P. Pradel, K.H. Sloth, C. Cirié, E. Delval, M.M. Mialon, and I. Veissier. 2017. Image analysis to refine measurements of dairy cow behaviour from a real-time location system. *Biosyst. Eng.* 173: 32-44. <https://doi.org/10.1016/j.biosystemseng.2017.08.019>.
83. Mewu, M.M., G. Fournié, T. Halasa, N. Toft, and S.S. Nielsen. 2013. Temporal characterization of the network of Danish cattle movements and its implication for disease control: 2000-2009. *Prev. Vet. Med.* 110: 379-387. <https://doi.org/10.1016/j.prevetmed.2013.02.015>.
84. Mohr, S., M. Deason, M. Churakov, T. Doherty, and R.R. Kao. 2018. Manipulation of contact network structure and the impact of foot-and-mouth disease transmission. *Prev. Vet. Med.* 157: 8-18. <https://doi.org/10.1016/j.prevetmed.2018.05.006>.

85. Myers, J.P. 1983. Space, time and the pattern of individual associations in a group living species: Sanderlings have no friends. *Behav. Ecol. And Sociobiol.* 12: 129-134.
86. Natale, F., A. Giovanni, L. Savini, D. Palma, L. Possenti, G. Fiore, and P. Calistri. 2009. Network analysis of Italian cattle trade patterns and evaluation of risks for potential disease spread. *Prev. Vet. Med.* 92: 341-350.
<https://doi.org/10.1016/j.prevetmed.2009.08.026>.
87. Negre, C.F.A., U.N. Morza, H.P. Hendrickson, R. Pal, G.P. Lisi, J.P. Loria, I. Rivalta, J. Ho, and V.S. Batista. 2018. Eigenvector centrality for characterization of protein allosteric pathways. *Proc. Natl. Acad. Sci.* 115(52): E12201–E12208.
<https://doi.org/10.1073/pnas.1810452115>.
88. Newman, M.E. 2004. Fast algorithm for detecting community structure in networks. *Phys. Rev.* 69(6): 066133.
89. Newman, M.E.J. 2005. A measure of betweenness centrality based on random walks. *Soc. Networks.* 27(1): 39-54.
90. Newman, M.E.J. 2006. Modularity and community structure in networks. *Proc. Natl. Acad. Sci.* 103(23): 8577-8582.
91. Newman, M.E.J., 2010. *Networks: An Introduction*. Oxford University Press, New York, USA.
92. Nicolas, G., B. Durand, R. Duboz, R. Rakotondravao, and V. Chevalier. 2013. Description and analysis of the cattle trade network in the Madagascar highlands: Potential role in the diffusion of Rift Valley fever virus. *Acta. Tropic.* 126: 19-27.
<https://doi.org/10.1016/j.actatropica.2012.12.013>.

93. Noopataya, S., S. Thongratsakul, and C. Poolkhet. 2015. Social network analysis of cattle movement in Sukhothai province, Thailand: a study to improve control measures. *Vet. Med. Int.* 2015: 587275. <https://doi.org/10.1155/2015/587252>.
94. Opsahl, T., F. Agneessens, and J. Skvoretz. 2010. Node centrality in weighted networks: Generalizing degree and shortest paths. *Soc. Networks.* 32: 245-251.
95. Ortiz-Pelaez, A., D. Pfeiffer, R. Soares-Magalhaes, and F. Guitian. 2006. Use of social network analysis to characterize the pattern of animal movements in the initial phases of the 2001 foot and mouth disease (fmd) epidemic in the UK. *Prev. Vet. Med.* 76: 40-55. <https://doi.org/10.1016/j.prevetmed.2006.04.007>.
96. Otte, E., R. Rousseau, 2002. Social network analysis: a powerful strategy, also for the information sciences. *J. Inf. Sci.* 28(6):441-453.
97. Ozkan-Canbolat, E., and A. Beraha. 2016. Configuration and innovation related network topology. *J. Innov. Knowl.* 14. <https://doi.org/10.1016/j.jik.2016.01.013>.
98. Page, L., S. Brin, R. Motwani, and T. Winograd. 1999. The pagerank citation ranking: bringing order to the web: Technical Report. Stanford University. 1999-66.
99. Palisson, A., A. Courcoul, and B. Durand. 2016. Role of cattle movements in bovine tuberculosis spread in France between 2005 and 2014. *PloS One.* 11(3): eo52578. <https://doi.org/10.1371/journal.pone.0152578>.
100. Pica-Ciamarra, U., D. Baker, N. Morgan, C. Ly, and S. Nouala. 2013. Investing in African livestock: Business opportunities in 2030-2050. Rome FAO. <https://www.fao.org/3/al757e/al757e.pdf>.

101. Poolkhet, C., S. Kasemsuwan. S. Seng, C. Keartha, C. Sokmao, M. Shin, W. Kalpravidh, and J. Hinrichs. 2016. Social network analysis of cattle movement in Kampong Cham, Kampong Speu and Takeo, Cambodia. *Acta. Tropica*. 159: 44-49.
<https://doi.org/10.1016/j.actatropica.2016.03.027>.
102. Portacci, K., R.S. Miller, P.D. Riggs, M.G. Buhnerkempe, and L.M. Abrahamsen. 2013. Assessment of paper interstate certificates of veterinary inspection used to support disease tracing in cattle. *J. Am. Vet. Med. Assoc.* 243(4): 555-560.
<https://doi.org/10.2460/javma.243.4.555>.
103. Pritchard, A. 1984. On the structure of information transfer networks. PhD Dissertation. School of Librarianship, Polytechnic of North London.
104. Provan, K.G., A. Fish, and J. Sydow. 2007. Interorganizational networks at the network level: a review of the empirical literature on whole networks. *J. Manage.* 33(3): 479-516.
105. Raghavan, P. 2001. Social networks on the web and in the enterprise. In: N. Zhong, Y.Y. Yao, J. Liu, and S. Ohsuga (eds), *Web Intelligence: Research and Development. Lecture Notes in Artificial Intelligence* 2198. Springer, Berlin. 58-60.
106. Rautureau, S., B. Dufour, and B. Durand. 2011. Vulnerability of animal trade networks to the spread of infectious disease: a methodological approach applied to evaluation and emergency control strategies in cattle, France, 2005. *Transb. Emerg. Dis.* 58: 110-120.
<https://doi.org/10.1111/j.1865-1682.2010.01187.x>.
107. Reinhardt, V., and A. Reinhardt. 1981. Cohesive relationships in a cattle herd (*Bos indicus*). *Behav.* 77(3): 121-150.

108. Richeson, J.T., T.E. Lawrence, and B.J. White. 2018. Using advanced technologies to quantify beef cattle behavior. *Transl. Anim. Sci.* 2: 223-229.
<https://doi.org/10.1093/tas/txy004>.
109. Robert, B., B.J. White, D.G. Renter, and R.L. Larson. 2009. Evaluation of three-dimensional accelerometers to monitor and classify behavior patterns in cattle. *Comp. Electron. Agric.* 67(1-2): 80-84. <https://doi.org/10.1016/j.compag.2009.03.002>.
110. Robinson, S.E., and R.M. Christley. 2007. Exploring the role of auction markets in cattle movements within Great Britain. *Prev. Vet. Med.* 81: 21-37.
<https://doi.org/10.1016/j.prevetmed.2007.04.011>.
111. Robinson, T.P., G.R.W. Wint, G. Conchedda, T.P. Van Boeckel, V. Ercoli, E. Palamara, G. Cinardi, L. D'Aietti, S.I. Hay, and M. Gilbert. 2014. Mapping the global distribution of livestock. *PLoS ONE*. 9(5): e96084. <https://doi.org/10.1371/journal.pone.0096084>.
112. Rocha, L.E.C., O. Terenius, I. Veissier, B. Meunier, and P.P. Nielsen. 2020. Persistence of sociality in group dynamics in dairy cattle. *Appl. Anim. Behav. Sci.* 233: 104921.
<https://doi.org/10.1016/j.applanimal.2019.104921>.
113. Rogalski, M. 1972. Hierarchy in a cattle herd. *Prz. hod.* 11: 13-15.
114. Sabidussi, G. 1966. The centrality index of a graph. *Pyschometrika*. 31(4): 581-603.
<https://doi.org/10.1007/bf02289527>.
115. Salathé, M., and J.H. Jones. 2010. Dynamics and control of diseases in networks with community structure. *PLoS Comput. Biol.* 65: e1000736.
<https://doi.org/10.1371/journal.pcbi.1000736>.

116. Schofield, S.A. 1998. Estrus detection methods and estrus behavior of dairy cows in different environment. [Unpublished PhD Thesis]. University College of North Wales.
117. Sembiring, F.A., F. Zuska, B. Ginting, R. Ismail, and H. Sitorus. 2019. Ego-centric social network analysis in marketing of fish from cage culture system in Simalungun District, Indonesia. *Int. J. Res. Bus. Soc. Sci.* 8(4): 105-113.
<https://doi.org/10.20525/ijrbs.v8i4.282>.
118. Shane, D.D., B.J. White, R.L. Larson, D.E. Amrine, and J.L. Kramer. 2016. Probabilities of cattle participating in eating and drinking behavior when located at feeding and watering locations by real time location system. *Comput. Electron. Agric.* 127: 460-466.
<https://doi.org/10.1016/j.compag.2016.07.005>.
119. Sołtysiak, T., and Z. Nogalski. The effects of social hierarchy in a dairy cattle herd on milk yield. 2010. *Pol. J. Natur. Sc.* 25(1):22-30. <https://doi.org/10.2478/v10020-010-0002-1>.
120. Sosa, S., C. Sueur, and I. Puga-Gonzalez. 2020. Network measures in animal social network analysis: Their strengths, limits, interpretations and uses. *Methods Ecol. Evol.* 12(1): 10-21. <https://doi.org/10.1111/2041-210X.13366>.
121. Stankovski, S., G. Ostojic, I. Senk, M. Rakic-Skokovic, S. Trivunovic, and D. Kucevic. 2012. Dairy cow monitoring by RFID. *Sci. Agric.* 69(1): 75-80.
122. Sueur, C., A. Jacobs, F. Amblard, O. Petit, and A.J. King. 2011. How can social network analysis improve the study of primate behavior? *Am. J. Primatol.* 73(8): 703-719.
<https://doi.org/10.1002/ajp.20915>.
123. Umemura, K. 2013. Technical note: Monitoring grazing bites and walking activity with pedometers. *J. Dairy. Sci.* 96(2): 1090-1093. <https://doi.org/10.3168/jds.2012-5872>.

124. VanderWaal, K.L., C. Picasso, E.A. Enns, M.E. Craft, J. Alvarez, F. Fernandez, A. Gil, A. Perez, and S. Wells. 2016. Network analysis of cattle movements in Uruguay: Quantifying heterogeneity for risk-based disease surveillance and control. *Prev. Vet. Med.* 123: 12-22. <https://doi.org/10.1016/j.preventmed.2015.12.003>.
125. Venon, M.C., and M.J. Keeling. 2009. Representing the UK's cattle herd as static and dynamic networks. *Proc. R. Soc. B: Biol. Sci.* 276: 469-476. <https://doi.org/10.1098/rspb.2008.1009>.
126. Vimalajeewa, D., S. Balasubramaniam., B. O'Brian, C. Kulantunga, and D.P. Berry. 2019. Leveraging social network analysis for characterizing cohesion of human-managed animals. *IEEE Trans. Comput. Soc.* 6(2): 333-344. <https://doi.org/10.1109/TCSS.2019.2902456>.
127. Vindondo, B. and B. Voelkl. 2018. Dynamic network measures reveal the impact of cattle markets and alpine summering on the risk of epidemic outbreaks in Swiss cattle population. *BMC Vet. Res.* 14. <https://doi.org/10.1186/s12917-018-1406-3>.
128. Vinueza, R.L., B. Durand, and G. Zanella. 2022. Network analysis of cattle movements in Ecuador. 2022. *Prev. Vet. Med.* 201:105608. <https://doi.org/10.1016/j.prevetmed.2022.105608>.
129. Volkova, V.V., R. Howey, N.J. Savill, and M.E.J. Woolhouse. 2010. Potential for transmission of infections in networks on cattle farms. *Epidemics.* 2: 116-122. <https://doi.org/10.1016/j.epidem.2010.05.004>.
130. Wasserman, S., and K. Faust. 1994. *Social Network Analysis: Methods and Applications*. Cambridge University Press, New York, New York.

131. Watts, D.J., and S.H. Strogatz. 1998. Collective dynamics of “small-world” networks. *Nature*. 393: 440-442.
132. Wellman, B, and S.D. Berkowitz. 1988. *Structural Analysis in Social Sciences 2: Social Structures: A Network Approach*. Cambridge University Press, Cambridge.
133. Wey, T., D.T. Blumstein, W. Shen, and F. Jordán. 2008. Social networks analysis of behavior: a promising tool for the study of sociality. *Anim. Behav.* 75(2): 333-344. <https://doi.org/10.1016/j.anbehav.2007.06.020>.
134. Whitehead, H. 2008. *Analyzing animal societies: quantitative methods for vertebrate social analysis*. University of Chicago Press.
135. Woolhouse, M.E.J., D.J. Shaw, L. Matthews, W.C. Liu, D.J. Mellor, and M.R. Thomas. 2005. Epidemiological implications of the contact network structure for cattle farms and the 20-80 rule. *Biol. Lett.* 1: 350-352. <https://doi.org/10.1098/rbssl.2005.0331>.
136. Yang, J., and J. Leskovec. 2013. Overlapping community detection at scale: a nonnegative matrix factorization approach. In *Proceedings of the sixth ACM international conference on Web search and data mining*. 587-596.
137. Zhou, Y., H. Cheng, and J.X. Yu. 2009. Graph clustering based on structural/attribute similarities. *Proceedings of the VLDB Endowment*. 2(1): 717-729.

Table 1-1. Glossary of Network Terminology.

Term	Definition
Closed Triplet	Three nodes fully interconnected between each other
Closeness	Reciprocal of farness
Clique	A subgraph of a network in which all nodes are connected. The term “complete subgraph” is a synonym in literature.
Community	A group of densely interconnected nodes that share similar properties. The term “cluster” is used as a synonym in literature.
Density	Number of connections present in a network over the total amount of possible connections
Diameter	The longest path of the shortest paths in a network
Degree	Number of individual connections a node has
Directed link	Connection within a network that considers directionality. Term arc is used as a synonym in literature.
Farness	Sum of distances from a particular node to all other nodes in a network
Fragmentation	Number of pairs of nodes that are disconnected from each other
Giant Strongly Connected Component (GSCC)	In a directed network, the largest group of nodes that are mutually connected
Giant Weakly Connected Component (GWCC)	In a directed network, those nodes that are captured in the GSCC plus the nodes that have links into and out of the GSCC
In-degree	In a directed network, the number of edges entering a node. Term “incoming link” is used as a synonym in literature.
Node	Element of a network representing an individual. The term “vertex” is used as synonym in literature.
Open Triplet	Three nodes interconnected to each other by only two connections
Out-degree	In a directed network, the number of edges leaving a node. The term “outgoing link” is used as a synonym in literature.
Path length	The distance between two nodes. The term “natural distance” is used as a synonym in literature.
Strength	Sum of edge weights of a particular node. The term weighted degree is used as a synonym in literature.
Subgraph	A subset of nodes within a network

Undirected link	Connection within a network that does not consider directionality. The term “edge” is used as a synonym in literature.
Weight	Value of a link representing the frequency of the interaction/association

Chapter 2 - Nonpathogenic *Escherichia coli* strains as a surrogate for Shiga toxin-producing *E. coli*- fecal shedding dynamics and pen environmental contamination in weaned calves and feedlot steers

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Abstract

Objective: The purpose of this study was to use non- pathogenic *Escherichia coli* as a surrogate to assess the transmission dynamics of Shiga toxin–producing *E. coli* in cattle.

Materials and Methods: Two studies were performed. For the first study, 3 nonpathogenic *E. coli* strains were orally inoculated into 12 weaned Holsteins calves. Calves were sampled daily the first week and 3 times a week for the following 4 wk. The nonpathogenic *E. coli* strain with the highest average fecal shedding concentration was chosen for use in the second study. The second study was performed twice over consecutive summers. In each summer, 5 steers were inoculated in a pen of 70 feedlot steers and shedding and transmission were monitored.

Results and Discussion: For the first study, all inoculated strains were shed in the feces of the calves and were detected in the hide, oral, and pen surface samples. During both years of the second study, fecal shedding in the steers was established, as well as transmission of the

inoculated strain between steers and into the pen environment. The inoculated strain was also detected in hide surface and oral cavity samples, as well as pen surface, water, and feed samples.

Implications and Applications: These studies provide baseline data on shedding and transmission of a non-pathogenic *E. coli* strain in dairy calves and feedlot steers and detection in the environment. These data will be useful for studying host-to-host and host-to-environment contact structures and to develop a modeling framework for *E. coli* transmission in a commercial feedlot setting.

Introduction

Cattle have been recognized as a major reservoir of *Escherichia coli* O157:H7 (Ferens and Hoyde, 2011), a Shiga toxin-producing serotype (STEC), which has been of concern to the commercial beef industry. Other STEC have been identified, and cattle have been shown to be a reservoir of STEC, with the prevalence of *E. coli* O157 in individual pens of feedlot cattle ranging from 10 to 28% but occasionally as high as 80% in the summer months (Callaway et al., 2003). Shiga toxin-producing *E. coli* have also been found on the pen floors and hides of cattle, as well as cattle feed, making these important potential environmental sources for STEC transmission between animals (Cobbold and Desmarchelier, 2002; Dodd et al., 2003). Water troughs that have been contaminated with feces are another important environmental source that may contribute to the transmission of STEC (Faith et al., 1996; Hancock et al., 1997; Shere et al., 1998; LeJeune et al., 2001).

Though STEC are important human pathogens with significance to the beef cattle industry, the transmission dynamics of STEC in feedlot cattle are not fully understood (Van

Donkersgoed et al., 2001; Turner et al., 2003; Vosough Ahmadi et al., 2006). Current understanding of STEC transmission dynamics is dependent on the longitudinal study of naturally occurring STEC infections in cattle. In studies of naturally occurring STEC, the temporal initiation of infection is unknown as is the initial source. Inoculation studies where the temporal initiation and source is known must be done in a biosafety level 2 setting for biosafety reasons. As such, experiments to evaluate transmission in natural settings that account for the time of onset or exposure, environmental factors, cattle contact, and social dynamics have not been done. A well-characterized and easily detected nonpathogenic *E. coli* surrogate would facilitate research on direct and indirect transmission dynamics and potential identification of control methods for STEC in a non-biosafety-level, commercial feedlot setting. The use of a surrogate strain could also be used to establish baseline data for host-to- host and host-to-environment contact structures to better study pathogens with an environmental transmission component, such as *E. coli* and *Salmonella*. Our data provide a known initial onset and source as well as a known initially negative environment to collect data on transmission.

Our objective was to evaluate the use of nonpathogenic *E. coli* strains as surrogates for STEC shedding and transmission from a known initial exposure to describe the temporal progression of transmission. First, we describe shedding duration and concentrations of 3 nonpathogenic serotypes of *E. coli* in weaned Holstein calves and their pen environment. Second, we describe the shedding and transmission of one nonpathogenic *E. coli* strain in a 70-head pen research feedlot system as a surrogate for studying STEC transmission.

Materials and Methods

All procedures performed in studies involving animals were in accordance with the ethical standards of the Kansas State University College of Veterinary Medicine (IA- CUC #4065, IBC #1327).

Strain Selection and Classification

Wild strains of *E. coli* that were naturally nalidixic acid (50 µg/mL) resistant (**NalR**) were isolated from a group of commercial feedlot cattle in the summer of 2016. Eight isolates were submitted to the *E. coli* Reference Center at Pennsylvania State University to identify the serotype and verify virulence gene negative status for *estA*, *stx1*, *stx2*, *cnf1*, *cnf2*, *eae*, *K99*, *CS31A*, and *F41*. Rifampicin resistance was induced in virulence gene negative strains by incrementally increasing the concentration of rifampicin in Tryptic soy broth (ThermoFisher) over 10 d up to a concentration of 50 µg/mL. To compare the growth of the NalR parent strain and the nalidixic acid (50 µg/mL) and rifampicin (50 µg/mL) resistant strain (**NalR-RifR**), growth curves were done in triplicate (Figures A-1A–1C). Nalidixic acid is a quinolone antibacterial, and rifampicin is a macrocyclic antibiotic. Growth was quantitated by optical density readings every 15 min for 7.5 h, and the standard growth curves were then plotted (Table A-1). Strains selected for study 1 were chosen based on the similarity in the growth curves between the parent strain and the dual-resistant strain. The single strain selected for studies 2A and 2B was done based on the highest area under the curve concentration of the inoculated strain shed in the feces of the calves in study 1.

Study 1—Dairy Calf

Calf Arrival and Randomization. Twelve weaned Holstein calves were purchased from a commercial dairy producer in September of 2016 and transported to outdoor pens at the Large Animal Research Center at Kansas State University. Mean calf BW was 134 kg (SD = 19.5 kg). Calves were randomly assigned to 1 of 3 pens based on a random number generator, with 4 calves being assigned to each pen and all calves in each pen assigned one strain of *E. coli* for inoculation. Pens were selected so that there would be no fenceline contact between pens. Calves were fed commercial calf pelleted feed and provided ad libitum access to long-stem hay. All calves were given a single dose of enrofloxacin (Baytril 100, Bayer Corporation) by s.c. injection at 10 mg/kg on the second day after arrival. Enrofloxacin was chosen to control bovine respiratory disease risk. Furthermore, it can significantly decrease the concentration of *E. coli* in the feces during peak drug concentrations (Ferguson et al., 2019), thereby increasing likelihood of establishing colonization with the inoculated strain.

Inoculations. Calves were inoculated using an esophageal tube with an average of 6.6×10^8 cfu (95% CI 2.8×10^8 to 1.4×10^9 cfu) of their assigned *E. coli* strain each day for 5 consecutive days. Inoculations were done first thing in the morning, before calves were fed. The first 2 d of inoculations used 10 mL of fresh culture inoculum, and the final 3 d of inoculations were with 10 mL of frozen, premade culture that was thawed just before the inoculations. The inoculum was flushed through the esophageal tube with approximately 300 mL of distilled water. To control cross contamination of strains between groups, Tyvek suits and boot covers were worn and changed between each group. Gloves were changed between each calf.

Sample Collection. Fecal and pen surface samples were taken 1 d before the first day of inoculation to establish that all calves and their pen environment were negative for natural nalidixic acid– and rifampicin-resistant *E. coli*. Following the first day of inoculation, calf and pen surface and water tank samples were taken daily for 6 d. For the following 4 wk, samples were taken 3 times a week. Fecal samples were collected from the rectum, and oral and hide swabs were collected using a Speci-Sponge (Nasco) premoistened with 10 mL of buffered peptone water. Hide samples were collected by swabbing an area of approximately 400 cm² (20 cm wide × 20 cm long). Due to the frequency of sampling, the hide sampling sites were rotated between the right shoulder, right ribcage, right hip, left shoulder, left ribcage, or left hip. The sites were rotated between each sampling day in the order just listed, and all calves had the same location sampled on the same day. To control cross contamination of strains between groups and calves, Tyvek suits and boot covers were worn and changed between each group and gloves were changed between each calf.

For each pen on each sampling day, pen surface samples and a water sample from the water trough were collected. Pen surface samples were collected in 3 locations around the water tank. Approximately 10 g of soil was collected from each site using plastic spoons that were scooped into sterile Whirl-Pak bags (Nasco). The water tank area was selected because it was a high traffic area for the calves and to optimize the probability of detecting the presence and survival of the strains in the environment. For each pen, 10 mL of water was collected from the water trough using a screw-top conical tube.

Studies 2A and 2B—Feedlot

Calf Arrival and Randomization. Seventy Angus/ Angus-cross steers were purchased through commercial channels in May of each of the years 2018 (study 2A) and 2019 (study 2B). Steers were housed in an outdoor pen at a commercial research feedlot. The pen measured 38 by 30.5 m, providing approximately 17 m² of area per steer. The pen had a centrally located water tank, a 24.5-m-long fence-line bunk, and a 3-m-wide concrete apron behind the bunk and around the water tank (Figure A-2). Steers were fed a high forage-based diet consisting of ground hay, wet distillers' grain, and a mineral pack. Steers were fed a measured ration that was increased as needed by monitoring the feed bunk for consumption. All steers were given a single s.c. injection of enrofloxacin (Baytril 100) at 10 mg/kg the day after arrival to control bovine respiratory disease risk and improve inoculation success, as in study 1. All calves received 2 ear tags for visual identification, as well as a real-time location system ear tag. For both studies, pen surface soil samples were taken before steer arrival and fecal samples were collected from each steer on the day after arrival during tagging and metaphylaxis to determine that the pen environment and each steer were negative for *E. coli* resistant to nalidixic acid and rifampicin.

Inoculations. Five steers were randomly selected for inoculation. These steers were identified with a uniquely colored ear tag to facilitate identification and removal from the group on the days of inoculation. Inoculations were done each day for 5 consecutive days using an esophageal tube. For study 2A, the average concentration of freshly prepared culture was 1×10^9 cfu (95% CI 1.2×10^8 to 1.9×10^9). For study 2B, the average concentration of freshly prepared culture was 2.5×10^9 (95% CI 1.5×10^9 to 3.4×10^9). The 10 mL of inoculum culture was flushed through the esophageal tube with approximately 500 mL of distilled water. Gloves were

changed between each steer. Inoculations were done at approximately the same time every morning before the calves were fed.

Timeline. Fecal samples of the 5 selected steers used in the inoculation were collected on d 2 through 5 of inoculation. For study 2A, all steers and their pen surface, water tank, and feed were sampled once a week for 10 wk. For study 2B, all steers and their pen surface, water tank, and feed were sampled once a week for 9 wk.

Sample Collection. Following inoculation, all calves were sampled weekly starting on d 7. From each steer on each sampling day, 3 samples were taken: a fecal sample was collected from the rectum, an oral swab was taken using a Speci-Sponge premoistened with 10 mL of buffered peptone water, and a hide swab was taken using a Speci-Sponge premoistened with 10 mL of buffered peptone water. Hide samples were collected on an area of approximately 400 cm² (20 cm wide × 20 cm long) on the right upper hip of the steer. This site was the same during each weekly sampling. Gloves were changed between steers.

Environment samples were collected weekly on the same day as steers were sampled. Three types of pen environment samples were taken during each sampling day: pen surface, water, and feed samples. Pen surface samples were collected in 25 locations distributed systematically around the pen. At each pen surface site, approximately 10 g of soil was collected into sterile Whirl-Pak bags using plastic spoons. Ten locations along the concrete feed apron at the front of the pen and 15 samples equally spaced in a grid fashion throughout the rest of the pen were sampled, including around the water tank (Figure A-2). These areas were selected to cover the high traffic area around the concrete apron, containing the feed bunk and water trough, and to cover the rest of the pen environment to give an estimate of the strain presence and

concentration in the whole pen environment. Three 20-mL samples of the water from the water tank were collected into screw-top 50-mL conical tubes. The trough was stirred between samples, mixing up any residue on the sides and bottom of the trough. Five feed samples, evenly spaced along the bunk, were collected approximately a third of the way through the feeding duration of the calves. Gloves were changed between each sample.

Sample Processing

All samples were placed on ice and transported to the Pre-Harvest Food Safety laboratories at the Kansas State University College of Veterinary Medicine for same-day processing. Samples were initially processed for enumeration of NalR-RifR *E. coli*. Samples that were negative by enumeration were subjected to enrichment.

Fecal Sample Processing. Approximately 1 g of feces was transferred to 9 mL of *E. coli* broth (**EC broth**; ThermoFisher). Samples were then vortexed for 30 s, and 1 mL of solution was diluted in another 9 mL of EC broth. Both dilutions were again vortexed for 30 s before being spiral plated for enumeration. All 1:10 dilutions were incubated for enrichment at 37°C for 6 h, after which sample tubes were transferred to a refrigerator for storage.

Hide and Oral Sample Processing. Liquid contents were squeezed from sponges and transferred to sterile 15-mL sterile glass tubes. Samples were then vortexed for 30 s and spiral plated for enumeration. A total of 1 mL of the sample solution was added to 9 mL of EC broth and incubated at 37°C for 6 h before being transferred to a refrigerator for storage pending enumeration results.

Pen Soil Surface Sample Processing. Approximately 1 g of pen surface was added to 9 mL of EC broth. Samples were vortexed for 30 s and then spiral plated for enumeration. All

sample tubes were incubated for enrichment at 37°C for 6 h and then transferred to a refrigerator for storage pending enumeration results.

Water Sample Processing. The screw-cap conical tubes were vortexed for 30 s, and approximately 5 mL was transferred to a 15-mL sterile empty tube. These tubes were then vortexed for 30 s before being plated. A total of 1 mL of the original sample was added to 9 mL of EC broth and incubated for enrichment at 37°C for 6 h before being transferred to a refrigerator for storage pending enumeration results.

Feed Sample Processing (Studies 2A and 2B Only). Approximately 1 g of feed contents was added to 9 mL of EC broth. Samples were vortexed for 30 s and then spiral plated for enumeration. All sample tubes were incubated for enrichment at 37°C for 6 h and then transferred to a refrigerator for storage pending enumeration results.

A total of 100 µL of all sample suspensions was plated for enumeration onto MacConkey agar containing nalidixic acid (50 µg/mL) and rifampicin (50 µg/mL) using a spiral plater (Eddy Jet V. 1.23, IUL S. A.). Plates were incubated at 37°C for 18 to 24 h. After incubation, plates were counted by overlaying a counting grid on the agar plate that relates the colonies on the plate to the volume deposited in that area. Colonies were counted according to the manufacturer's instructions and guidelines outlined in the Bacteriological Analytical Manual (Maturin and Peeler, 2001).

After completion of sample enumeration, if the sample was recorded as no growth, the enrichment of that same sample was retrieved from the refrigerator and vortexed for 30 s and then approximately 10 µL was plated onto new MacConkey agar containing nalidixic acid (50

µg/ mL) and rifampicin (50 µg/mL). Plates were incubated for 18 to 24 h at 37°C, and plates were recorded as having “growth” or “no growth” of morphological *E. coli*.

Weather Data (Studies 2A and 2B Only)

Daily temperature and precipitation data were downloaded from the National Oceanic and Atmospheric Administration National Centers for Environmental Information (NOAA, 2021). The data set details maximum and minimum 24-h temperature (in °C) and daily precipitation (in cm) in Manhattan, Kansas.

Statistical Methods

Descriptive statistics for all studies were produced using Microsoft Excel. The R package flux 0.3-0 was used to evaluate area under the curve following the trapezoidal rule for fecal shedding of each calf during study 1.

Results and Discussion

Strain Selection

Six different O groups were represented in the 8 selected isolates that were submitted to the *E. coli* Reference Center at The Pennsylvania State University. Naturally nalidixic acid-resistant strains selected and adapted to rifampicin for study 1 were *E. coli* O28:H43, *E. coli* O19:H+, and *E. coli* O101:H10. Growth curves, maximum optical density, and mean specific growth rate for the 3 selected strains (NalR-RifR) used in study 1 were similar to their parent strains (NalR; Figures A-1A–1C), suggesting that the dual antibiotic resistance did not change the growth rate of the strain.

Study 1—Dairy Calves

In study 1, all calves had an enumerable level of the inoculated strain in their feces at least one sample day during the study. On the first day after inoculation (d 1), the average pen-level fecal shedding concentrations was 1.8 log₁₀CFU/g (O19:H+), 2.9 log₁₀CFU/g (O101:H10), and 2.4 log₁₀CFU/g (O28:H43; Figure A-3). The highest pen-level average fecal shedding for each strain was 3.9 log₁₀CFU/g (O19:H+, d 6), 4.4 log₁₀CFU/g (O101:H10, d 4), and 5.2 log₁₀CFU/g (O28:H43, d 32). Strain O28:H43 yielded the highest concentration in the feces by an individual calf at 5.8 log₁₀CFU/g on study d 32 (Table 2-1). Strain O19:H+ and strain O101:H10 were detected in the feces of at least one calf by enumeration on 17 (94%) and 16 (89%) of the sampling points, respectively (Table 2-2). Strain O28:H43 was enumerable in the feces of at least one calf for all sampling points. Strains O19:H+ and O28:H43 were last enumerable on d 34. Strain O101:H10 was last enumerable in calf feces on d 30 but was positive by enrichment in at least one calf through the end of the study. The average area under the curve was greatest for strain O28:H43 (Table 2-3).

All strains were detected on hide samples either by enumeration or enrichment. Strain O28:H43 had the highest concentration detected (2.9 log₁₀CFU/400 cm², d 3) on an individual hide sample (Table 2-1). Strain O19:H+ was enumerable on at least one individual calf hide on 6 (33%) sampling days and was last enumerable on d 30. Strain O101:H10 was detected by enumeration on at least one individual calf hide on 10 (56%) sampling days. Strain O28:H43 was enumerable on at least one individual calf hide sample on 8 (44%) sampling days. Strains O101:H10 and O28:H43 were last enumerable on study d 34.

All strains were detected in oral samples either by enumeration or enrichment. The highest oral concentration detected in an individual oral sample was strain O101:H10 ($3.0 \log_{10}\text{CFU/mL}$, d 4). Strain O19:H+ was enumerable in at least one oral sample on 5 (28%) sampling days, and all 4 oral samples were enumerable on d 3 and 4. Strain O101:H10 was enumerable in at least one oral sample on 10 (56%) sampling days, and all 4 oral samples were enumerable on study d 4 and 13. Strain O28:H43 was enumerable in at least one oral sample on 9 (50%) sampling days. The last day strains O19:H+ and O101:H10 were detected in an oral sample by enumeration was d 27. Strain O28:H43 was enumerable in one oral sample and detected by enrichment in another oral sample on d 34.

All strains were detected in the pen surface soil samples by either enumeration or enrichment. For all strains, the highest concentration detected was on study d 27. All 3 strains were enumerable in the soil samples on 13 (72%) sampling days. All soil samples from strain O19:H+ remained enumerable from study d 23 through the end of the study. All 3 soil samples for strain O101:H10 were enumerable on d 30; however, on d 32 and 34, only 2 of the soil samples were enumerable. All soil samples from strain O28:H43 remained enumerable from study d 27 through the end of the study.

Only 2 water samples were positive during the study period. A water sample of strain O19:H+ was detected positive by enrichment on study d 18, and a water sample of strain O28:H43 was enumerable at a concentration of $1.0 \log_{10}\text{CFU/mL}$ on study d 27.

In study 1, we established that 3 verified nonpathogenic *E. coli* strains were able to colonize the gastrointestinal tract of weaned Holstein calves, shed in their feces, and spread to their pen environment. Fecal shedding was established in the calves, though shedding was

variable between calves and days. In previous studies of STEC, day-to-day fecal shedding has been noted to be variable (Howe et al., 1976; Cray and Moon, 1995; Sanderson et al., 1999). This variability can be due to the range of shedding seen on an individual basis, as well as the transient nature of colonization of *E. coli* in the gut and the potential for reinfection. Though our inoculated strains established shedding in the calves, their shedding curves were more variable than those seen with calves that are experimentally inoculated with *E. coli* O157 (Sanderson et al., 1999). Our inoculated strains lack the *stx*- and LEE-associated gene communities. Though the functional associations between these genes are only speculated to confer adaptation benefits in the bovine intestinal environment (Arimizu et al., 2019), their absence could have disadvantaged our strains in the bovine hindgut. The variability may also be due to the intermittent shedding interacting with the lower limit of detection for both of our detection methods: spiral plating and EC broth enrichment.

For each calf in study 1, the total area under the curve was used to describe the concentration of fecal shedding and potential environmental transmission. This measure has been used previously to describe the concentration of *E. coli* shedding in calves in challenge studies (Naylor et al., 2007). Strain O28:H43 NaIR-RifR was chosen for use in studies 2A and 2B based on the greatest average area under the curve for fecal shedding.

Studies 2A and 2B—Feedlot

Study 2A—2018. In study 2A, 70 steers were enrolled in the study, and 5 were inoculated with *E. coli* O28:H43 NaIR-RifR. The mean arrival BW of the steers was 271.7 kg (SD = 19.4 kg). Steers were then sampled once a week for 10 wk (70-d study period). One steer was removed from the study on d 35 due to septic laminitis.

Fecal samples from all steers during initial processing (study d 0) were negative for *E. coli* resistant for both nalidixic acid and rifampicin antibiotics. The feces of 5 randomly selected steers used for the initial inoculations were positive by enrichment for *E. coli* O28:H43 NalR-RifR during the inoculation period (d 2 through 5). The highest concentration of the strain detected in the feces of an individual steer was $3.7 \log_{10}\text{CFU/g}$ (d 56, Table A-2). Feces of at least 1 of the 70 steers in the pen was enumerable for 7 (70%) of the sampling weeks (Table A-2). The first week (d 7) yielded the highest number of positive fecal samples detected by enumeration or enrichment with 34 positives (49%). No fecal samples were enumerable or positive by enrichment on d 63, and only 5 fecal samples were positive by enrichment on d 70. Mean shedding count of enumerable samples was $2.6 \log_{10}\text{CFU/g}$ of feces.

The average number of positive fecal samples detected throughout the entire study period per steer by either method (enumeration or enrichment) was 2 (Table A-2). Throughout the entire sampling period, 7 steers were positive in their feces for at least 2 consecutive weeks by either detection method (i.e., positive 1 wk via enumeration and positive 1 wk by enrichment or vice versa). Seventeen steers had at least 2 consecutive weeks in which their fecal samples were positive via enrichment. No steers had fecal samples that were enumerable for 2 consecutive sampling weeks. Seven steers were never fecal positive during the entire study period. Six of these 7 negative steers were enrolled in the entire study period, and 1 was removed on study d 35 because of septic laminitis.

The inoculated strain was detected on the hide samples of the individual steers by both enumeration and enrichment methods. The highest concentration detected on the hide of an individual steer was $2.3 \log_{10}\text{CFU}/400 \text{ cm}^2$ on study d 28. At least one hide sample was

enumerable for 5 (50%) of the sampling weeks (Table 2-4). Twelve hide samples were positive by enrichment on study d 70.

The inoculated strain was detected in the oral cavities of the individual steers by both enumeration and enrichment methods. The highest concentration detected in an individual steer was $3.1 \log_{10}\text{CFU/mL}$ on study d 7. At least one oral sample was positive by enrichment on 4 (40%) sampling weeks (Table 2-4). The last day an oral sample was positive by either enumeration or enrichment was study d 56.

All pen surface samples taken before steer arrival were negative for *E. coli* resistant to both nalidixic acid and rifampicin antibiotics. After steer inoculation, the inoculated strain was detected in the soil of the pen surface at the first sampling time (study d 7). The highest concentration detected in an individual pen surface sample was $3.79 \log_{10}\text{CFU/g}$ on study d 49. At least one pen surface sample was enumerable for 5 wk (50%; Table 2-5). The highest number of pen surface samples positive by either method occurred on d 42 with 23 (92%) positive samples. Day 49 was the last day a pen surface sample was enumerable with 2 (8%) of the samples positive. The last day a pen surface sample was positive by enrichment was d 56 with one (4%) sample being positive. No samples were positive via enumeration or enrichment on the last 2 wk of the study.

One water sample of the 3 taken on study d 42 was positive by enrichment. No other water samples were positive via enumeration or enrichment throughout the study.

All feed samples ($n = 5$) were positive by enrichment on study d 42. No other feed samples were positive via enumeration or enrichment throughout the study.

Local weather data obtained from the National Oceanic and Atmospheric Administration was downloaded for each sampling week, with the week starting on Monday (day of sampling) and ending on Sunday (the day before sampling; NOAA, 2021). The highest and lowest temperature recorded during the study period was 40.6°C (wk 6) and 12.2°C (wk 1; Table 2-6). The weekly total precipitation ranged from 0 cm (wk 3) to 3.9 cm (wk 2). The total precipitation for the study period was 13.6 cm, with an average weekly precipitation of 1.4 cm.

Study 2B—2019.

In Study 2B, 70 steers were enrolled in the study, and 5 were inoculated with *E. coli* O28:H43 NalR-RifR. The mean arrival BW of the steers in study 2B was 285.2 kg (SD = 26.7 kg). Steers were then sampled once a week for 9 week (63-d study period). Two steers were removed from the study due to chronic lameness: one on d 35 and another on d 56. Fecal samples taken from all steers during initial processing (study d 0) were negative for *E. coli* resistant to both nalidixic acid and rifampicin antibiotics. The feces of the 5 randomly selected steers used for the inoculation were all positive for *E. coli* NalR-RifR via enumeration throughout the entire inoculation period (d 2 through 5). The concentrations detected in the feces of the 5 steers throughout the inoculation period ranged from 3.7 log₁₀CFU/g to 5.7 log₁₀CFU/g (Table A-3). During the study, the highest concentration detected in a fecal sample was 4.9 /g on study d 7. All steers sampled yielded an enumerable count of the inoculated strain in the feces on d 56 (Table 2-7). On d 14 and 28, 45 and 44% of the steer fecal samples were enumerable, respectively. The last day an enumerable sample was detected in the feces was d 56, though samples remained positive by enrichment through the last week of the study. Mean shedding count of enumerable samples was 3.5 log₁₀CFU/g of feces.

All steers at some point in the study were positive in their feces for at least 2 consecutive weeks (Table A-3). The average number of positive fecal samples detected per calf by either enumeration or enrichment was 6. Thirty-three steers were positive by enrichment for at least 2 consecutive sampling weeks, and 21 steers were positive by enumeration for at least 2 consecutive sampling weeks. Fifty-two steers were positive by either method for at least 3 consecutive sampling weeks. Six steers were positive by either method for 6 consecutive sampling weeks.

The inoculated strain, O28:H43 Nal^R-Rif^R, was detected by both enumeration and enrichment methods on individual steer hide samples. Positive hide samples were detected during 6 of the 9 study weeks. The highest concentration detected on an individual hide sample was 3.3 log₁₀ cfu/400 cm² on d 14. On d 7, 97% of hide samples were positive, with 64% of samples enumerable (Table 2-7). On d 21 and 56, 90 and 99% of the hide samples, respectively, were quantifiable. The last day an enumerable sample was detected on the hide of the steers was study d 56, though 97% of samples remained positive by enrichment through the end of the study.

The inoculated strain was detected by both enumeration and enrichment in the oral cavities of the steers. The highest concentration detected in a steer oral sample was 3.5 log₁₀CFU/mL on study d 42. On d 7, 79% of oral samples were positive, with 43% enumerable (Table 2-7). On d 56, 100% of oral samples were positive and 97% were enumerable. Oral samples were enumerable during 6 of the 9 study weeks. The last study day enumerable samples were detected in the oral swabs of the steers was d 56, though 49% of samples remained positive via enrichment through the end of the study.

All pen surface samples taken before steer arrival were negative for *E. coli* that was resistant to both nalidixic acid and rifampicin antibiotics. After steer inoculation, the inoculated strain was detected by both enumeration and enrichment methods in the pen surface soil samples. The highest concentration detected in an individual pen surface sample was 3.9 log₁₀CFU/g on both study d 28 and 56. Enumerable levels were found in the pen surface samples during 6 of the 9 wk with the highest number of enumerable samples being detected on d 14 (96%; Table 2-8). All soil samples were positive via either enumeration or enrichment during 5 of the 9 wk. The last study day enumerable samples were detected in the pen surface samples was study d 56, though 100% samples remained positive by enrichment through the end of the study.

The inoculated strain was found in the feed samples during 5 of the 9 sampling weeks (Table 2-8). The highest concentration detected in a feed sample was 2.5 log₁₀CFU/g on study d 14. The last study day enumerable samples were detected in the feed samples was study d 28, though samples were positive via enrichment during d 49, 56, and 63. The inoculated strain was detected in the water samples taken from the water tank during 5 wk of the study (Table 2-8). The highest concentration detected in an individual water sample was 2.0 log₁₀ cfu/mL on study d 56. On d 21 and 56, all water samples taken were enumerable. The last day enumerable samples were detected in the water samples was d 56, though samples remained positive via enrichment through the end of the study period.

Weather data obtained from the National Oceanic and Atmospheric Administration was downloaded for each sampling week, with the week starting on Monday (day of sampling) and ending on Sunday (the day before sampling; NOAA, 2021). The highest and lowest temperatures recorded during the study period were 36.1°C (wk 8) and 5°C (wk 1; Table 2-9). The weekly

total precipitation ranged from 0.9 cm (wk 6) to 16.7 cm (wk 1). The total precipitation for the study period was 59 cm, with an average of 6.6 cm per week.

In both studies 2A and 2B, we established that one verified nonpathogenic *E. coli* strain, O28:H43, was able to colonize, shed in the feces of feedlot steers, spread to their pen environment, and transmit between steers. For both studies, all fecal and pen surface soil samples taken before each inoculation were negative for any *E. coli* strain resistant to nalidixic acid and rifampicin antibiotics. Thus, any *E. coli* found throughout the study is assumed to be our inoculated strain. Our results also indicated that transmission between steers occurred during the study periods, and the strains persisted in steers and the environment over an extended period. Mean shedding of the enumerable-only positive samples for the feedlot steer fecal samples were 2.6 log₁₀CFU/g (Study 2-A) and 3.5 log₁₀CFU/g (Study 2-B). These means are comparable to the mean enumerable fecal counts of *E. coli* O157:H7 in the study by Cobbold et al. (2007). As mentioned before, our inoculated strain lacked the *stx*- and LEE-associated gene communities, which are speculated to confer adaptation benefits in the bovine intestinal environment (Arimizu et al., 2019). Their absence, particularly the genes involved in adhesion of *E. coli* to intestinal epithelial cells, could have disadvantaged our strains in the bovine hindgut; however, our mean shedding levels in comparison with longitudinal STEC shedding in Cobbold et al. (2007) suggest this disadvantage was not a factor. In studies 2A and 2B steers that were not initially selected for inoculation became positive with the inoculated strain in their feces. In all studies, there was variability in the fecal shedding of the dairy calves and steers between sampling weeks. The lowest concentration of *E. coli* quantifiable by spiral plate method was 1.0 log₁₀CFU/400 cm² in hide samples, 1.0 log₁₀CFU/mL in oral samples, and 2.0 log₁₀CFU/g in feces. We commonly

detected *E. coli* NalR-RifR using enrichment in samples that were negative for enumeration on spiral plates. The enrichment method of detection would have detected samples shedding below the detection limit of the spiral plate method ($<1.0 \log_{10}\text{CFU}/400 \text{ cm}^2$ in hide samples, $<1.0 \log_{10}\text{CFU}/\text{mL}$ in oral samples, and $<2.0 \log_{10}\text{CFU}/\text{g}$ in feces).

Variability in week-to-week detection could be due to individual variability in shedding concentration. The enrichment process in EC broth would improve the ability to detect any *E. coli* compared with direct spiral plating methods. Additionally, the detection of *E. coli* resistant to nalidixic acid and rifampicin in a selective medium containing the antibiotics, to some extent, is affected by the competing bacterial flora. However, the variability in shedding concentration is not surprising because it is well known that fecal shedding of STEC, and possibly any *E. coli*, in cattle is intermittent and highly variable (Berry and Wells, 2010).

In both years, the inoculated strain was detected in the feed, water, and pen surface soil samples, though in 2019 there was a greater proportion of positive samples. The detection of the inoculated strain in these sample types shows the survival of the strain in different environmental samples. Similar to that of STEC O157, which has been isolated from feedlot water tanks and feedlot-cattle feed (Dodd et al., 2003; Sargeant et al., 2004), *E. coli* O157 has been shown to persist in feedlot soil environments, over a wide range of water and manure contents (Berry and Miller, 2005). Our inoculated strain showed a similar persistence in the pen surface samples. These environmental reservoirs could act as transmission points between animals and should be taken into consideration when modeling *E. coli* transmission within a feedlot setting.

All strains in study 1 and the one strain used in both studies 2A and 2B were detected in the oral samples collected from the calves. STEC O157 has been found in the oral cavity of both

naturally colonized (Keen and Elder, 2002) and experimentally inoculated (Buchko et al., 2000) animals. STEC O157 has also been found naturally occurring on the hide surface of beef feedlot cattle (Keen and Elder, 2002). The hide and oral surfaces may serve as ecological reservoirs for STEC O157 that should be accounted for when studying direct or indirect animal-to-animal or animal-to-environment transmission.

Another factor that could be influencing the shedding variability of the calves and shedding differences seen between studies 2A and 2B is the weather. Though both years experienced a similar range of temperatures, study 2B (2019) experienced over 4 times the total precipitation than the study in 2018. It is a generally accepted view that pathogen survival is usually extended in moist soil environments over drier environments (Rudolfs et al., 1950; Van Donsel et al., 1967; Reddy et al., 1981). Study 2B saw a greater proportion of positive strain detection in all sample types taken from the calves (fecal, hide, and oral) and the pen environment (soil, feed, and water) compared with 2018. The extra moisture seen in 2019 compared with 2018 may explain why the inoculated strain survived longer in the environment and was detected at greater concentrations. Nalidixic acid-resistant strains of *E. coli* have been validated as a marker regarding chemical interventions in food safety (Blackburn and Davies, 1994; Taormina and Beuchat, 1999). These studies demonstrated that nalidixic acid-resistant strains had similar growth rates and stress tolerances as nalidixic acid-susceptible parent strains. However, no such validation has been done for the growth and survival of a nalidixic acid-resistant and rifampicin-resistant strain in a feedlot production environment.

Applications

These studies describe the shedding duration and concentration of 3 nonpathogenic *E. coli* strains in weaned Holstein calves and the shedding and transmission of one nonpathogenic strain in 2 groups of feedlot steers and their pen environment. Overall, these studies support use of a surrogate strain to establish baseline data for host-to-host and host-to-environment enteric pathogen transmission. The use of a nonpathogenic *E. coli* strain with known temporal onset and source as a surrogate to generate data on shedding and transmission dynamics could help to understand enteric pathogen transmission and to model STEC transmission in commercial US feedlot systems to explore effective control options in real-world situations.

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Literature Cited

1. Arimizu, Y., Y. Kirino, M. P. Sato, K. Uno, T. Sato, Y. Gotoh, F. Auvray, H. Brugere, E. Oswald, J. G. Mainil, K. S. Anklam, D. Döpfer, S. Yoshino, T. Ooka, Y. Tanizawa, Y. Nakamura, A. Iguchi, T. Morita-Ishihara, M. Ohnishi, K. Akashi, T. Hayashi, and Y. Ogura. 2019. Large-scale genome analysis of bovine commensal *Escherichia coli* reveals that bovine-adapted *E. coli* lineages are serving as evolutionary sources of the emergence

- of human intestinal pathogenic strains. *Genome Res.* 29:1495–1505.
<https://doi.org/10.1101/gr.249268.119>.
2. Berry, E. D., and D. N. Miller. 2005. Cattle feedlot soil moisture and manure content: II. Impact on *Escherichia coli* O157. *J. Environ. Qual.* 34:656–663.
<https://doi.org/10.2134/jeq2005.0656>.
 3. Berry, E. D., and J. E. Wells. 2010. *Escherichia coli* O157:H7: Recent advances in research on occurrence, transmission, and control in cattle and the production environment. *Adv. Food Nutr. Res.* 60:67–117. [https://doi.org/10.1016/S1043-4526\(10\)60004-6](https://doi.org/10.1016/S1043-4526(10)60004-6).
 4. Blackburn, C. W., and A. R. Davies. 1994. Development of antibiotic- resistant strains for the enumeration of foodborne pathogenic bacteria in stored foods. *Int. J. Food Microbiol.* 24:125–136. [https://doi.org/10.1016/0168-1605\(94\)90112-0](https://doi.org/10.1016/0168-1605(94)90112-0).
 5. Buchko, S. J., R. A. Holley, W. O. Olson, V. P. Gannon, and D. M. Veira. 2000. The effect of different grain diets on fecal shedding of *Escherichia coli* O157:H7 by steers. *J. Food Prot.* 63:1467–1474. <https://doi.org/10.4315/0362-028X-63.11.1467>.
 6. Callaway, T. R., R. O. Elder, J. E. Keen, R. C. Anderson, and D. J. Nisbet. 2003. Forage feeding to reduce preharvest *Escherichia coli* populations in cattle, A review. *J. Dairy Sci.* 86:852–860. [https://doi.org/10.3168/jds.S0022-0302\(03\)73668-6](https://doi.org/10.3168/jds.S0022-0302(03)73668-6).
 7. Cobbold, R., and P. Desmarchelier. 2002. Horizontal transmission of Shiga toxin-producing *Escherichia coli* within groups of dairy calves. *Appl. Environ. Microbiol.* 68:4148–4152.
<https://doi.org/10.1128/AEM.68.8.4148-4152.2002>
 8. Cobbold, R. N., D. D. Hancock, D. H. Rice, J. Berg, R. Stilborn, C. J. Hovde, and T. E. Besser. 2007. Rectoanal junction colonization of feedlot cattle by *Escherichia coli*

- O157:H7 and its association with supershedders and excretion dynamics. *Appl. Environ. Microbiol.* 73:1563–1568. <https://doi.org/10.1128/AEM.01742-06>.
9. Cray, W. C. Jr., and H. W. Moon. 1995. Experimental infection of calves and adult cattle with *Escherichia coli* O157:H7. *Appl. Environ. Microbiol.* 61:1586–1590. <https://doi.org/10.1128/aem.61.4.1586-1590.1995>.
10. Dodd, C. C., M. W. Sanderson, J. M. Sargeant, T. G. Nagaraja, R. D. Oberst, R. A. Smith, and D. D. Griffin. 2003. Prevalence of *Escherichia coli* O157 in cattle feeds in midwestern feedlots. *Appl. Environ. Microbiol.* 69:5243–5247. <https://doi.org/10.1128/AEM.69.9.5243-5247.2003>.
11. Faith, N. G., J. A. Shere, K. Brosch, K. W. Arnold, S. E. Ansay, M. S. Lee, J. B. Luchansky, and C. W. Kaspar. 1996. Prevalence and clonal nature of *Escherichia coli* O157:H7 on dairy farms in Wisconsin. *Appl. Environ. Microbiol.* 62:1519–1525. <https://doi.org/10.1128/aem.62.5.1519-1525.1996>.
12. Ferens, W. A., and C. J. Hoyde. 2011. *Escherichia coli* O157:H7: Animal reservoir and sources of human infection. *Foodborne Pathog. Dis.* 8:465–487. <https://doi.org/10.1089/fpd.2010.0673>.
13. Ferguson, K. M., M. E. Jacob, C. M. Theriot, B. J. Callahan, T. Prange, M. G. Papich, and D. M. Foster. 2019. Dosing regimen of enrofloxacin impacts intestinal pharmacokinetics and the fecal microbiota in steers. *Front. Microbiol.* 9:1–15. <https://doi.org/10.3389/fmicb.2018.02190>.

14. Hancock, D. D., T. E. Besser, D. H. Rice, D. E. Herriott, and P. I. Tarr. 1997. A longitudinal study of *Escherichia coli* O157 in fourteen cattle herds. *Epidemiol. Infect.* 118:193–195. <https://doi.org/10.1017/S0950268896007212>.
15. Howe, K., A. H. Linton, and A. D. Osborne. 1976. A longitudinal study of *Escherichia coli* in cows and calves with special reference to the distribution of O-antigen types and antibiotic resistance. *J. Appl. Bacteriol.* 40:331–340. <https://doi.org/10.1111/j.1365-2672.1976.tb04181.x>.
16. Keen, J. E., and R. O. Elder. 2002. Isolation of Shiga-toxigenic *Escherichia coli* O157 from hide surfaces and the oral cavity of finished beef feedlot cattle. *J. Am. Vet. Med. Assoc.* 220:756–763. <https://doi.org/10.2460/javma.2002.220.756>.
17. LeJeune, J. T., T. E. Besser, and D. D. Hancock. 2001. Cattle water troughs as reservoirs of *Escherichia coli* O157. *Appl. Environ. Microbiol.* 67:3053–3057. <https://doi.org/10.1128/AEM.67.7.3053-3057.2001>.
18. Maturin, L., and J. T. Peeler. 2001. Aerobic plate count. Ch. 3 in *Bacteriological Analytical Manual*. US Food and Drug Administration. Accessed Jan. 31, 2016. <https://www.fda.gov/food/laboratory-methods-food/bam-chapter-3-aerobic-plate-count>.
19. NOAA (National Oceanic and Atmospheric Administration). 2021. National centers for environmental information: Climate data online. Accessed Jun. 29, 2021. <https://www.ncdc.noaa.gov/cdo-web/>.
20. Naylor, S. W., A. Flockhart, P. Nart, D. G. E. Smith, J. Huntley, D. L. Gally, and J. C. Low. 2007. Shedding of *Escherichia coli* O157:H7 in calves is reduced by prior colonization

- with the homologous strain. *Appl. Environ. Microbiol.* 73:3765–3767.
<https://doi.org/10.1128/AEM.02670-06>.
21. Reddy, K. R., R. Khaleel, and M. R. Overcash. 1981. Behaviour and transport of microbial pathogens and indicator organisms in soils treated with organic wastes. *J. Environ. Qual.* 10:255–266. <https://doi.org/10.2134/jeq1981.00472425001000030001x>.
 22. Rudolfs, W., L. L. Falk, and R. A. Ragotzkie. 1950. Literature review on the occurrence and survival of enteric, pathogenic, and related organisms in soil, water, sewage and sludges and on vegetation. I. Bacterial and virus diseases. *Sewage Ind. Waste.* 22:1261–1281.
 23. Sanderson, M. W., T. E. Besser, J. M. Gay, C. C. Gay, and D. D. Hancock. 1999. Fecal *Escherichia coli* O157:H7 shedding patterns of orally inoculated calves. *Vet. Microbiol.* 69:199–205. [https://doi.org/10.1016/S0378-1135\(99\)00106-6](https://doi.org/10.1016/S0378-1135(99)00106-6).
 24. Sargeant, J. M., M. W. Sanderson, D. D. Griffin, and R. A. Smith. 2004. Factors associated with the presence of *Escherichia coli* O157 in feedlot-cattle water and feed in the midwestern USA. *Prev. Vet. Med.* 66:207–237.
<https://doi.org/10.1016/j.prevetmed.2004.09.006>.
 25. Shere, J. A., K. J. Bartlett, and C. W. Kaspar. 1998. Longitudinal study of *Escherichia coli* O157:H7 dissemination on four dairy farms in Wisconsin. *Appl. Environ. Microbiol.* 64:1390–1399. <https://doi.org/10.1128/AEM.64.4.1390-1399.1998>.
 26. Taormina, P. J., and L. R. Beuchat. 1999. Comparison of chemical treatments to eliminate enterohemorrhagic *Escherichia coli* O157:H7 on alfalfa seeds. *J. Food Prot.* 62:318–324.
<https://doi.org/10.4315/0362-028X-62.4.318>.

27. Turner, J., M. Begon, R. G. Bowers, and N. P. French. 2003. A model appropriate to the transmission of a human food-borne pathogen in a multigroup managed herd. *Prev. Vet. Med.* 57:175–198. [https://doi.org/10.1016/S0167-5877\(03\)00006-0](https://doi.org/10.1016/S0167-5877(03)00006-0).
28. Van Donkersgoed, J., J. Berg, A. Potter, D. Hancock, T. Besser, D. Rice, J. LeJeune, and S. Klashinsky. 2001. Environmental sources and transmission of *Escherichia coli* O157 in feedlot cattle. *Can. Vet. J.* 42:714–720.
29. Van Donsel, D. J., E. E. Geldreich, and N. A. Clarke. 1967. Seasonal variations in survival of indicator bacteria in soil and their contribution to storm water pollution. *Appl. Microbiol.* 15:1362–1370. <https://doi.org/10.1128/am.15.6.1362-1370.1967>.
30. Vosough Ahmadi, B., A. G. J. Velthuis, H. Hogeveen, and R. B. M. Huirne. 2006. Simulating *Escherichia coli* O157:H7 transmission to assess effectiveness of interventions in Dutch dairy-beef slaughterhouses. *Prev. Vet. Med.* 77:15–30. <https://doi.org/10.1016/j.prevetmed.2006.05.011>.

Table 2-1. Study 1- Highest Enumerable Concentration Detected by Strain and by Sample Type.

	Highest Enumerable Concentration (Sampling Day)		
	O19:H+	O101:H10	O28:H43
Feces (log ₁₀ CFU/g)	4.46 (day 6)	4.97 (day 4)	5.76 (day 32)
Hide (log ₁₀ CFU/400 cm ²)	1.85 (day 13)	2.86 (day 2)	2.88 (day 3)
Oral (log ₁₀ CFU/mL)	2.90 (day 3)	2.95 (day 4)	2.91 (day 3)
Pen surface (log ₁₀ CFU/g)	4.00 (day 27)	4.56 (day 27)	4.55 (day 27)

Study 1 (dairy calf) Highest enumerable concentration of the inoculated strain detected in fecal, hide, oral, and pen surface soil samples.

Table 2-2. Study 1- Count of Positive Fecal Samples by Study Day.

Study Day	O19:H+		O101:H10		O28:H43	
	Enumeration	Enrichment	Enumeration	Enrichment	Enumeration	Enrichment
1	0	4	3	0	3	1
2	4	-	4	-	2	2
3	4	-	4	-	4	-
4	3	1	4	-	4	-
5	4	-	4	-	4	-
6	3	1	3	1	4	-
9	3	0	4	-	3	1
11	4	-	2	2	2	2
13	2	1	1	3	4	-
16	3	0	3	1	1	3
18	1	2	2	2	4	-
20	2	2	4	-	4	-
23	2	1	2	1	2	2
25	3	1	3	0	1	3
27	1	2	1	1	4	-
30	3	1	1	3	4	-
32	1	2	0	1	4	-
34	1	1	0	3	4	-

Study 1 (dairy calf) Number positive for fecal samples by strain, method, and day. For each strain n=4. Enumerable positive samples are those defined as positive via spiral plating methods. Enrichment positive samples are those defined as negative via spiral plating methods, but positive via 6h incubations in EC broth.

Table 2-3. Study 1- Fecal AUC by Calf and Pen Averages.

Strain	Calf Tag Number	AUC (log ₁₀ CFU)	Strain Average AUC (log ₁₀ CFU)
O19:H+	2	57.75	77.17
	4	92.38	
	11	81.87	
	175	76.68	
O101:H10	9	74.86	79.52
	12	81.57	
	171	108.10	
	173	54.54	
O28:H43	5	86.91	102.67
	6	103.47	
	7	90.47	
	172	129.83	

Study 1 (dairy calf) Total Area under the curve (AUC) data for fecal concentrations of each individual calf, as well as a strain average. Results shown in total log₁₀CFU for the entire study period.

Table 2-4. Study 2-A- Sample Counts for Fecal, Hide, and Oral Samples.

Study Day	Fecal Number (Percent Pos)			Hide Number (Percent Pos)			Oral Number (Percent Pos)		
	Enumeration	Enrichment	Total Positive	Enumeration	Enrichment	Total Positive	Enumeration	Enrichment	Total Positive
7	4 (6%)	30 (43%)	34 (49%)	0	4 (6%)	4 (6%)	9 (13%)	8 (11%)	17 (24%)
14	2 (3%)	10 (14%)	12 (17%)	0	24 (34%)	24 (34%)	2 (3%)	3 (4%)	5 (7%)
21	2 (3%)	17 (24%)	19 (27%)	1 (1%)	0	1 (1%)	0	6 (9%)	6 (9%)
28	0	4 (6%)	4 (6%)	7 (10%)	4 (6%)	11 (16%)	5 (7%)	0	5 (7%)
35	7 (10%)	9 (13%)	16 (23%)	15 (22%)	4 (6%)	19 (28%)	0	0	0
42	2 (3%)	29 (42%)	31 (45%)	2 (3%)	19 (28%)	21 (31%)	0	0	0
49	2 (3%)	24 (35%)	26 (38%)	0	1 (1%)	1 (1%)	0	0	0
56	13 (19%)	0	13 (19%)	4 (6%)	15 (22%)	19 (28%)	4 (6%)	15 (22%)	19 (28%)
63	0	0	0	0	8 (12%)	8 (12%)	0	0	0
70	0	5 (7%)	5 (7%)	0	12 (17%)	12 (17%)	0	0	0

Study2-A (Feedlot, 2018) Number positive (%) in the samples taken from the individual steers by day, sample, and method. (n=70

through day 28, then n=69). Enumerable positive samples are those defined as positive via spiral plating methods. Enrichment positive samples are those defined as negative via spiral plating methods, but positive via 6h incubations in EC broth. Total positives are the total number of samples positive via enumeration and enrichment.

Table 2-5. Study 2-A- Sample Counts for Pen Surface Soil Samples.

Study Day	Enumeration Number (Percent Pos)	Enrichment Number (Percent Pos)	Total Positive Number (Percent Pos)
7	2 (8%)	1 (4%)	3 (12%)
14	0	1 (4%)	1 (4%)
21	0	3 (12%)	3 (12%)
28	4 (16%)	3 (12%)	7 (28%)
35	8 (32%)	10 (40%)	18 (72%)
42	2 (8%)	21 (84%)	23 (92%)
49	2 (8%)	6 (24%)	8 (32%)
56	0	1 (4%)	1 (4%)
63	0	0	0
70	0	0	0

Study 2-A (Feedlot, 2018) Number positive (%) in the pen surface soil samples by day and method. (n=25). Enumerable positive samples are those defined as positive via spiral plating methods. Enrichment positive samples are those defined as negative via spiral plating methods, but positive via 6h incubations in EC broth. Total positives are the total number of samples positive via enumeration and enrichment.

Table 2-6. Study 2-A- Weekly Temperature Range and Total Precipitation.

Study Week	Weekly Temperature (°C)		Weekly Total Precipitation (cm)
	High	Low	
1	37.2	12.2	0.1
2	35.0	13.3	3.9
3	35.6	14.4	0.0
4	37.8	20.0	0.8
5	35.6	16.1	0.3
6	40.6	18.3	1.9
7	38.9	16.7	2.9
8	38.3	18.3	2.6
9	34.4	19.4	0.8
10	32.8	16.7	0.5

Study 2-A (Feedlot, 2018) Weekly temperature in Celsius (high and low) and total precipitation in centimeters data obtained from NOAA for the study period. Study weeks defined as the 7 days prior to that week's sampling point

Table 2-7. Study 2-B- Sample Counts for Fecal, Hide, and Oral Samples.

Study Day	Fecal Number (Percent Pos)			Hide Number (Percent Pos)			Oral Number (Percent Pos)		
	Enumeration	Enrichment	Total Positive	Enumeration	Enrichment	Total Positive	Enumeration	Enrichment	Total Positive
7	23 (32%)	34 (49%)	57 (81%)	45 (64%)	23 (33%)	68 (97%)	30 (43%)	25 (36%)	55 (79%)
14	38 (54%)	2 (3%)	40 (57%)	54 (77%)	0	54 (77%)	41 (59%)	0	41 (59%)
21	0	12 (17%)	12 (17%)	63 (90%)	5 (7%)	68 (97%)	15 (21%)	50 (71%)	65 (92%)
28	31 (44%)	12 (17%)	43 (61%)	16 (23%)	12 (17%)	28 (40%)	23 (33%)	22 (31%)	45(64%)
35	0	68 (97%)	68 (97%)	0	19 (27%)	19 (27%)	0	27 (39%)	27 (39%)
42	2 (3%)	8 (11%)	10 (14%)	0	1 (1%)	1 (1%)	32 (46%)	1 (1%)	33 (47%)
49	4 (6%)	61 (88%)	65 (94%)	7 (10%)	18 (26%)	25 (36%)	0	36 (52%)	36 (52%)
56	68 (100%)	0	68 (100%)	67 (99%)	1 (1%)	68 (100%)	66 (97%)	2 (3%)	68 (100%)
63	0	43 (63%)	43 (63%)	0	66 (97%)	66 (97%)	0	33 (49%)	33 (49%)

Study 2-B (Feedlot, 2019) Number positive (%) in the samples taken from the individual steers by day, sample, and method. (n=70 through day 35, n=69 on day 42 and 49, n=68 on day 56 and 63. Enumerable positive samples are those defined as positive via spiral plating methods. Enrichment positive samples are those defined as negative via spiral plating methods, but positive via 6h incubations in EC broth. Total positives are the total number of samples positive via enumeration and enrichment.

Table 2-8. Study 2-B- Sample Counts for Pen Surface, Feed, and Water Samples.

	Pen Surface (n=25) Number (Percent Pos)			Feed (n=5) Number (Percent Pos)			Water (n=3) Number (Percent Pos)		
Study Day	Enumeration	Enrichment	Total Positive	Enumeration	Enrichment	Total Positive	Enumeration	Enrichment	Total Positive
7	19 (76%)	4 (16%)	23 (92%)	0	0	0	2 (67%)	1 (33%)	3 (100%)
14	24 (96%)	0	24 (96%)	2 (40%)	0	2 (40%)	0	0	0
21	23 (92%)	2 (8%)	25 (100%)	0	0	0	3 (100%)	-	3 (100%)
28	20 (80%)	5 (20%)	25 (100%)	3 (60%)	2 (40%)	5 (100%)	0	0	0
35	0	18 (72%)	18 (72%)	0	0	0	0	0	0
42	3 (12%)	1 (4%)	4 (16%)	0	0	0	0	0	0
49	0	25 (100%)	25 (100%)	0	4 (80%)	4 (80%)	0	2 (67%)	2 (67%)
56	22 (88%)	3 (12%)	25 (100%)	0	3 (60%)	3 (60%)	3 (100%)	-	3 (100%)
63	0	25 (100%)	25 (100%)	0	5 (100%)	5 (100%)	0	3 (100%)	3 (100%)

Study 2-B (Feedlot, 2019) Number positive (%) in the environmental pen samples day, sample, and method. Enumerable positive samples are those defined as positive via spiral plating methods. Enrichment positive samples are those defined as negative via spiral plating methods, but positive via 6h incubations in EC broth. Total positives are the total number of samples positive via enumeration and enrichment.

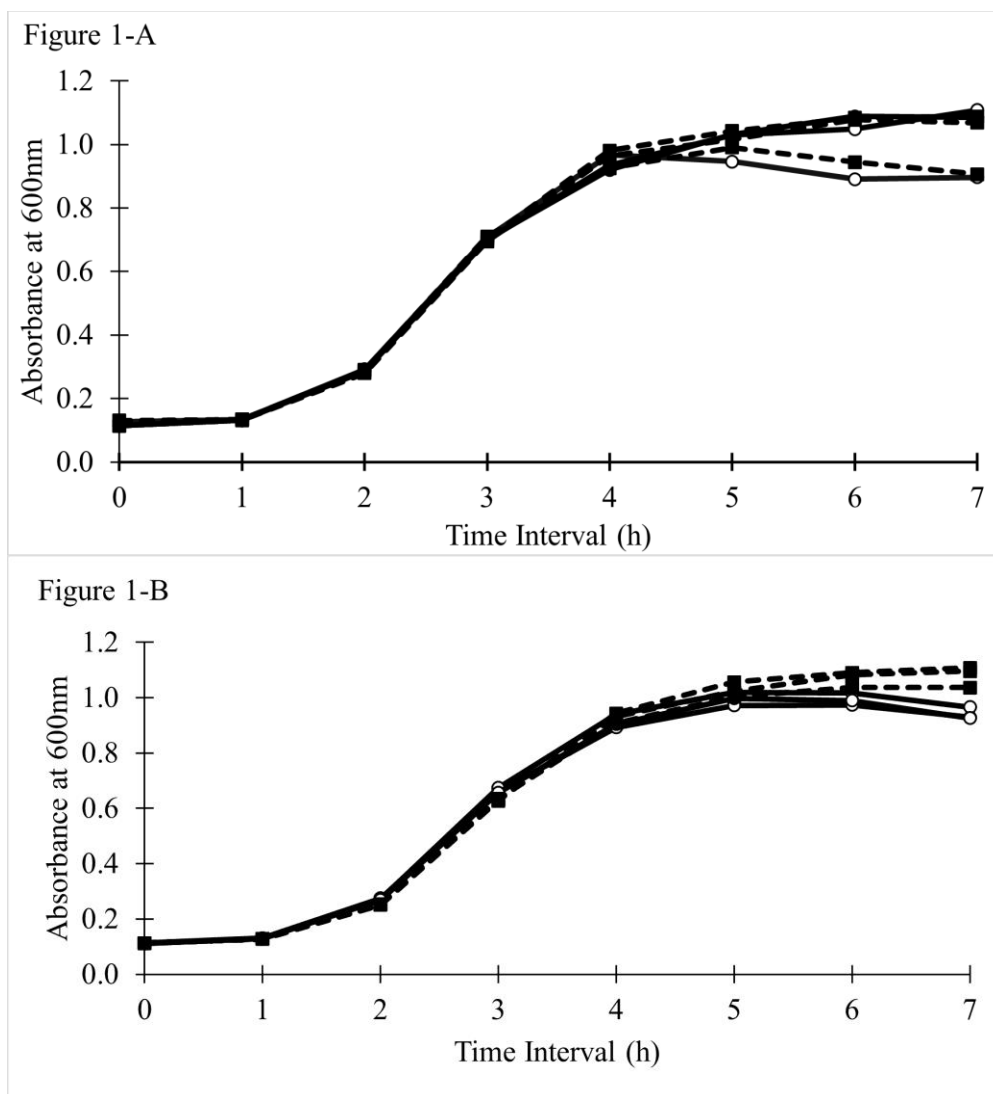
Table 2-9. Study 2-B- Weekly Temperature Range and Total Precipitation.

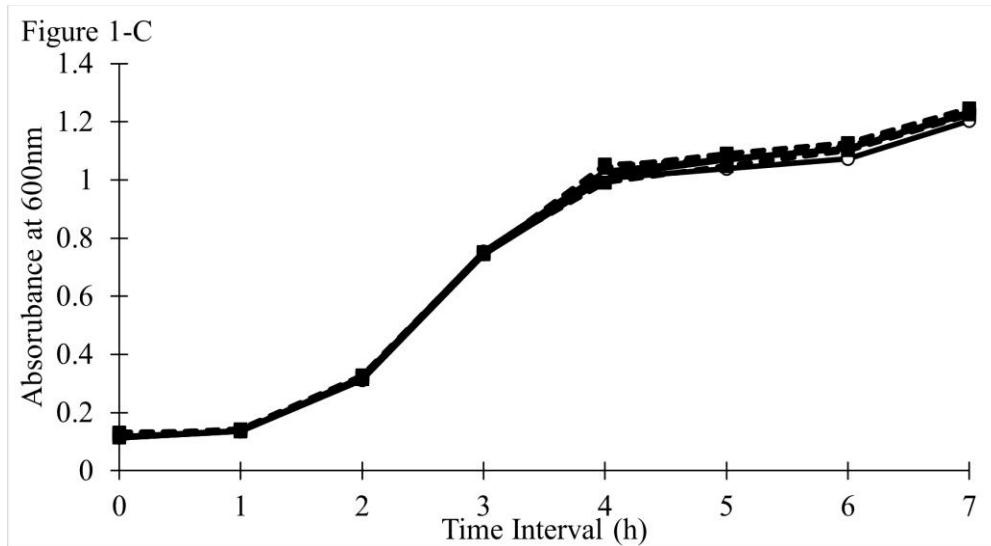
Study Week	Weekly Temperature (°C)		Weekly Total Precipitation (cm)
	High	Low	
1	25.6	5.0	16.7
2	32.8	7.2	6.2
3	25.6	7.8	7.3
4	30.0	11.1	2.3
5	31.7	13.3	1.7
6	31.1	8.9	0.9
7	32.8	15.6	13.4
8	36.1	12.8	2.1
9	32.8	19.4	8.3

Study 2-B (Feedlot, 2019) Weekly temperature in Celsius (high and low) and total precipitation in centimeters data obtained from NOAA for the study period. Study weeks are the 7 days prior to that week's sampling.

Appendix A - Supplementary Material

Figure A-1A-C. Microbial Growth Curve for NalR-RifR Strains and Parent NalR Strains.





Microbial growth curve obtained for *E. coli* O19:H⁺ NalR and *E. coli* O19:H⁺ NalR-RifR

(Figure 1A), *E. coli* O101:H10 NalR and *E. coli* O101:H10 NalR-RifR (Figure 1B), *E. coli*

O28:H43 and *E. coli* O28:H43 NalR-RifR (Figure 1C). All parents (NalR only) strains

represented by the solid circle lines. All NalR-RifR strains are represented by the dashed square

lines. Optical density was measured by absorbance at a wavelength of 600 nm every 15 minutes

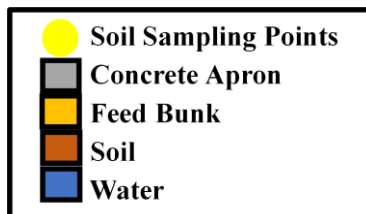
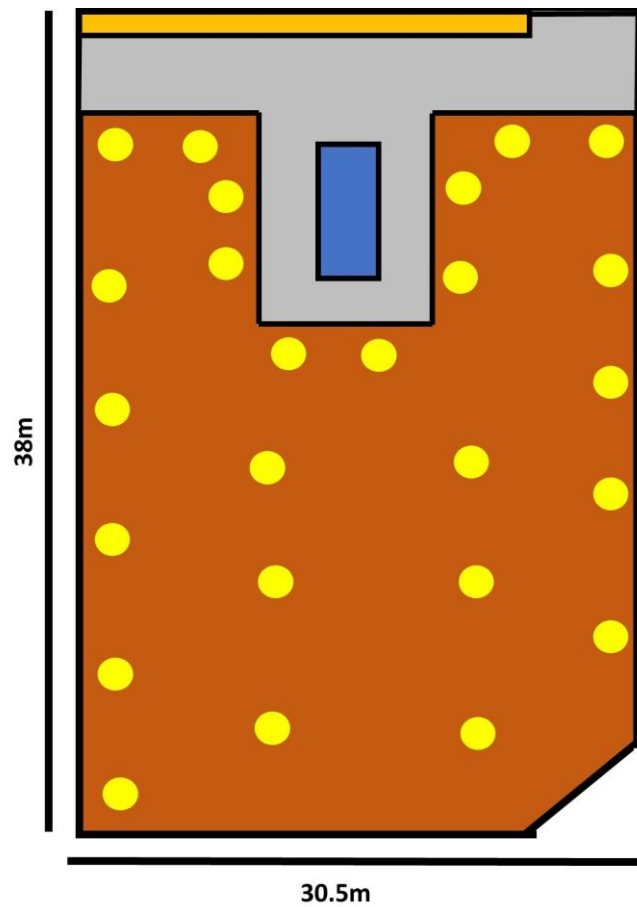
for 7.5 hours. The parent strain and the NalR-RifR strain were done in triplicate.

Table A-1. Highest Maximum Optical Density Reading and Mean Specific Growth Curve by Strain.

Strain	Maximum Optical Density	Mean Specific Growth Rate (Absorbance at 600nm/min)
O19:H+ NalR	1.11	0.005
O19:H+ NalR-RifR	1.09	0.005
O101:H10 NalR	1.04	0.005
O101:H10 NalR-RifR	1.11	0.006
O28:H43 NalR	1.23	0.006
O28:H43 NalR-RifR	1.25	0.006

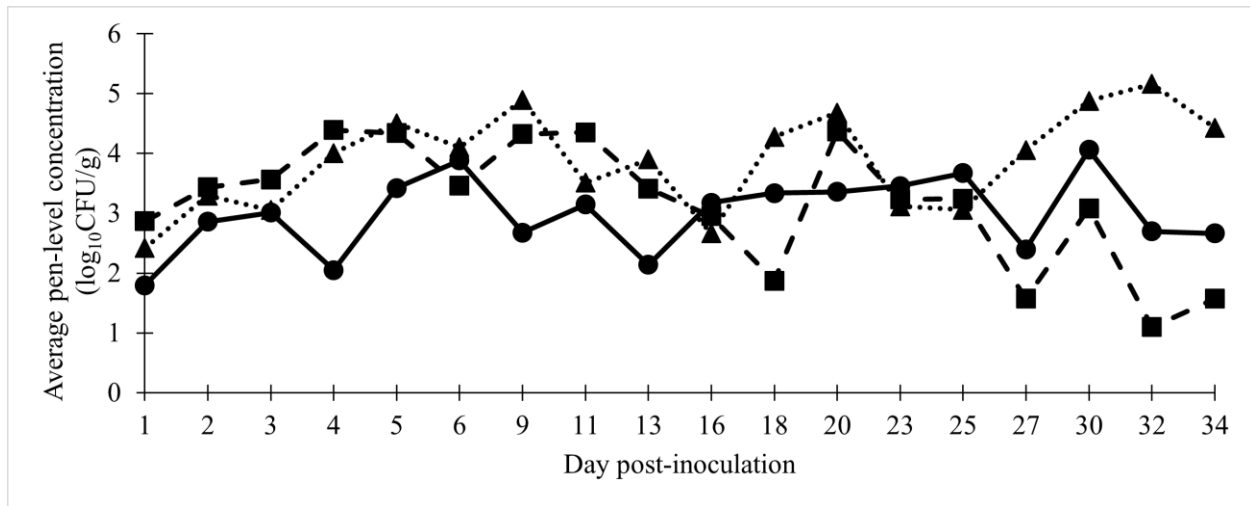
Highest maximum optical density read at 600nm and mean specific growth rate in absorbance at 600nm/min for *E. coli* O19:H+ NalR, *E. coli* O19:H+ NalR-RifR, *E. coli* O101:H10 NalR, *E. coli* O101:H10 NalR-RifR, *E. coli* O28:H43 and *E. coli* O28:H43 NalR-RifR. Optical density was measured by absorbance at a wavelength of 600 nm every 15 minutes for 7.5 hours. The parent strain (NalR only) and the NalR-RifR strain were done in triplicate.

Figure A-2. Study 2-A and B Pen Map.



Pen map for Study 2-A and B, feedlot studies, outlining the areas of the feed bunk, concrete apron, water tank, and the 25 locations around the pen that weekly pen surface soil samples were collected from.

Figure A-3. Average Pen-Level Fecal Concentrations by Day Post-Inoculation.



The average pen-level fecal concentrations in log₁₀CFU/g for each strain group for each sample collection day post-first day of inoculations for Study 1 (dairy calf). (Circle: *E. coli* strain O19:H+ NalR-RifR; Square: *E. coli* strain O101:H10 NalR-RifR; Triangle: *E. coli* strain O28:H43 NalR-RifR)

Table A-2. Study 2-A- Enumerable and Enrichment Positive Fecal Samples.

Calf ID	Study Day													
	2	3	4	5	7	14	21	28	35	42	49	56	63	70
1502														
1503														
1504														
1505														
1507														
1509												2.6		
1510														
1511														
1512												3.0		
1515												2.7		
1516														
1517					2.8							2.0		
1518														
1521														
1523							2.00					2.0		
1525									NS	NS	NS	NS	NS	NS
1526														
1527												3.5		
1528														
1529														
1533														
1536														
1537									2.7					
1538										2.3		3.4		
1541														
1543														
1545												2.0		
1548														
1549														
1552														
1553														
1557												3.0		
1558									2.3					
1560														
1561							2.3		2.0					
1562												2.5		
1564														
1565														

	Study Day													
Calf ID	2	3	4	5	7	14	21	28	35	42	49	56	63	70
1569														
1570														
1573									2.3					
1575											2.5			
1576									2.3					
1577						2.7								
1578														
1579														
1580					3.5							2.9		
1582														
1583									2.0					
1584												3.7		
1585														
1587														
1590														
1594														
1599														
1600														
1601									2.0					
1602					2.9									
1604														
1621														
1623					2.5									
1661														
1662														
1675														
1680												2.3		
1683						2.3						2.9		
1684														
1709														
1739														
1812											2.0			

Study 2-A (Feedlot, 2018): Fecal samples taken from the individual steers by day (n=70 through day 28, then n=69). Enumerable positive samples (green) are those defined as positive via spiral plating methods. Concentrations listed for enumerable positive samples are in log₁₀CFU/g of feces. Enrichment positive samples (gold) are those defined as negative via spiral plating

methods, but positive via 6h incubations in EC broth. No sample (NS). The 5 steers randomly selected for the initial inoculation are highlighted in red.

Table A-3. Study 2-B-Enumerable and Enrichment Positive Fecal Samples.

Calf ID	Study Day												
	2	3	4	5	7	14	21	28	35	42	49	56	63
1	4.6	5.1	5.2	5.3	3.7	2.5		3.3		2.3		3.6	
2						2.8						3.5	
3						2.8				2.0		3.3	
4					2.8			2.6				4.4	
5											2.5	3.8	
6						2.0						3.1	
7					2.0	2.0						3.0	
8					4.8			2.3				3.4	
9						2.7		2.8				2.9	
10						2.9		2.3				3.0	
11						2.5		2.5				3.0	
12						2.3						3.9	
13						2.3						2.9	
14						2.5		2.9			2.0	4.0	
15												2.9	
16						2.0						3.6	
17												4.0	
18												3.1	
19												2.9	
20						2.0		2.0				4.3	
21					2.0	2.5						3.4	
22					4.8	3.0		2.6				3.6	
23					3.3	2.0						3.6	
24												3.4	
25						2.6		2.6				2.8	
26								2.6				2.8	
27						2.6		2.6			3.5	3.9	
28					2.9							3.3	NS
29												3.7	
30								2.8				3.3	
31						2.0						2.9	
32					2.0							3.7	
33												3.3	
34					4.8	2.8		3.5				3.0	
35												4.0	
36					4.7	2.0		2.0				3.1	
37						2.0		2.5				3.3	
38	4.5	5.3	4.8	5.7	2.9	2.0		3.6				4.3	

	Study Day												
Calf ID	2	3	4	5	7	14	21	28	35	42	49	56	63
39												3.3	
40						2.8						3.4	
41					4.9	2.3		2.0				2.8	
42					2.6	2.5		2.0				3.1	
43								2.3			2.0	3.3	
44						2.2						3.9	
45												3.0	
46					3.3	2.0						2.8	
47					4.8							4.4	
48						2.9		2.6				3.6	
49						2.3		2.3				3.3	
50						2.0						3.2	
51						2.0		2.7	NS	NS	NS	NS	NS
52					4.7	2.5					NS	3.2	
53	4.5	4.9	5.5	4.7	3.0	2.3						3.3	
54								3.1				4.0	
55					4.9	2.9		2.9				3.2	
56	4.7	4.9	4.9	4.7	2.7	2.7						3.3	
57												3.0	
58								2.0				3.2	
59								3.0				3.0	
60												2.8	
61												3.5	
62					3.0			2.0				3.4	
63	3.7	4.9	5.0	5.0	3.6	3.5						3.3	
64												3.9	
65					4.9	2.8						NS	NS
66												4.1	
67								3.4				3.9	
68												3.2	
69								2.6				3.2	
70								2.0				3.7	

Study 2-B (Feedlot, 2019) Fecal samples taken from the individual steers by day (n=70 through day 35, n=69 on day 42 and 49, n=68 on day 56 and 63). Enumerable positive samples (green) are those defined as positive via spiral plating methods. Concentrations listed for enumerable positive samples are in log₁₀CFU/g of feces. Enrichment positive samples (gold) are those

defined as negative via spiral plating methods, but positive via 6h incubations in EC broth. No sample (NS). The five steers randomly selected for initial inoculation are highlighted in red.

Chapter 3 - The effect of temporal resolution and contact duration on Real-Time Location System-based contact networks for confined feedlot cattle

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Abstract

Contact network-based disease transmission models are critical for describing disease transmission dynamics and identifying control measures for specific diseases; however, empirical data on animal contact networks are scarce and most models use idealized contact networks. The objective of this study was to use contact network analysis to quantify contacts within three pens of feedlot cattle across three consecutive years at varying temporal resolutions to better inform the construction of network-based disease transmission models for cattle within confined-housing systems. We also aimed to describe the influence of the variation in Real-Time Location System (RTLS) average tag read rates and the effect of increasing minimum contact duration (MCD) on the contact networks of feedlot cattle. Three pens of feedlot steers were tagged with RTLS, one pen in each of three consecutive summers from 2017 to 2019. Contacts

were defined with a spatial threshold of 0.71 m and an MCD of either 10, 30, or 60 seconds. Static, undirected, weighted contact networks were created for the full study duration and then split into daily (24-h), 6-h period, and hourly networks to better assess network heterogeneity. For the full study duration time scale, all three networks were found to be densely connected. The networks showed more heterogeneity in network density and clustering coefficient when smaller time scales (6-h period and hourly) were applied. When contacts were defined with a MCD of 30 or 60 seconds, the total number of contacts seen in each network decreased, indicating that most of the contacts observed in our networks may have been transient passing contacts. For example, the total defined contacts for the 2017 native read rate network were 930,843 at 10 s MCD, 95,570 at 30 s MCD, and 19,135 at 60 s MCD. Though the same system was used for all three years, variation in average tag read rate was observed (range: 2.2 readings/min (2018) to 7.4 readings/min (2017)). When the networks were down-scaled from higher average tag read rates to match lower tag read rates, the overall networks maintained similar network density and clustering, though the average edge weight between pairs decreased. Overall, the networks created here from high-resolution spatial and temporal contact observation data provide estimates for a contact network within a commercial US feedlot pen that can be used to better inform pathogen transmission models on social contact networks.

Introduction

Infectious disease transmission is highly related to the structure of contact networks in populations, and heterogeneity or clustering in contacts among hosts is highly influential in disease transmission within populations (Eames, 2008; Lloyd-Smith et al., 2005; Nold, 1980). The common infectious disease modeling transmission assumption of homogenous random

mixing among all individuals may lead to misspecified models and inaccurate predictions of epidemic behavior and the impact of interventions (Keeling and Eames, 2005). Contact patterns may be driven by population density and individual behavior. Knowledge of heterogeneity in contact patterns in populations is critical to proper specification of transmission models and correct understanding and prediction of infectious disease spread (Ames et al., 2011; Craft, 2015; Kao et al., 2007; Keeling, 2005).

Contact networks have been applied to various wildlife animal populations at varying temporal and spatial resolutions, including examining the shifts in social network structure in colobus monkeys (Adams et al., 2021), social patterns and demographic factors of capuchin monkeys (Gazes et al., 2022), the dynamic contact networks of raccoons (Reynolds et al., 2015), contact networks of hummingbirds (Bandivadekar et al., 2018), dynamics of social networks among Asian and African elephants (de Silva et al., 2011; Murphy et al., 2020), the individual variation in behavior among ants (Hui and Pinter-Wollman, 2014; Pinter-Wollman et al., 2011), and exploring the dynamics of disease across the wildlife-domestic interface of badgers and cattle (Moustakas and Evans, 2017). The majority of wild animals are challenging to track individually and consistently with a high sampling rate, this poses a significant challenge as the resulting networks have a lower temporal resolution, usually a day or longer. These lower temporal resolution networks may not provide the most complete picture regarding the contact network between individuals in the observed population.

Networks have been used extensively in both human and animal settings at varying temporal and spatial scales. Recently, networks have been used to describe the spread of diseases that require close contact within confined environments such as: communities, schools, hospitals,

and households (Goeyvarts et al., 2018; Grijalva et al., 2015; Luo et al., 2022; Rodríguez et al., 2017; Salathé et al., 2010). To better predict livestock disease transmission dynamics and identify interventions, it is essential to characterize contact networks in confined environments, such as feedlot pens.

In production animal settings, contact networks have been used primarily to describe farm-to-farm disease spread at large spatial scales (Bigras-Poulin et al., 2006; Ciccolini et al., 2012; de Koeijer et al., 2020; Keeling et al., 2001; Keeling and Eames, 2005; Lindström et al., 2010; Ortiz-Pelaez et al., 2006; Rossie et al., 2017; Webb, 2005; Woolhouse et al., 2005). There is limited data available on contact structure of individuals within premises at smaller defined spatial scales, so the networks are poorly understood despite their potential influence on within herd or between herd disease transmission (Chirs Ster et al., 2012; Duncan et al., 2012; Nicbakhsh et al., 2013). Additionally, there are Recently, the contact networks for small groups of Holstein calves in confined pen environments have been described over a 2-week period (Chen et al., 2014; Chen et al., 2015), but larger populations of confined feedlot cattle over multiple months have not been described.

Feedlot cattle in the U.S. are typically housed in confined, dry lot pens. This housing system allows their location to be explicitly and accurately tracked using a Real-Time Location System (RTLS). These systems have become increasingly accurate, with positional accuracies often less than 2 m (Chen et al., 2013; Dawson et al., 2019; King et al., 2012; Schiffner et al., 2018), and the ability to fix individuals' locations over increasingly small temporal intervals (e.g., 1-10s) (Dawson et al., 2019; Kays et al., 2015; Schiffner et al., 2018). The high resolution of this system allows for multiple location readings per minute for each tagged animal within

their confined pen space, resulting in high-resolution networks, both temporally and spatially. Creating an individual steer-to-steer contact network develops a more representative description and enhances our understanding of animal dynamics. This characterization of high-resolution contact networks can be incorporated into future research including more accurate pathogen transmission on contact networks.

The aim of this study was to use contact network analysis to quantify contacts within pens of feedlot cattle across multiple years at varying timescales (24-h, 6-h, and h) to better inform the construction of network-based disease transmission models for cattle within confined-housing systems. We also describe the influence of RTLS read rate (average readings per minute) and extended minimum contact duration (10 seconds (10s), 30 seconds (30s), and 60 seconds (60s)) on the realized description of contact networks of feedlot cattle.

Materials and Methods

All procedures performed in studies involving animals were in accordance with the ethical standards of Kansas State University College of Veterinary Medicine, IACUC #4065.

Study Populations

Contact network data were collected over three distinct study periods during the summer months of 2017 to 2019. In May of each year, seventy Angus/Angus-cross steers were purchased through commercial channels. Each cohort of steers was housed in an outdoor pen at a commercial research feedlot (Figure 3-1). In 2017, steers were housed in Pen 1. In 2018 and 2019, steers were housed in Pen 2. All pens measured 38 m by 30.5 m, providing approximately 17 m² area per steer, which are typical stocking rates of commercial US feedlots. Each pen had a centrally located water tank, a 24.5 m long fence line feed bunk, and a 3 m wide concrete apron

behind the feed bunk and around the water tank (Figure 3-1). All steers were unfamiliar with the enclosure prior to the experiment, and no individuals were retained from year to year.

Point Location Data

All steers received two ear tags for visual identification, as well as a RTLS ear tag placed centrally in the left ear (Smartbow GmbH, Weibers, Austria). These radio-transmitting ear tags communicated with receivers positioned around the pens. These receivers were hardwired to a server located in an enclosed shed that was used to store and transmit the location data (Figure 3-1). Steers' positions (X,Y) were triangulated and recorded on the server. The coordinate pairs (X,Y) obtained were 90% accurate to within ± 0.5 m of individuals' true location (Farthing et al., 2021). A flow chart of location data processing and subsequent contact network creation can be found in Figure 3-2. As previously outlined in Dawson et al. 2019 and Farthing et al. 2021, raw location data were filtered and smoothed to decrease error-induced noise and standardize temporal resolution. Data points were first filtered and removed if location points fell outside the designated pen area or if sequential locations indicated that the individual was moving at speeds greater than 10 meters per second (mps). Resulting location data were then smoothed to represent individuals' average location (X,Y) at 10 second intervals over the course of that study period.

Due to the differences in realized read rates (number of location (X,Y) readings per time period by individual tags) between study years, we down-scaled the 2017 and 2019 data sets to produce common read rates and better assess the effect of tag read rate on the creation of contact networks. The 2017 and 2019 datasets were down-scaled by sorting the raw location readings in chronological order by individual steer and then applying a random process to drop individual

readings to match the desired expected read rate. For example, to decrease the 2017 read rate to match the 2018 read rate we randomly dropped each line in the 2017 data set using the following probability equation:

$$\text{Probability of location point being dropped} = 1 - \left(\frac{2018 \text{ Tag Read Rate}}{2017 \text{ Tag Read Rate}} \right)$$

The three down-scaling procedures include two done on the 2017 data (2017 read rate to match the 2018 read rate and the 2019 read rate) and one done on the 2019 data to match the 2018 read rate. Each down-scaling was repeated for 50 iterations, and variation in total contacts and network parameters at all time scales were compared across iterations to ensure convergence to a stable estimate of the down-scaled networks. The median network measure of all 50 iterations is reported for each down-scaled network.

Model Construction

Using the procedures described in Dawson et al., 2019 and Farthing et al., 2021, all contact networks were created with a spatial threshold (SpTh) for identifying contacts of 0.71 m and a minimum contact duration (MCD) of 10s. The smoothed data representing each individual's average location for each 10s temporal sampling window (TSW) was used to determine if a contact occurred in each temporal window. Distances between each steer for each temporal window were calculated and the spatial threshold of 0.71 m was applied to determine whether a contact occurred in that given TSW. Matrices of distance between each steer pair were created for each temporal window. Individual contacts were then identified by applying a spatial threshold of 0.71 m to determine whether a contact occurred between each pair of steers in that given TSW generating an adjacency matrix for each temporal sampling window. Contact

duration was defined as the total number of consecutive TSW in which two steers were within the 0.71 m spatial threshold. Contact was considered broken when individuals were observed outside of the specified 0.71 m spatial threshold for any given TSW. These defined contacts were then used to create weighted undirected networks by summing the total contact duration for every pair of steers over a specified period duration of time. Overall networks as reported here included the entire study period. For daily (24 h), 6-hour, and hourly networks the first day of each study (when the calves were getting tags put on at different times) and the last day (when the calves were getting tags taken off at different times) were removed. The 6-h networks were defined in Central Daylight time from 00:00-05:59 (night), 06:00-11:59 (morning), 12:00-17:59 (afternoon), and 18:00-23:59 (evening).

Additionally, we generated weighted, undirected contact networks for each study year at all timescale durations and read rate combinations (native and down-scaled) based on an alternate minimum contact duration (MCD, the duration of the temporal sampling windows that defined a contact). To examine an MCD of 30s (those contacts between two steers that were 30 seconds or longer) the contact networks were created as described above with the same SpTh and TSW and the adjacency matrix was generated by keeping only contacts that were 3 TSW or larger. To examine a MCD of 60s (those contacts between two steers that were 60 seconds or longer) the contact networks were created as described above with the same SpTh and TSW and the adjacency matrix was generated by keeping only contacts that were 6 TSW or larger.

Software

All data processing was conducted in RStudio (RStudio Team, 2022, v. 2022.02.3) running R (R Core Team, 2020, v. 4.1.1). We used the contact package (v. 1.2.8), (Farthing et

al., 2020) to clean point-location data and create contact networks. The dplyr package (v. 1.0.9) was used to randomly sample rows in the down-scaled networks for the years 2017 and 2019. The Igraph package (v. 1.2.6) was used to visualize and obtain network metrics of the contact networks. Network modularity was calculated using the fast-greedy algorithm in Igraph (v. 1.2.6). The Hmisc package (v. 4.7-0) was used to visualize the distributions of the total edge weight for each contact network. Stata 16.1 was used to analyze the skewness and kurtosis of the full network and median daily network edge weights (StataCorp, 2019).

Results

Section 1: Native Read Rate Networks

Study 1 was conducted in the summer of 2017 over 50 days. Seventy steers were enrolled in the study with a mean arrival body weight of 339.1 kg (sd = 31.3 kg). No steers were removed from the study. A total of 39,132,528 location (X,Y) readings were collected during the study period. A median of 11,847 readings per tag per day were recorded (range: 0-18,282, Table B-1) with an average tag read rate of 7.4 readings/min. A total of 930,843 contacts at 0.71 m with a 10s TSW were identified between dyad pairs. All steers (n=70) and connections between pairs (n=2,415) were identified in the full network, yielding a network density of 100%, modularity of 0 and clustering coefficient of 1 (Table 3-1). The median overall edge weight contacts between dyad pairs were 328 TSW (range: 45-71,319, Table B-2). When initially examined, the edge weights between pairs of steers showed a positive skewness of 17.2 and kurtosis of 321.7. When total edge weights were ranked, one calf accounted for the top 30 total edge weights with a range of connections from 1,420 TSW to 71,319 TSW. This calf was removed from the visualization of the total edge weights (Figure 3-3). When this calf was removed the distribution showed a slight

positive skewness of 0.9 and kurtosis of 4.7. The next highest ranking edge weight between any two steers was 1,380 TSW. Daily (24-h) networks had a median density of 92% (range: 81%-98%, Table 3-2). The median daily edge weight between dyad pairs was 5 TSW (range: 1-1,861, Table B-3). When networks were examined on a daily (24-h) basis, they still showed a high level of clustering with a median clustering coefficient of 0.9 (range: 0.8-1), but no community structure was detected with a median daily modularity of 0 (range: 0-0, Table 3-2). The 6-hour period networks had median density of 49% (range: 0%-90%, Table 3-2) with morning and afternoon 6-hour periods having the highest median density (Table 3-3). On an hourly timescale, the median density was 13% (range: 0%-42%, Table 3-2) with the network density trending higher in the daylight hours compared to those in the evening and nighttime (Table B-4).

Study 2 was conducted in the summer of 2018 over 71 days. Seventy steers were initially enrolled in the study, with a mean arrival body weight of 271.7 kg (sd = 19.4 kg). One steer was removed from the study on day 35 due to lameness. A total of 16,099,395 location (X,Y) readings were collected during the study period. A median of 3,138 readings per tag per day were recorded (range: 0-10,952, Table B-1) with an average tag read rate of 2.2 readings/min. A total of 68,757 contacts were identified between dyad pairs. All steers (n=70) were identified in the full network. However, only 2,331 connections (edges) between steers or 97% density were identified for the full network (Table 3-1). The median overall edge weight (number of contacts between dyad pairs) was 26 (range:1-319, Appendix Table 3-2). The edge weights between pairs of steers showed a positive skewness of 2.2 and kurtosis of 10 (Figure 3-3). There was evidence of slight clustering for the daily networks with a median clustering coefficient of 0.3 (range: 0.2-0.4), but limited community structure was detected with a median modularity of 0.2 (range: 0.1-

0.2, Table 3-2). The median daily (24-h) and 6-hour network densities were 19% (range: 9%-30%) and 5% (range: 0%-15%), respectively (Table 3-2). The median daily edge weight between dyad pairs was 1 (range: 1-47, Table B-3) There was little variation between the median 6-h period densities (Table 3-3) and median hourly densities (Table B-4).

Study 3 was conducted during the summer of 2019 over 64 days. Seventy steers were initially enrolled in the study with a mean arrival body weight of 285.2 kg (sd = 26.7 kg). Two steers were removed from the study due to lameness: 1 on day 35 and another on day 56. A total of 26,788,833 location (X,Y) readings were collected during the study period. A median of 5,065 readings per tag per day were recorded (range: 0-18,418, Table B-1) with an average tag read rate of 3.3 readings/min. A total of 230,655 contacts were identified between dyad pairs. One tagged steer was present in the study with pen location readings but had no defined contacts. All remaining tagged steers (n=69) and 2,273 connections between pairs were identified in the overall network. Based on the 69 steers with location data, the full network density was 97%. The median overall edge weight (number of total contacts between dyad pairs) was 110 (range: 1-1181, Table B-2). The edge weights between pairs of steers showed a positive skewness of 2.5 and kurtosis of 14.5 (Figure 3-3). On a daily (24-h) basis there was evidence of moderate clustering with a median clustering coefficient of 0.5 (range: 0.4-0.8), but there was limited community structure detected with a median modularity of 0.1 (range, 0-0.3, Table 3-2). The median daily (24-h) and 6-hour period network density was 37% (range: 10%-72%) and 11% (range: 0%-52%), respectively (Table 3-2). The median daily edge weight was 2 TSW (range: 1-143, Table B-3). There was little variation seen in the median densities for the 6-h period networks (Table 3-3) and hourly network densities (Table B-4).

Section 2: Down-scaled Read Rate Networks

When the 2017 network (average 7.4 readings/tag/min) was down-scaled down to the read rate of 2018 (average 2.2 readings/tag/min) a total of 27,692,305 location (X,Y) readings were randomly dropped for each iteration. Across all iterations, a median of 71,704 total contacts at a SpTh of 0.71 m and 1 TSW were identified (Table 3-1). For all iterations, the full network contained 70 steers and all 2415 edges, yielding a network density of 100%. The median of the iteration specific overall median edge weight dropped by approximately 92% from 328 to 28 TSW (range: 1-120 TSW, Table 3-1). Across all iterations, the median overall edge weight between pairs of steers showed a slight positive skewness of 1 and kurtosis of 5.7 (Figure 3-3). Across all iterations, the median daily modularity was 0.1 (range: 0.1-0.1) and the median daily clustering coefficient was 0.6 (range: 0.5-0.8, Table 3-2). The median of the iteration specific daily median edge weight dropped by 60% from 5 to 2 TSW (range: 1-31). The median of the iteration specific daily, 6-hour, and hourly median network density decreased from 92% to 37% (range: 27%-51%), from 49% to 11% (range: 0%-29%), and from 11% to 2% (range: 0%-9%), respectively (Table 3-2).

For the 2017 network (7.4 read rate) down-scaled to the read rate of 2019 network (average 3.3 readings/tag/min) a total of 21,972,350 location (X,Y) readings were randomly dropped for each iteration. Across all iterations, a median of 155,957 total contacts at 0.71m SpTh and 1 TSW were defined across the iterations (Table 3-1). Across all iterations, the overall network identified 70 steers and 2415 edges with a network density of 100%. The median of the iteration specific overall median edge weight between pairs dropped by approximately 80% from 328 to 64 TSW (range: 6-307 TSW, Table 3-1). Across all iterations, the median modularity for

the daily networks was 0.1 (range: 0.1-0.1) and the median daily clustering coefficient was 0.4 (range: 0.3-0.5, Table 3-2). Across all iterations, the median overall edge weights between pairs of steers showed a slight positive skewness of 1 and kurtosis of 5.3 (Figure 3-3). The median of the iteration specific daily median edge weight dropped by 80% from 5 to 1 TSW (range: 1-15). The median of the iteration specific daily network density decreased from 92% to 58% (range: 43%-75%, Table 3-2). On a 6-hour and hourly timescale the median of the iteration specific network density decreased from 49% to 19% (range: 0%-50%) and from 11% to 3% (range: 0%-17%), respectively.

When the 2019 network (3.3 read rate) was down-scaled to the read rate of 2018 (2.2 read rate) a total of 8,623,693 location (X,Y) readings were randomly dropped for each iteration. Across all iterations, a median of 142,656 total contacts were defined (Table 3-1). Across all iterations, all steers (n=69) and a median of 2,265 total edges were identified. The median iteration specific overall density was 97%. The median of the iteration specific overall median edge weight between pairs decreased by 55% from 110 to 50 TSW (range: 1-529 TSW, Table 3-1). Across all iterations, the median modularity of the daily networks was 0.2 (range: 0.2-0.2, Table 3-2). Across all iterations, the median overall edge weights between pairs of steers showed a positive skewness of 2.5 and kurtosis of 14.5 (Figure 3-3). The median of the iteration specific daily median edge weight remained at 2 TSW (range: 1-63, Table 3-2). The median of the iteration specific network density decreased from 37% to 26% (range: 0%-63%) for the daily (24-h) networks, from 12% to 8% (range: 0%-46%) for the 6-hour period networks and remained at 2% (range: 0%-31%) for the hourly networks (Table 3-2).

When the down-scaled networks are compared to the matching native read rate network, the median edge weight for each network is similar, ranging from 1 to 2 TSW per study day. For those networks with a native or down-scaled read rate of 2.2 readings/min the median daily network density ranged from 19% to 37%. For the 2019 native read rate and 2017 down-scaled network with a read rate of 3.3 readings/min the daily, 6-h, and hourly networks were similar in network density and clustering metrics. The median daily network density was 37% and 58% for those networks with a native or down-scaled read rate of 3.3 readings/min.

Section 3: Native Read Rate Networks at 30s and 60s Minimum Contact Duration

For Study 1 in 2017, when contacts were defined as with a MCD of 30s, a total of 95,570 contacts were defined (Table 3-3). All steers ($n = 70$) were identified in the full network, meaning that all steers had at least 1 contact that was greater than 30s with another steer during the study period. The overall network yielded 2393 edges with a density of 99%, modularity of 0, and clustering coefficient of 1 (Table 3-4). The median overall edge weight between pairs was 45 TSW (range: 3-46,751 TSW). On a daily (24-h) network basis there was limited community structure detected with a median modularity 0.2 (range: 0.2-0.3, Table 3-4). When contacts were defined as those 30s or longer the median network density was 18% (range: 12%-26%) for the daily (24-h) networks, 5% (range: 0%-11%) for the 6-h period networks, and 1% (range: 0%-4%) for the hourly networks (Table 3-4). A visual representation of community structure detected at varying MCD for study day 1 of 2017 can be found in Figure B-5.1-3.

For Study 1, when contacts were defined as a MCD of 60s, a total of 19,135 contacts were defined (Table 3-4). All steers ($n = 70$) were identified in the full network, showing that all steers had at least 1 contact that was greater than 60s with another steer. The overall network had

1,330 edges with a density of 55% and modularity of 0.1 and clustering of 0.7 (Table 3-4). The median overall edge weight between pairs was 14 TSW (range: 6-24,950 TSW). For the daily (24-h) network there was moderate community structure detected with a median modularity of 0.6 (range: 0.4-0.7, Table 3-4). When contacts were defined as those 60s or longer, the median density of networks was 3% (range: 2%-4%) for the daily (24-h) networks, 1% (range: 0-2%) for the 6-h period networks, and 0.2% (range: 0-1%) for the hourly networks (Table 3-4).

During Study 2 in 2018, a total of 3,317 contacts were found when the MCD was set to 30s (Table 3-4). The full network included 68 steers and a total of 1,085 edges (density: 48%, modularity: 0.1, clustering: 0.6). Two steers were not identified, meaning that these two steers had no identifiable contacts with any other steer within the entire study period that were longer than 30s. The median overall edge weight between pairs was 7 TSW (range: 3-96 TSW). On a daily (24-h) network basis there was high to moderate community structure detected with a median modularity of 0.8 (0.6-0.9, Table 3-4). When the minimum contact duration was 30s or greater, the median network density was 1% (range: 0%-3%) for the daily (24-h) networks, 0.3% (range: 0%-1%) for the 6-h period networks, and 0% (range: 0%-0.4%) for the hourly networks (Table 3-4).

For summer 2018, when contacts were defined with a MCD of 60s, a total of 301 contacts were defined (Table 3-4). The full network had 61 steers and a total of 196 edges (density: 11%, modularity: 0.1, clustering: 0.6). Nine steers were not identified in the full network, meaning that these steers had no identifiable contacts with any other steer within the entire study period that was longer than 60s. The median overall edge weight between pairs was 7 TSW (range: 6-55 TSW). On a daily (24-h) basis there was moderate to high community

structure detected with a median modularity of 0.8 (range: 0.6-0.9, Table 3-4). When the minimum contact duration was 60s or longer, the median daily network density was 0% (range: 0%-0.5%, Table 3-4).

In Study 3 (2019), a total of 17,741 contacts were defined when the MCD was set to 30s (Table 3-4). All steers (n=69) and 2,024 edges were identified in the full network. The full network density was 86%, modularity 0, and clustering of 0.9. The median overall edge weight between pairs was 21 TSW (range: 3-461 TSW). When a contact was defined as being at least 30s, the median network density was 5% (range: 0%-12%) for the daily (24-h) networks, 1% (range: 0%-7%) for the 6-h period networks, and 0.2% (range: 0%-6%) for the hourly networks (Table 3-4). On a daily (24-h) basis there was moderate community structure detected with a median modularity of 0.5 (range: 0.3-0.7, Table 3-4).

For summer 2019, a total of 1,825 contacts were defined when the MCD was 60s (Table 3-4). A total of 68 steers were identified in the full network. One steer did not have any contact with any other steer that was recognized as at least 60s in length. The full network contained 798 edges with a density of 35%, modularity of 0.1, and clustering of 0.4. The median overall edge weight between pairs was 10 TSW (range: 6-173 TSW). On a daily (24-h) basis there was moderate to high community structure detected with a median modularity of 0.8 (range: 0.6-0.9). When contacts were defined as being 60s or longer, the median daily density was 1% (range: 0-2%, Table 3-4).

Section 4: Down-scaled Read Rate Networks at 30s and 60s Minimum Contact

Duration

For the 2017 network (7.4 read rate) that was down-scaled to the read rate of summer 2018 (2.2 read rate) and contacts were defined as those being 30s or longer (MCD = 3), a median of 869 contacts were defined across all iterations (Table B-6). Across all 50 iterations, all 70 steers and a median of 651 edges were identified, with median full network density of 27% and median modularity of 0.5 and median clustering coefficient of 0.4. The median of the iteration specific overall median edge weight between pairs of steers was 4 TSW (range: 3-18 TSW, Table B-6). The median of the iteration specific daily network density was 0% (range: 0%-0%). On a daily (24-h) network basis there was moderate community structure detected with a median modularity across all iterations was 0.5 (range: 0-0.50). The median of the iteration specific 6-hour period and hourly median density was 0% (range: 0%-0%, Table B-6).

When summer 2017 (7.4 read rate) was down-scaled to the read rate of summer 2018 (2.2 read rate) and contacts were defined as those being 60s or longer (MCD = 6), a median of 2 contacts were defined across all iterations (Table B-6). Across all iterations, a median of 4 steers and 2 edges were identified in the full network. The median iteration specific overall network density was 0.1%.

The 2017 network (7.4 read rate) down-scaled to the read rate of 2019 (3.3 read rate) and contacts were defined as those 30s or longer, a median of 93 contacts were defined across all iterations (Table B-6). Across all iterations, a median of 60 total steers and 91 edges were identified in the full network, with the full network density of 4%, modularity of 0.1, and clustering of 0.1. The median of the iteration specific overall median edge weight between pairs

of steers was 3 TSW (range: 3-6 TSW). On a daily (24-h) network basis there is a high amount of community structure detected with a median daily modularity across all iterations of 0.8 (range: 0.8-0.8, Table B-6). When the 2017 network was down-scaled to the read rate of 2019 and a contact was defined as 60s or longer, no contacts were identified. No contacts were identified in any iteration when the 2019 network (3.3 read rate) was down-scaled to reflect the read rate of 2018 (2.2 read rate) and contacts were defined as those 30s or 60s.

Discussion

In this study, we presented high-resolution contact networks for three independent pens of feedlot steers across three consecutive summers. These networks provide estimates for a contact network within a commercial US feedlot pen that can be used to better inform pathogen transmission models on contact networks.

The cattle observed in these studies were confined within a limited pen space and our resulting networks on a longer timescale were dense, with the majority of potential connections possible between pairs of steers present over each study period. All three pen networks had high network density when considered for the full study period. This was true for all observed read rates. At a daily (24-h) time scale, the density remained high for the year 2017. This dense network at the 24-hour time scale could be due to the high read rate seen in 2017 compared to 2018 and 2019. The confined space for the cattle in this study is likely influential in the observed measures. Additional heterogeneity or evidence of social affinity may be more likely observed in pasture cattle with more space to roam.

Though the same system was used for all three years, variation in the contact networks was seen from year to year. This variation in the networks may be normal pen-to-pen variation in feedlot cattle, however the wide discrepancy in average read rate also contributed to the variation in the observed contact networks. The highest tag read rate was seen in 2017 and the networks created in 2017 may be most complete due to this high tag read rate, however they may contain more transient contacts than 2018 and 2019. The lowest tag read rate was seen in 2018 with an average of 2.2 readings/min for individual tags and this low read rate may not have picked up all possible contacts creating a potentially incomplete network. When the 2017 and 2019 networks were down-scaled to match the average read rate of the 2018 network, both networks maintained their overall full study density. However, even though the 2017 network was only down-scaled by approximately 70% (7.4 to 2.2) the median edge weight for the full study dropped by over 90% (328 to 28). When the 2019 network was down-scaled by approximately 33% the median edge weight for the full study dropped by approximately 55%. However, when the 2017 and 2019 network were standardized to match the read rate of the 2018 network the median daily edge weight was comparable between all three networks at 1 to 2 TSW/day. The median daily network density for all three networks ranged from 19% to 37%, indicating that there may be some inherent variation in daily network density between pens of feedlot cattle. When the 2017 network was down-scaled to match the average read rate of the 2019 network (3.3 readings/min), the overall network density remained unchanged. The median overall edge weight for 2017 decreased by approximately 80% even though the network was down-scaled by only 55% and was approximately 40% lower than 2019. The 2019 native read rate network and the 2017 down-scaled network with an average read rate of 3.3 readings/min showed similar median

daily edge weight of 1 to 2 TSW per study day. The two networks had a median daily network density between 19% and 37%, highlighting the potential inherent variability in daily network density between pens of feedlot cattle.

Many pathogens have complex transmission dynamics, with multiple direct and indirect transmission routes. Homogenous-mixing mean-field compartmental models with random contact distribution through time and among individuals are commonly used to model direct transmission events (Bansal et al., 2007). However, the contact structure within the intended populations may be heterogenous. Heterogeneity in individual contact patterns can profoundly shape the population-level disease dynamics (Bansal et al., 2007). However, despite their influence on disease transmission, the contact structure in larger confined livestock populations is not well understood. For all three of our studies, the total edge weights found between pairs of steers was shown to have a positive skewness. This skewness in distribution for the total edge weights shows that not all pairs of steers contact each other evenly and this should be considered when modeling disease transmission between individuals in a population. Increases in modularity in 24-hour networks and at longer contact duration support community structure within the population.

Though these networks are considered high resolution with an estimated accuracy of 90% within ± 0.5 m of individuals' true location (Farthing et al., 2021) and an observed average tag read rate between 2.2 and 7.4 readings per minute, they are still static in nature. These networks presented here are only created for aggregated defined timescales, and a dynamic contact structure was not applied at this time. Dynamic networks can be used for simulating disease transmission, both within and between populations (Duncan et al., 2012; Vernon and Keeling,

2009) and are important if contact structures change over time. Chen et al. showed dynamic changes in cattle contact structure were able to change the disease dynamics at a pen level (2014). By applying a dynamic network structure, individual behavior events such as disease-induced changes, as well as pen-level events such as weather-related events and cyclical feeding events can be modeled and their influences estimated (Farine, 2018). Though we are capturing some of these event influences in our contact structure, more work is needed to estimate their influence on the network.

To determine the influence of the required duration of contact on the number of contacts created in the network we looked at the minimum duration in which a contact was defined, either a 10s, 30s, or 60s MCD. The three contact durations were chosen to better differentiate between passing transient contacts and longer contacts made between cattle, such as grooming events. For all studies, a larger number of contacts were found when the MCD was 10s, compared to 30s and 60s. The difference in total number of contacts created based on the MCD could be a natural phenomenon, meaning the majority of contacts created in our feedlot pens on a day-to-day basis are truly short in nature. There could also be some effect due to how the data was filtered and smoothed in processing. In 2017, we saw a higher number of longer (30s and 60s) contacts compared to 2018 and 2019, suggesting the higher read rate may more effectively capture longer contact durations. Additionally, when the down-scaled networks (lower read rates) were examined at longer MCD (30s and 60s) no real network was observed. Depending on the assumed amount of contact needed for transmission of a particular disease, a higher location sampling rate may be needed to capture this MCD. The ability to generate different contact networks based on TSW, MCD and spatial threshold is an advantage of the RTLS system we

used. We also saw an increase in modularity for daily networks and as the MCD increased. The increased modularity at a daily time period coupled with near zero for the full study period suggests that group structure may exist over a short time period, but that those groups may be transient resulting in little community structure over an extended period. The increased modularity as the MCD increased suggests that any community structure present is associated with longer term interactions. These observations require additional data analysis to resolve.

Instead of using contact occurrence to model disease transmission probability in a contact network, it may be more useful to use total edge weight created between dyad pairs combined with an appropriate time scale for modeling the transmission dynamics of a particular disease. For example, modeling applications for Foot and Mouth Disease (FMD) in cattle, edge weight count over a shorter time interval may be appropriate as it is highly transmissible. However, for a pathogen such as *Mycobacterium bovis* where extended contact duration is required for transmission an edge weight counts over a longer timescale may be more appropriate for modeling. Though a wide spectrum of transmission rates for *M.bovis* have been reported in field studies, it is considered to be a relatively slow process (Menzies and Neill, 2000). An appropriate time scale relative to that pathogen's transmissibility should be taken into consideration for modeling transmission events.

Conclusions

In summary, our studies used high temporal and spatial resolution observation data to construct contact networks for three pens of feedlot steers across three consecutive years. The resulting contact networks were dense when examined at longer time scales. To better account for contact heterogeneity between individuals, accounting for total contact time between

individuals for the appropriate timescale for transmission of a particular disease may be beneficial. These findings can lead to better construction of network-based disease transmission models within confined-housing systems.

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Literature Cited

1. Adams, F.V., Arseneau-Robar, T.J.M., Bonnell, T.R., Stead, S.M., Teichroeb, J.A., 2021. Temporal patterns in the social network of core units in Rwenzori Angolan colobus monkeys: effects of food availability and interunit dispersal. *Ecol. Evol.* 11:3251-3268. <https://doi.org/10.1002/ece3.7274>.
2. Ames, G.M., George, D.B., Hampson, C.P., Kanarek, A.R., McBee, C.D., Lockwood, D.R., Achter, J.D., Webb, C.T., 2011. Using network properties to predict disease dynamics on human contact networks. *Proc. R. Soc. B.* 278:3544-3550. <https://doi.org/10.1098/rspb.2011.0290>.

3. Bandivadekar, R.R., Pandit, P.S., Sollmann, R., Thomas, M.J., Logan, S.M., Brown, J.C., Klimely, A.P., Tell, L.A., 2018. Use of RFID technology to characterize feeder visitations and contact networks of hummingbirds in urban habitats. PLoS One. 13(12):e0208057. <https://doi.org/10.1371/journal.pone.020857>.
4. Bansal, S., Grenfell, B.T., Meyers, L.A., 2007. When individual behavior matters: homogenous and network models in epidemiology. J. R. Soc. Interface. 4:879-891. <https://doi.org/10.1098/rsif.2007.1100>.
5. Bigras-Poulin, M., Thompson, R.A., Chriel, M., Mortensen, S., Greiner, M., 2006. Network analysis of Danish cattle industry trade patterns as an evaluation of risk potential for disease spread. Prev. Vet. Med. 76:11-39. <https://doi.org/10.1016/j.prevetmed.2006.04.004>.
6. Ciccolini, M., Dahl, J., Chase-Topping, M.E., Woolhouse, M.E.J., 2012. Disease transmission on fragmented contact networks: livestock-associated Methicillin-resistant *Staphylococcus aureus* in the Danish pig-industry. Epidemics. 4:171-178. <https://dx.doi.org/10.1016/j.epidem.2012.09.001>.
7. Chen, S., Ilany, A., White, B.J., Sanderson, M.W., Lanzas, C., 2015. Spatial-temporal dynamics of high-resolution animal networks: what can we learn from domestic animals? PLoS One. 10(6):e0129253. <https://doi.org/10.1371/journal.pone.0129253>.
8. Chen, S., Sanderson, M.W., White, B.J., Amrine, D.E., Lanzas, C., 2013. Temporal-spatial heterogeneity in animal-environmental contact: Implications for exposure and transmission of pathogens. Sci. Rep. 3:3112. <https://doi.org/10.1038.srep03112>.

9. Chen, S., White, B.J., Sanderson, M.W., Amrine, D.E., Ilany, A., Lanzas, C., 2014. Highly dynamic animal contact network and implications on disease transmission. *Sci. Rep.* 4:4472. <https://doi.org/10.1038/srep04472>.
10. Chris-Ster, I., Dodd, P.J., Ferguson, N.M., 2012. Within-farm transmission dynamics of foot and mouth disease as revealed by the 2001 epidemic in Great Britain. *Epidemics.* 4:158-169. <https://dx.doi.org/10.1016/j.epidem.2012.07.002>.
11. Craft, M.E., 2015. Infectious disease transmission and contact networks in wildlife and livestock. *Phil. Trans. R. Soc. B.* 370:20140107. <https://dx.doi.org/10.1098/rstb.2014.0107>.
12. Dawson, D.E., Farthing, T.S., Sanderson, M.W., Lanzas, C., 2019. Transmission of empirical dynamic contact networks is influenced by data processing decisions. *Epidemics.* 26:32-42. <https://doi.org/10.1016/j.epidem.2018.08.003>.
13. de Koeijer, A.A., Hagenaaars, T.J., van Leuken, J.P.G., Swart, A.N., Boender, G.J., 2020. Spatial transmission risk during the 2007-2010 Q fever epidemic in The Netherlands: Analysis of the farm-to-farm and farm-to-resident transmission. *PLoS One.* 15(2): e0227491. <https://doi.org/10.1371/journal.pone.0227491>.
14. de Silva, S., Ranjeewa, A.D.G., Kryazhimskiy, S., 2011. The dynamics of social networks among female Asian elephants. *BMC Ecol.* 11:17.
15. Duncan, A.J., Gunn, G.J., Lewis, F.I., Umstatter, C., Humphry, R.W., 2012. The influence of empirical contact networks on modeling diseases in cattle. *Epidemics.* 4:117-123. <https://dx.doi.org/10.1016/j.epidem.2012.04.003>.

16. Eames, K.T.D., 2008. Modelling disease spread through random and regular contacts in clustered populations. *J. Theor. Biol.* 73:104-111.
<https://doi.org/10.1016/j.tpb.2007.09.007>.
17. Farine, D.R., 2017. When to choose dynamic vs. static social network analysis. *J. Anim. Ecol.* 87: 128-138. <https://doi.org/10.1111/1365-2656.12764>.
18. Farthing, T.S., Dawson, D.E., Sanderson, M.W., Lanzas, C., 2020. Accounting for space and uncertainty in real-time location system-derived contact networks. *Ecol. Evol.* 10:4702-4715. <https://doi.org/10.1002/ece3.6225>.
19. Farthing, T.S., Dawson, D.E., Sanderson, M.W., Seger, H., Lanzas, C., 2021. Combining epidemiological and ecological methods to quantify social effects on *Escherichia coli* transmission. *R. Soc. Open Sci.* 8: 210328. <https://doi.org/10.1098/rsos.210328>.
20. Gazes, R.P., Schrock, A.E., Leard, C.N., Lutz, M.C., 2022. Dominance and social interaction patterns in brown capuchin monkey (*Cebus [Sapajus] apella*) social networks. *Am. J. Primatol.* 83: e23365. <https://doi.org/10.1002/ajp.23365>.
21. Goeyvaerts, N., Santermans, E., Potter, G., Torneri, A., Van Kerckhove, K., Willem, L., Aerts, M., Peutels, P., Hens, N., 2018. Household members do not contact each other at random: implications for infectious disease modelling. *Proc. R. Soc. B.* 285:20182201.
<https://dx.doi.org/10.1098/rspb.2018.2201>.
22. Grijalva, C.G., Goeyvaerts, N., Verastegui, H., Edwards, K.M., Gil, A.I., Lanata, C.F., Hens, N., 2015. A house-based study of contact networks relevant for the spread of infectious diseases in the highlands of Peru. *PLoS One.* 10(3):e0118457.
<https://doi.org/10.1371/journal.pone.0118457>.

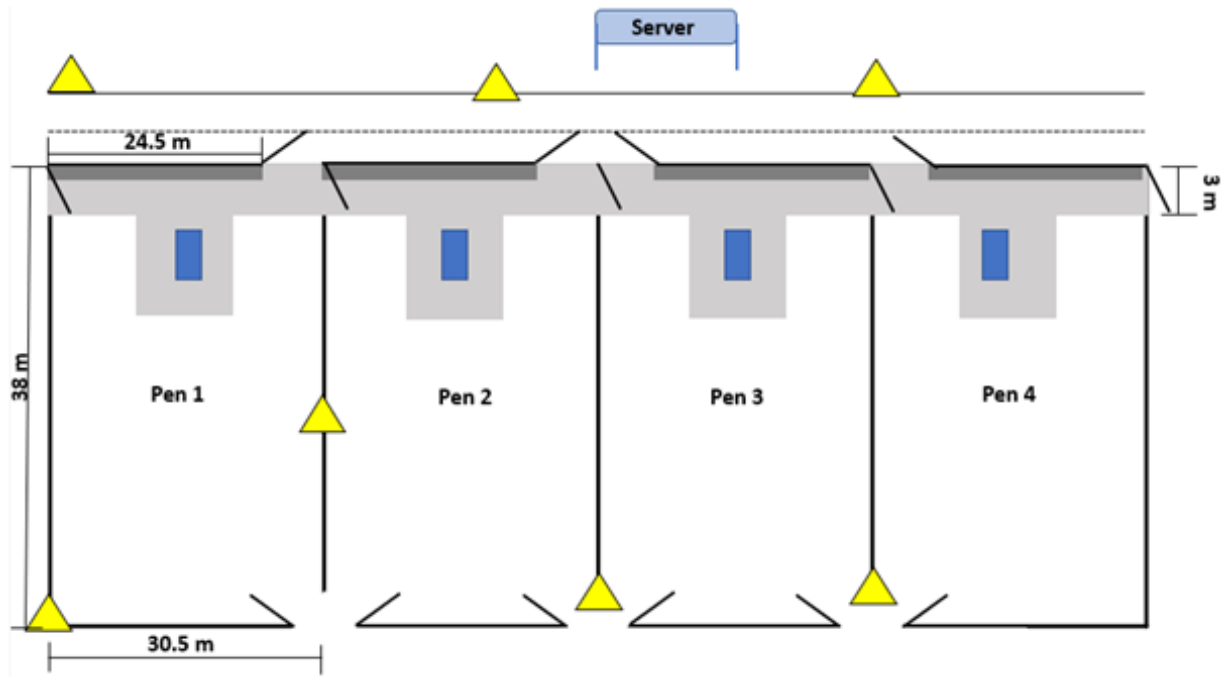
23. Hui, A., Pinter-Wollman, N., 2014. Individual variation in exploratory behavior improves speed and accuracy of collective nest selection by Argentine ants. *Anim. Beh.* 93:261-266. <https://dx.doi.org/10.1016/j.anbehav.2014.05.006>.
24. Kao R.R., Green, D.M., Johnson, J., Kiss, I.Z., 2007. Disease dynamics over very different time-scales: food-and-mouth disease and scrapie on the network of livestock movements in the UK. *J. R. Soc. Interface.* 4:907-916. <https://doi.org/10.1098/rsif.2007.1129>.
25. Kays, R., Crofoot, MC., Jetz, W., Wikelski, M., 2015. Terrestrial animal tracking as an eye on life and planet. *Sci.* 348(6240):aaa2478. <https://doi.org/10.1126/science.aaa2478>.
26. King, A.J., Wilson, A.M., Wilshin, S.D., Lowe, J., Haddadi, H., Hailes, S., Morton, A.J., 2012. Selfish-herd behavior of sheep under threat. *Curr. Biol.* 22(14):R561-R562. <https://doi.org/10.1016/j.cub.2012.05.008>.
27. Keeling, M.J., Woolhouse, M.E.J., Shaw, D.J., Matthews, L., Chase-Topping, M., Haydon, D.T., Carnell, S.J., Kappey, J., Wilesmith, J., Grenfell, B.T., 2001. Dynamics of the 2001 UK foot and mouth epidemic: stochastic dispersal in a heterogenous landscape. *Science.* 294:813-817. <https://doi.org/10.1126/science.1065973>.
28. Keeling, M.J., 2005. The implications of network structure for epidemic dynamics. *Theor. Popul. Biol.* 67:1–8. <https://doi.org/10.1016/j.tpb.2004.08.002>.
29. Keeling, M.J., Eames, K.T.D., 2005. Networks and epidemic models. *J. R. Soc. Interface.* 2:295-307. <https://doi.org/10.1098/rsif.2005.0051>.
30. Lindström, T., Sisson, S.A., Lewerin, S.S., Wennergren, U., 2010. Estimating animal movement contacts between holdings of different production types. *Prev. Vet. Med.* 95:23-31. <https://doi.org/10.1016/j.prevetmed.2010.03.002>.

31. Lloyd-Smith, J.O., Schreiber, S.J., Kopp, P.E., Getz, W.M., 2005. Superspreading and the effect of individual variation on disease emergence. *Nature*. 48(17):355-359.
<https://doi.org/10.1038/nature04153>.
32. Luo, T., Cao, Z., Wang, Y., Zeng, D., Zhang, Q., 2022. Role of asymptomatic Covid-19 cases in viral transmission: findings from a hierarchical community contact network model. *IEEE Trans. Autom. Sci. Eng.* 19(2):576-585.
<https://doi.org/10.1109/TASE.2021.3106782>.
33. Menzies, F.D., Neill, S.D., 2000. Cattle-to-cattle transmission of Bovine Tuberculosis. *Vet. J.* 160(2):92-106. <https://doi.org/10.1053/tvjl.2000.0482>.
34. Moustakas, A., Evans, M.R., 2017. A big-data spatial, temporal and network analysis of bovine tuberculosis between wildlife (badgers) and cattle. *Stoch. Environ. Res. Risk Assess.* 31:315-328. <https://doi.org/10.1007/s00477-016-1311-x>.
35. Murphy, D., Mumby, H.S., Henley, M.D., 2020. Age differences in the temporal Stability of a male African elephant (*Loxodonta Africana*) social network. *Beh. Ecol.* 31(1):21-31.
<https://doi.org/10.1093/beheco/arz152>.
36. Nickbakhsh, S., Matthews, L., Dent, J.E., Innocent, G.T., Arnold, M.E., Reid, S.W.J., Kao, R.R., 2013. Implications of within-farm transmission for network dynamics: consequences for the spread of avian influenza. *Epidemics*. 5:67-76.
<https://dx.doi.org/10.1016/j.epidem.2013.03.001>.
37. Nold, A., 1980. Heterogeneity in disease-transmission modeling. *Math. Biosci.* 52:227-240.
38. Ortiz-Pelaez, A., Pfeiffer, D.U., Soares-Magalhães, R.J., Guitian, F.J., 2006. Use of social network analysis to characterize the pattern of animal movements in the initial phases of

- the 2001 foot and mouth disease (FMD) epidemic in the UK. *Prev. Vet. Med.* 76:40-55.
<https://doi.org/10.1016/j.prevetmed.2006.04.007>.
39. Pinter-Wollman, N., Wollman, R., Guetz, A., Holmes, S., Gordon, D.M., 2011. The effect of individual variation on the structure and function of interaction networks in harvester ants. *J. R. Soc.* 8:1562-1573. <https://doi.org/10.1098/rsif.2011.0059>.
 40. R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>. (Last accessed 15 June 2022).
 41. RStudio Team (2022). RStudio: Integrated Development Environment for R. RStudio, PBC, Boston, MA. <http://www.rstudio.com/>. (last accessed 15 June 2022).
 42. Reynolds, J.J.H., Hirsch, B.T., Gehrt, S.D., Craft, M.E., 2015. Raccoon contact networks predict seasonal susceptibility to rabies outbreaks and limitations of vaccination. *J. Anim. Ecol.* 84:1720-1731. <https://doi.org/10.1111/1365-2656.12422>.
 43. Rodríguez, J.P., Ghanbarnejad, F., Eguíluz, V.M., 2017. Risk of coinfection outbreaks in temporal networks: a case study of a hospital contact network. *Front. Phys.* 5(46).
<https://doi.org/10.3389/fphys.2017.00046>.
 44. Rossi, G., Smith, R.L., Pongolini, S., Bolzoni, L., 2017. Modelling farm-to-farm disease transmission through personnel movements: from visits to contacts, and back. *Sci. Rep.* 7:2375. <https://doi.org/10.1038/s41598-017-02567-6>.
 45. Salathé, M., Kazandjieva, M., Lee, J.W., Levis, P., Feldman, M.W., Jones, J.H., 2010. A high-resolution human contact network for infectious disease transmission. *PNAS.* 107(51):22020-22025. <https://doi.org/10.1071/pnas.1009094108>.

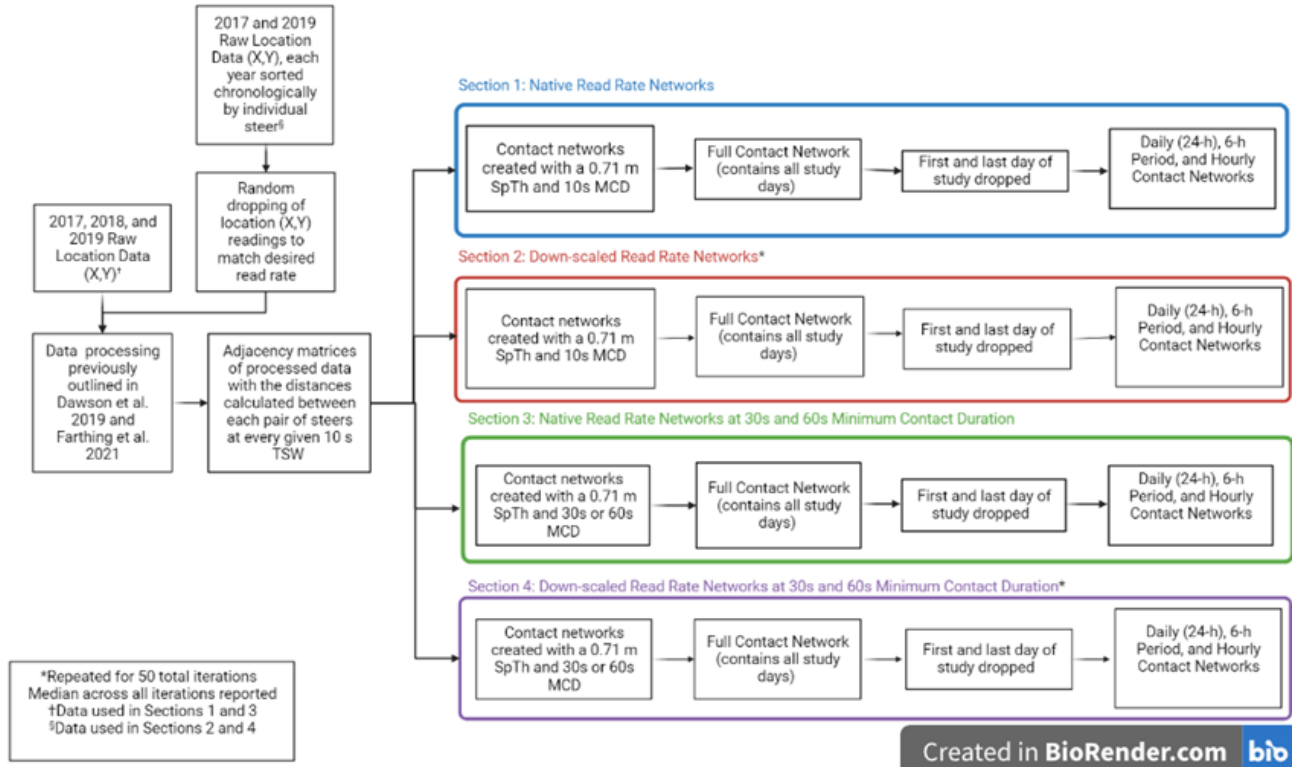
46. Schiffner, I., Fuhrmann, P., Reimann, J., Wiltshko, R. 2018. Behavioral traits of individual homing pigeons, *Columba livia f. domestica*, in their homing flights. PLoS ONE. 13:e0201291. <https://doi.org/10.1371/journal.pone.0201291>.
47. StataCorp. 2019. Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC. (Last accessed 1 January 2023).
48. Vernon, M.C., Keeling, M.J., 2009. Representing the UK's cattle herd as static and dynamic networks. Proc. R. Soc. B: Biol. Sci. 276:469-476. <https://doi.org/10.1098/rspb.2008.1009>.
49. Webb, C.R., 2005. Farm animal networks: unraveling the contact structure of the British sheep population. Prev. Vet. Med. 68:3-17. <https://doi.org/10.1016/j.prevetmed.2005.01.003>.
50. Woolhouse, M.E.J., Shaw, D.J., Matthews, L., Lui, W.-C., Mellor, D.J., Thomas, M.R., 2005. Epidemiological implications of the contact network structure for cattle farms and the 20-80 rule. Biol. Lett. 1:350-352. <https://doi.org/10.1098/rsbl.2005.0331>.

Figure 3-1. Map of Study Area.



Map of the study area containing four identical feedlot pens measuring 38 m by 30.5 m. Each pen contains a 24.5 m long feed bunk (dark grey), an automatic waterer (dark blue), and a concrete apron surrounding the feedbunk and waterer (light grey). Fences are outlined in solid dark lines, the feed alley is represented by the dashed line, and gates are represented as angled lines. Pens 1, 2, and 3 were equipped with the Real-Time Location system (RTLS). Receivers (yellow triangles) were positioned around the pens and hardwired to a server, which was housed in an enclosed space inside a shed. Steers in 2017 were housed in Pen 1. Steers in 2018 and 2019 were housed in Pen 2.

Figure 3-2. Flowchart of the Data Processing and Creation of Contact Networks.



Flowchart contact network creation of the native read rate networks for the 2017, 2018, and 2019 location data and down-scaled networks for 2017 and 2019. Down-scaled tag read rate was created to compare to the native read rate. Down-scaled networks were created to compare to native read rate networks, this was done by creating a new set of location readings based on the new average tag read rate was done by organizing all location readings for the original study in chronological order by individual steer and then dropping a defined amount at random based on the probability of the location point being dropped being equal to 1 minus (Lower Tag Read Rate / Higher Tag Read Rate). This process was done in sets of 50 iterations; the median of the 50 iterations is reported. Once the required amount of location points was dropped to match the native read rate to the desired down-scaled read rate the data was processed the same as the

native read rate location data. With the processed data, adjacency matrices were created by calculating the distance between all pairs of steers for every given 10 s temporal sampling window (TSW). These adjacency matrices were used to create contact networks at a spatial threshold (SpTh) of 0.71 m and either a 10 second (10s) minimum contact duration (MCD, Results Section 1 and 2) or at 30 second (30s) or 60 second (60s) MCD (Results Section 3 and 4). Networks were examined as full networks containing all study days. The first and last day of each study were dropped to create the daily (24-h), 6-h period, and hourly contact networks.

Table 3-1. Full Network Comparison for Native Read Rate and Down-scaled Read Rate Networks.

Year	2017	2017	2017	2018	2019	2019
Native Read Rate/min	7.4	7.4	7.4	2.2	3.3	3.3
Down-scaled Read Rate/min	-	3.3	2.2	-	-	2.2
Total Study Days	50	50	50	71	64	64
Defined Contacts	930,843	155,957	71,704	68,757	230,655	142,656
Contacts/Study Day	18,617	3,119	1,434	968	3,604	2,229
Nodes	70	70	70	70	69	69
Edge Count	2,415	2,415	2,415	2,331	2,273	2,265
Density	100%	100%	100%	97%	97%	97%
Clustering Coefficient	1	1	1	0.99	0.99	0.99
Modularity (no. of communities)	0 (2)	0 (2)	0 (2)	0 (2)	0 (2)	0 (2)
Median Edge Weight (Range)	328 (45-71,319)	64 (6-307)	28 (1-120)	26 (1-319)	110 (1-1,181)	50 (1-529)

Full network metrics for native read rate and down-scaled read rate networks. Native tag read rate is defined as the average location readings (X,Y) by each individual tag per minute of the study. Down-scaled tag read rate was created to compare to the native tag read rate and across years. This was done by creating a new set of location readings based on the new average tag read rate was done by organizing all location readings for the original study in chronological order by individual steer and then dropping a defined amount at random based on the probability of the location point being dropped being equal to 1 minus (Lower Tag Read Rate / Higher Tag Read Rate). This process was done in sets of 50 iterations; the median of the 50 iterations is reported for downscaled networks. Contacts are defined as two steers in that study who were within a 0.71 m Spatial Threshold (SpTh) of each other within any given 10 second

(10s) Temporal Sampling Window (TSW). Nodes in the full network are defined as individual steers that had at least one defined contact within the study period. Edges in the network are the connection between two individual steers.

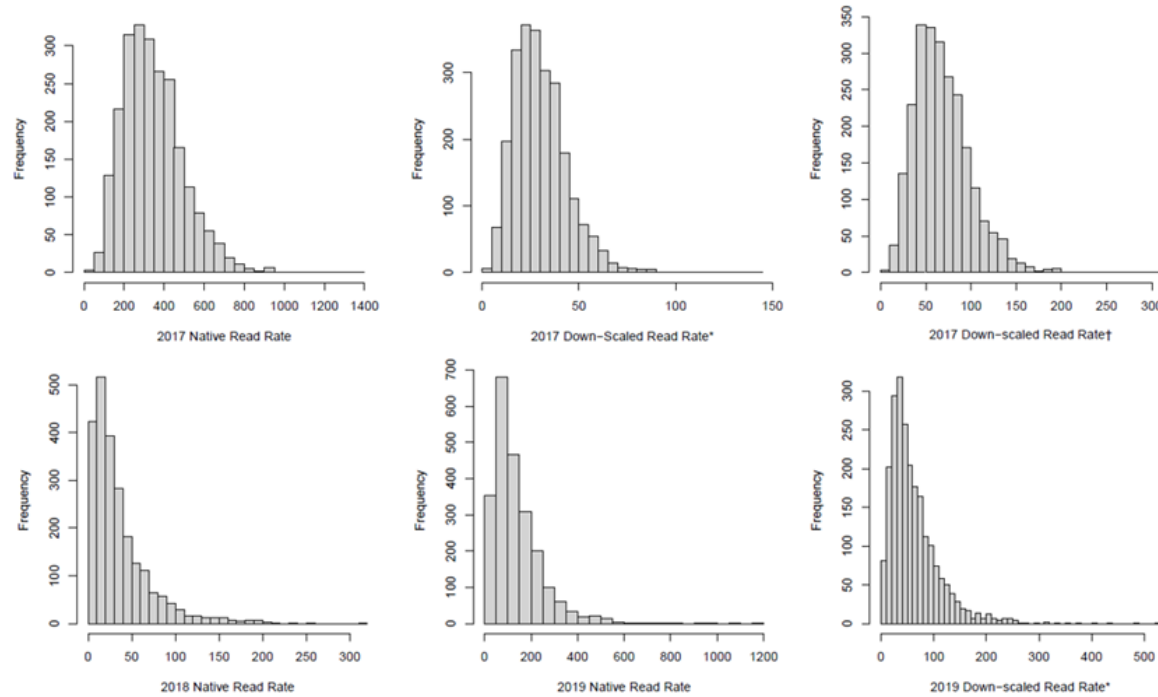
Table 3-2. Daily, 6-h Period, and Hourly Network Comparison for Native and Down-scaled Networks.

Year		2017	2017	2017	2018	2019	2019
Native Read Rate/min		7.4	7.4	7.4	2.2	3.3	3.3
Down-scaled Read Rate/min		-	3.3	2.2	-	-	2.2
Daily (24-h)	Median Edge Weight (Range)	5 (1-1,861)	1 (1-15)	2 (1-31)	1 (1-48)	2 (1-144)	2 (1-63)
	Median Density (Range, %)	92% (81-98)	58% (43-75)	37% (27-51)	19% (9-30)	37% (10-72)	26% (0-63)
	Median Clustering Coefficient (Range)	0.9 (0.8-1)	0.4 (0.3-0.5)	0.6 (0.5-0.8)	0.3 (0.2-0.4)	0.5 (0.4-0.8)	0.4 (0.2-0.7)
	Median Modularity (Range)	0 (0-0)	0.1 (0.1-0.1)	0.1 (0.1-0.1)	0.2 (0.1-0.2)	0.1 (0-0.3)	0.2 (0.2-0.2)
	Median No. Communities (Range)	2 (2-3)	3 (3-3)	3 (3-4)	4 (3-6)	3 (2-6)	3 (3-4)
6-h Period	Median Density (Range, %)	49% (0-90)	19% (0-50)	11% (0-29)	5% (0-15)	11% (0-52)	8% (0-46)
	Median Clustering Coefficient (Range)	0.5 (0.3-0.9)	0.2 (0-0.2)	0.3 (0.2-0.6)	0.1 (0-0.4)	0.3 (0.1-0.7)	0.2 (.1-0.6)
Hourly	Median Density (Range, %)	11% (0-42)	3% (0-17)	2% (0-9)	1% (0-5)	2% (0-36)	2% (0-31)
	Median Clustering Coefficient (Range)	0.2 (0-1)	0.1 (0-0.5)	0.1 (0-0.6)	0.1 (0-1)	0.2 (0-1)	0.2 (0-0.8)

Daily (24-h), 6-h period, and hourly network metrics for native read rate and down-scaled read rate networks. For daily (24-h), 6-h period, and hourly network metrics the first and last day of each study are not included in the analysis. The 6-h period networks were defined in Central Daylight time from 00:00-05:59 (night), 06:00-11:59 (morning), 12:00-17:59 (afternoon), and 18:00-23:59

(evening). Native tag read rate is defined as the average location readings (X,Y) by each individual tag per minute of the study. Down-scaled tag read rate was created to compare to the native tag read rate and across years. This was done by creating a new set of location readings based on the new average tag read rate was done by organizing all location readings for the original study in chronological order by individual steer and then dropping a defined amount at random based on the probability of the location point being dropped being equal to 1 minus (Lower Tag Read Rate / Higher Tag Read Rate). This process was done in sets of 50 iterations; the median of the 50 iterations is reported for each period (downscaled reported medians are the median of the daily, 6 hour or hourly medians across iterations). Contacts are defined as two steers in that study who were within a 0.71 m Spatial Threshold (SpTh) of each other within any given 10 second (10s) Temporal Sampling Window (TSW).

Figure 3-3. Histograms of Overall Edge Weight for Native and Down-Scaled Networks.



*Down-scaled networks with a read rate of 2.2 readings/min

†Down-scaled network with a read rate of 3.3 readings/min

Histograms demonstrating the total edge weight for the full study for each dyad pair in each study. Native tag read rate is defined as the average location readings (X,Y) by each individual tag per minute of the study. Down-scaled tag read rate was created to compare to the native tag read rate and across years. This was done by creating a new set of location readings based on the new average tag

read rate was done by organizing all location readings for the original study in chronological order by individual steer and then dropping a defined amount at random based on the probability of the location point being dropped being equal to $1 - (\text{Lower Tag Read Rate} / \text{Higher Tag Read Rate})$. This process was done in sets of 50 iterations; the median of the 50 iterations is reported. Edge weight is defined as the cumulative 10 second (10s) temporal sampling windows (TSW) that a pair of steers were in defined as being in contact for the duration of the study. A contact is defined as two steers having location (X,Y) readings within 0.71 m of each other within any given 10s TSW. Frequency is the number of dyad pairs that fell within that edge weight range. Due to high overall edge weights, one steer was removed from the 2017 Native Read Rate network for visualization purposes.

Table 3-3. Median Network Density for 6-hour Periods.

Period	Hours	2017	2018	2019
Night	00:00-05:59	32%	6%	10%
Morning	06:00-11:59	59%	6%	12%
Afternoon	12:00-17:59	58%	5%	12%
Evening	18:00-23:59	47%	3%	12%

The median density for all 6-hour periods in each study. The first and last day of each study are not included in this analysis. The 6-h period networks were defined in Central Daylight time from 00:00-05:59 (night), 06:00-11:59 (morning), 12:00-17:59 (afternoon), and 18:00-23:59 (evening). Contacts are defined as two steers in that study who were within a 0.71 m Spatial Threshold (SpTh) of each other within any given 10 second (10s) Temporal Sampling Window (TSW).

Table 3-4. Native Read Rate at 30 and 60 s Minimum Contact Duration (MCD) Network Metrics.

		2017		2018		2019	
MCD (seconds)		30	60	30	60	30	60
Full Network	Defined Contacts	95,570	19,135	3,317	301	17,741	1,825
	Contacts/Study Day	1,911	383	47	4	277	29
	Nodes	70	70	68	61	69	68
	Edge Count	2393	1330	1085	196	2024	798
	Density	99%	55%	48%	11%	86%	35%
	Median Edge Weight	45	14	7	7	21	10
	(Range)	(3-46,751)	(6- 24,950)	(3-96)	(6-55)	(3-461)	(6-173)
	Clustering Coefficient	1	0.7	0.6	0.1	0.9	0.4
	Modularity (no. of communities)	0 (2)	0.1(4)	0.1 (3)	0.3 (6)	0 (2)	0.1 (3)
Daily (24-h)	Median Density	18%	3%	1%	0.2%	5%	1%
	(Range, %)	(12-21)	(2-4)	(0-3)	(0-0.7)	(0-12)	(0-2)
	Median Clustering Coefficient (Range)	0.2 (0.2-0.3)	0.1 (0-0.2)	0 (0-0.3)	0 (0-0)	0.1 (0-0.2)	0 (0-0.5)
	Median Modularity (Range)	0.2 (0.2-0.3)	0.6 (0.4-0.7)	0.8 (0.6-0.9)	0.6 (0-0.9)	0.5 (0.3-0.7)	0.8 (0.6-0.9)
	Median No. of communities (Range)	4 (3-6)	8 (6-12)	9 (6-13)	3 (2-8)	7 (4-10)	9 (3-13)
6-h Period	Median Density (Range, %)	5% (0-11)	1% (0-2)	0.3% (0-1)	0% (0-0.5)	1% (0-7)	0.2% (0-1)
	Median Clustering Coefficient (Range)	0.1 (0-1)	0 (0-0.2)	0 (0-0.7)	0 (0-0)	0.1 (0-0.5)	0 (0-1)
Hourly	Median Density (Range, %)	1% (0-4)	0.2% (0-1)	0% (0-0.4)	0% (0-0.2)	0.2% (0-6)	0% (0-0.4)
	Median Clustering Coefficient (Range)	0.1 (0-1)	0 (0-0.8)	0 (0-1)	0 (0-0)	0 (0-1)	0 (0-1)

An overview of the full, daily (24-h), 6-hour period, and hourly network metrics for each study at 30 seconds (30s) and 60 seconds (60s) minimum contact duration (MCD). Full Network contains all study days. The first and last day of each study are not included in the analysis for the daily (24-h), 6-h period, or hourly networks. The 6-h period networks were defined in Central Daylight time from 00:00-05:59 (night), 06:00-11:59 (morning), 12:00-17:59 (afternoon), and 18:00-23:59 (evening). Contacts are defined as two steers in that study who were within a 0.71 m Spatial Threshold (SpTh) of each other within any given 10 second (10s) Temporal Sampling Window (TSW) for a minimum of 30s or 60s.

Appendix B - Supplementary Material

Table B-1. Summary of Tag Readings per Study Day.

	2017	2018	2019
Min	0	0	0
5 th Percentile	1472	608	0
25 th Percentile	7,929	1,894	2,108
Median	11,847	3,138	5,065
Mean	10,598	3,239	4,731
75 th Percentile	13,633	4,404	7,076
95 th Percentile	15,380	6,339	9,649
Max	18,282	10,952	18,418

Summary statistics of individual tag readings for each study day. A reading is defined as an individual location (X,Y) reading identified in the system by that individual tag for each day.

The first and last day of each study are not included.

Table B-2. Summary of Full Network Edge Weight.

	2017	2018	2019
Min	45	1	1
5 th Percentile	141	4	28
25 th Percentile	237	13	66
Median	328	26	110
Mean	579	37	139
75 th Percentile	438	48	181
95 th Percentile	645	107	338
Max	71,319	319	1,181

Summary statistics for edge weight for each study. Edge weight is defined as the cumulative 10 second (10s) temporal sampling windows (TSW) that a pair of steers were in defined as being in contact for the duration of the study. A contact is defined as two steers having location (X,Y) readings within 0.71 m of each other within any given 10s TSW.

Table B-3. Summary of Median Daily Edge Weight.

	2017	2018	2019
Min	1	1	1
5 th Percentile	1	1	1
25 th Percentile	3	1	1
Median	5	1	2
Mean	13	3	5
75 th Percentile	10	2	5
95 th Percentile	24	8	20
Max	1,861	47	143

Summary statistics for edge weight for each year by study day. Edge weight is defined as the cumulative 10 second (10s) temporal sampling windows (TSW) that a pair of steers were in defined as being in contact for that day. The first and last days of each study were excluded, due to being incomplete days. A contact is defined as two steers having location (X,Y) readings within 0.71 m of each other within any given 10s TSW. Median values are reported.

Table B-4. Median Density by Hour of Day.

	2017	2018	2019
00:00-00:59	8.2%	0.9%	2.4%
01:00-01:59	7.6%	1.1%	2.5%
02:00-02:59	7.4%	1%	2.2%
03:00-03:59	7.3%	1%	2.4%
04:00-04:59	7.2%	1.2%	2.0%
05:00-05:59	9.1%	1.3%	2.4%
06:00-06:59	10.1%	1.2%	2.1%
07:00-07:59	12.6%	1.2%	1.9%
08:00-08:59	12.8%	1.1%	2.1%
09:00-09:59	15.4%	1.1%	2.5%
10:00-10:59	17.3%	1.1%	2.8%
11:00-11:59	17.2%	1.1%	2.6%
12:00-12:59	16.1%	1%	2.5%
13:00-13:59	15.3%	1%	2.3%
14:00-14:59	13.5%	1%	2.2%
15:00-15:59	13.9%	0.9%	2.5%
16:00-16:59	14.1%	0.8%	2.8%
17:00-17:59	13.7%	0.7%	2.5%
18:00-18:59	13.4%	0.7%	2.3%
19:00-19:59	13.6%	0.7%	2.3%
20:00-20:59	13.2%	0.7%	2.3%
21:00-21:59	10.8%	0.7%	2.5%
22:00-22:59	8.1%	0.7%	2.9%
23:00-23:59	8.3%	0.7%	2.8%

The median density for individual hourly periods in each study. The first and last day of each study are not included in this analysis, as they were not complete days. Contacts are defined as two steers in that study who were within a 0.71 m Spatial Threshold (SpTh) of each other within any given 10 second (10s) Temporal Sampling Window (TSW). Density is the number of edges (connections) present out of all edges possible.

Figure B-5. Network Graphs for 2017 Study Day 1 At Varying Minimum Contact Durations
(10, 30, 60 s)

Figure B-5.1 - 10 s MCD

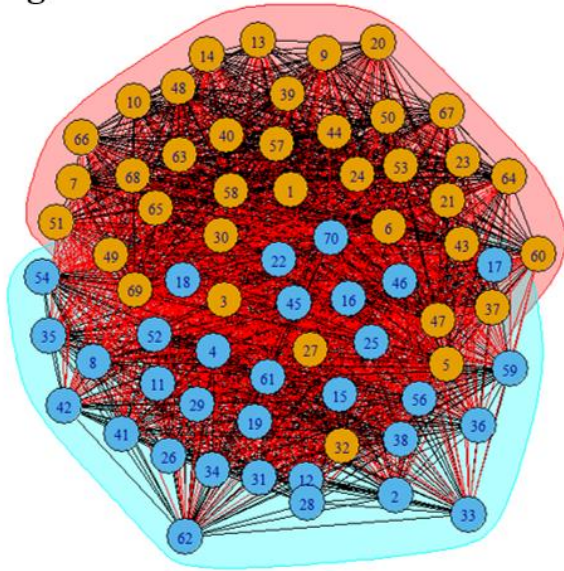


Figure B-5.2 - 30 s MCD

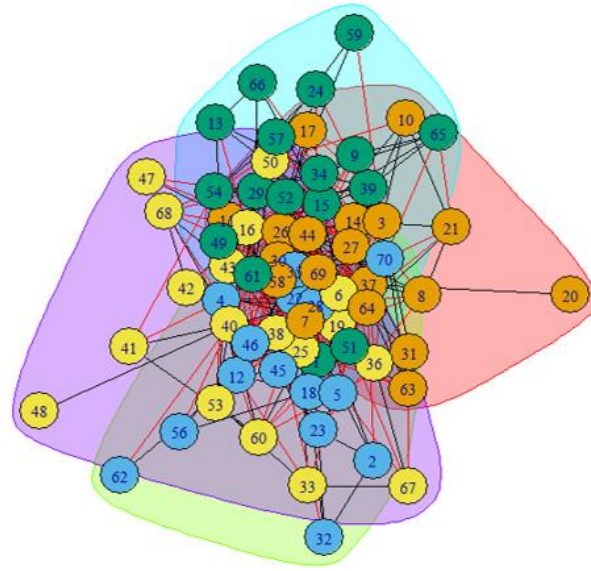
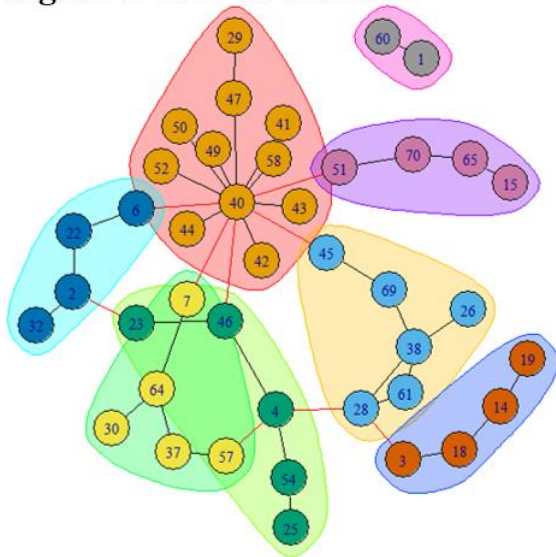


Figure B-5.3 - 60 s MCD



Network graphics representing the community structure detected with the `cluster_fast_greedy()` algorithm for the first study day of 2017 at its native average tag read rate at varying minimum contact duration (MCD). Individual steers within the network graph are represented by numbered circles. Connections between individual steers are represented as lines. These lines represent a defined contact between that pair of steers occurring in the first study day at that determined minimum contact duration of 10, 30, or 60 s. Contacts are defined as two steers in that study who were within a 0.71 m Spatial Threshold (SpTh) of each other within any given 10 second (10s) Temporal Sampling Window (TSW). Minimum contact duration is the number of consecutive TSW in which a defined contact occurred. Communities detected with the algorithm are represented as the larger colored areas.

Table B-6. Down-scaled Read Rate Network Metrics for 30 and 60 s Minimum Contact

Duration (MCD).

		2017		2017	
Down-scaled Read Rate/min		3.3		2.2	
MCD (seconds)		30	60	30	60
Full Network	Defined Contacts	93	0	869	2
	Contacts/Study Day	2	-	17	0
	Nodes	60	-	70	4
	Edge Count	91	-	651	2
	Density	4%	-	27%	0.1%
	Median Edge Weight (Range)	3 (3-6)	-	4 (3-18)	-
	Clustering Coefficient	0.1	-	0.4	-
	Modularity (no. of communities)	0.1 (4)	-	0.5 (8)	-
	Median Density (Range, %)	0 (0-0)	-	0 (0-0)	-
	Median Clustering Coefficient (Range)	0 (0-0)	-	0 (0-0.3)	-
Daily (24-h)	Median Modularity (Range)	0.8 (0.8-0.8)	-	0.5 (0-0.5)	-
	Median No. of communities (Range)	9 (8-10)	-	2 (2-2)	-
	Median Density (Range, %)	0 (0-0)	-	0 (0-0)	-
6-h Period	Median Clustering Coefficient (Range)	0 (0-0)	-	0 (0-0.8)	-
	Median Density (Range, %)	0 (0-0)	-	0 (0-0)	-
Hourly	Median Clustering Coefficient (Range)	0 (0-0)	-	0 (0-1)	-
	Median Density (Range, %)	0 (0-0)	-	0 (0-0)	-

An overview of the full, daily (24-h), 6-hour period, and hourly network metrics for the down-scaled 2017 networks at 30 seconds (30s) and 60 seconds (60s) minimum contact duration

(MCD). Down-scaled tag read rate was created to compare to the native tag read rate and across

years. This was done by creating a new set of location readings based on the new average tag read rate was done by organizing all location readings for the original study in chronological order by individual steer and then dropping a defined amount at random based on the probability of the location point being dropped being equal to $1 - (\text{Lower Tag Read Rate} / \text{Higher Tag Read Rate})$. This process was done in sets of 50 iterations; the median of the 50 iterations is reported. Full Network contains all study days. The first and last day of each study are not included in the analysis for the daily (24-h), 6-h period, or hourly networks. The 6-h period networks were defined in Central Daylight time from 00:00-05:59 (night), 06:00-11:59 (morning), 12:00-17:59 (afternoon), and 18:00-23:59 (evening). Contacts are defined as two steers in that study who were within a 0.71 m Spatial Threshold (SpTh) of each other within any given 10 second (10s) Temporal Sampling Window (TSW) for a minimum of 30s or 60s.

Chapter 4 - Analysis of within-pen and between-pen fenceline temporal contact networks in confined feedlot cattle

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Abstract

Though contact networks are important for describing the dynamics for disease transmission and intervention applications, individual animal contact and barriers between animal populations, such as fences, are not often utilized in the construction of these models. The objective of this study was to use contact network analysis to quantify contacts within two confined pens of feedlot cattle and the shared “fenceline” area between the two pens at varying temporal resolutions and contact duration requirements to better inform the construction of network-based disease transmission models for cattle within confined-housing systems. Two neighboring pens of feedlot steers were tagged with Real-Time Location System (RTLS) in November of 2017. Within-pen contacts were defined with a spatial threshold (SpTh) of 0.71 m and a minimum contact duration (MCD) of either 10 seconds (10s), 30 seconds (30s), or 60 seconds (60s). For the network created across the shared fenceline location readings were filtered

to be within an area extending from 1 m on either side of the shared fence. “Fenceline” contacts were defined with the same SpTh and MCD durations as within-pen contacts but could only occur between a steer from each pen. Static, undirected, weighted contact networks for two within-pen networks and one between-pen network for the full study duration and then split into daily (24-h), 6-h period, and hourly networks to better assess network heterogeneity. For the full study duration time scale, the two within-pen networks were found to be densely homogenous. The within-pen networks showed more heterogeneity when smaller timescales (6-h period and hourly) were applied. When contacts were defined with a MCD of 30s or 60s, the total number of contacts seen in each network decreased, indicating that most of the contacts observed in our networks may have been transient passing contacts. Of the 90 total tagged steers between the two pens, 86 steers (46 steers from Pen 2 and 40 steers from Pen 3) produced at least one contact across the shared fenceline. The total network density for the network created across the shared fenceline between the two pens was 17%, and limited contacts were defined in the shared fenceline network at smaller timescales. Similar to the within-pen networks, when the defined contacts had an MCD of 30s or 60s, the total number of fenceline contacts decreased. Overall, the contact networks created here from high-resolution spatial and temporal contact observation data provide estimates for a contact network within commercial US feedlot pens and the contact network created between two neighboring pens of cattle. These networks can be used to better inform pathogen transmission models on social contact networks.

Introduction

The U.S. has approximately 13 million cattle on feed distributed in over 30,000 feedlots across the 48 contiguous states (USDA-NASS, 2017). Cattle in U.S. feedlots are commonly

housed in confined populations of pens, with shared fencelines occurring between groups. These fencelines do not prevent direct nose-to-nose contact occurring between animals. Additionally, water tanks located on the fenceline may be shared between multiple pens.

Contagious agent transmission can occur from multiple routes within and between these confined populations. One of these contact routes is direct animal-to-animal contact between shared fencelines, resulting in potential infectious disease transmission. As previously predicted by modeling studies, a highly contagious disease event, such as a Foot and Mouth Disease (FMD) outbreak, would have disastrous economic consequences for the livestock and related industries (Paarlberg et al., 2002; Pendell et al., 2015; Schroeder et al., 2015; Tozer et al., 2015). Progression of an infectious disease through a feedlot could be enhanced by direct fenceline contacts, however little is known about the contact structure between adjacent pens.

A recent model projected the transmission, infection, and clinical manifestation dynamics of FMD within contemporary U.S. cattle feedlots and multiple intervention sceneries to control FMD within the modeled feedlots (Cabezas et al., 2020; Cabezas et al., 2021). With no explicit contact data available the role of fenceline contacts in disease transmission was modeled by scaling the beta transmission parameter for between pen direct contacts. This model highlights the importance for understanding the complex contact structure in cattle populations within feedlots. Better understanding of these contact structure dynamics within confined pens of feedlot cattle and between confined pens across shared fencelines would increase the realism of models for within-feedlot dynamics of highly transmissibility diseases, such as FMD.

The aim of this study was to use contact network analysis to quantify contacts within two pens of feedlot cattle and across a shared fenceline at varying temporal resolutions to better

inform the construction of network-based disease transmission models for cattle within confined-space systems with barriers to transmission.

Materials and Methods

All procedures performed in studies involving animals were in accordance with the ethical standards of Kansas State University College of Veterinary Medicine IACUC #4065 and IACUC #4014.

Study Populations

The collection of contact network data occurred as part of the larger research trial on Bovine Respiratory Disease (BRD) diagnostics from November 7th to 23rd, 2017. Prior to the start of the study, Angus/Angus-cross steers were purchased through commercial channels and housed in four outdoor pens each containing forty-eight steers at a commercial research feedlot (Figure 4-1). Contact data was collected on steers housed in Pen 2 and Pen 3. Pen 1 and 4 contained untagged steers. All pens measured 38 m by 30.5 m, providing approximately 24 m² area per steer. These stocking densities match the observed stocking rates of commercial US feedlots. Each pen had a centrally located water tank, a 24.5 m long fenceline bunk and a 3 m wide concrete apron behind the bunk and around the water tank (Figure 4-1). All steers were unfamiliar to the enclosure prior to the study.

Point Location Data

All steers received an ear tag for visual identification, as well as a RTLS ear tag placed centrally in the left ear (Smartbow GmbH, Weibern, Austria). These radio-transmitting ear tags communicated with receivers positioned around the pens. These receivers were hardwired to a

server located in an enclosed shed that was used to store and transmit the location data (Figure 4-1). Steers' positions (X,Y) were triangulated and recorded into the server. The coordinate pairs (X,Y) obtained were 90% accurate to within ± 0.5 m of individuals' true location (Farthing et al., 2021). A flowchart of data processing and contact network creation can be found in Figure 4-2. As previously outlined in Dawson et al. 2019 and Farthing et al. 2021, raw location data were filtered and smoothed to decrease error-induced noise and standardize temporal resolution. Data points were first filtered and removed if location points fell outside the designated pen area or if sequential locations indicated that the individual was moving at excess speeds of greater than 10 meters per second (mps). Resulting location data were then smoothed to represent individuals' average location (X,Y) at 10s intervals over the course of the study period.

Model Construction

Networks were created for both within-pens (Pen 2 and Pen 3), as well as the between-pen "Fenceline" contact network. Using the procedures described in Dawson et al., 2019 and Farthing et al., 2021, all contact networks were created with a spatial threshold (SpTh) for identifying contacts of 0.71 m with a temporal sampling window (TSW) of 10s. The smoothed data representing each individual's average location for each 10s TSW was used to determine if a contact occurred in each temporal window. Distances between each steer for each temporal window were calculated and the spatial threshold of 0.71 m was applied to determine whether a contact occurred in that given 10s TSW. These adjacency matrices were used for analysis of all contact networks.

The "Fenceline" network was created by filtering smoothed location data points to only those within 1 m of either side of the shared fenceline between Pen 2 and Pen 3. This area was

created for computational efficiency. A contact for the “Fenceline” network was only considered if it contained one steer from Pen 2 and one steer from Pen 3 within the “fenceline” area. With the filtered location data within the fenceline area, distances between each steer for each temporal window were calculated and the spatial threshold of 0.71 m was applied to determine whether a contact occurred in that given 10s TSW. These adjacency matrices were used for analysis of all contact networks.

Overall (full study) networks included the entire study period. For analysis of daily (24 h), 6-hour period, and hourly networks the first day of each study (when the steers were getting tags put on at different times) and the last day (when the steers were getting tags taken off at different times) were removed. The 6-h networks were defined in Central Daylight time from 00:00-05:59 (night), 06:00-11:59 (morning), 12:00-17:59 (afternoon), and 18:00-23:59 (evening). Additionally, we also generated contact networks for Pen 2, Pen 3 and the “Fenceline” using extended minimum contact duration (MCD, the duration of the temporal sampling windows that defined a contact). We defined contact networks with an MCD of 30s (those contacts between two steers that were 30 s or longer) and an MCD of 60s (those contacts between two steers that were 60 s or longer). Both networks used the same SpTh (0.71 m) and TSW (10 s) and a filter was applied to the contact duration keeping only contacts that were 3 TSW or longer (30 s) or only contacts that were 6 TSW or longer (60 s).

Software

All data processing and analyses were conducted in RStudio (RStudio Team, 2022, v. 2022.02.3) running R (R Core Team, 2020, v. 4.1.1). The contact package (v. 1.2.8), (Farthing et al., 2020) was used to clean point-location data and create contact networks. The Igraph package

(v. 1.2.6) was used to visualize and analyze network metrics. The `cluster_fast_greedy` algorithm within the Igraph package was used to estimate network modularity. The Hmisc package (v. 4.7-0) was used to visualize the distributions of the total edge weight for each contact network. Stata 16.1 was used to analyze the skewness and kurtosis of the full network edge weights (StataCorp, 2019).

Results

Pen 2 Network

Pen 2 contained 48 steers for the duration of the study. No steers were removed from the study and all steers within the pen were tagged with RTLS transmitter tags. A total of 9,265,677 location (X,Y) readings were collected during the study period (Table 4-1). A median of 14,104 readings per tag per full study day were recorded (range: 3,424-18,733, Table 4-2) with an average tag read rate of 8.9 readings/min. A total of 83,683 contacts were identified between dyad pairs. Two initially tagged steers were not identified in any network. Forty-six steers and all edges (connections) between pairs were identified in the network ($n=1,035$), yielding a network density of 100% for steers identified in the network, clustering coefficient of 1, and modularity of 0. The median overall edge weight contacts between dyad pairs were 88 TSW (range: 9-960, Table 4-3). The edge weights between pairs of steers showed a positive skewness of 2.7 and kurtosis of 20.7 (Figure 4-3). The median daily networks had a density of 86% (range: 73%-95%, Table 4-4). The median daily edge weight between dyad pairs was 4 TSW (range: 0-144, Table 4-5). Median daily clustering was 0.9 (range: 0.7-1) and modularity was 0 (range: 0-0). The 6-h period had median density of 30% (range: 13%-75%) with the morning 6-h period (06:00-11:59 Central Daylight time) had the highest median density (Table 4-6). On an hourly

timescale, the median density was 7% (range: 0%- 34%, Table 4-4) with the network density trending higher in the daylight hours compared to those in the evening and nighttime (Table 4-7). Median clustering for the hourly networks was 0.3 (range: 0-0.6, Table 4-4).

For Pen 2, when contacts were defined with a MCD of 30 s, a total of 5,235 contacts were defined (Table 4-8). All steers found in the network at an MCD of 10 s ($n = 46$) were identified in the overall network, meaning that all steers had at least 1 contact that was greater than 30 s with another steer during the study period. The overall network yielded 850 edges with a density of 82%, clustering coefficient of 0.9, and modularity of 0 (Table 4-8). The median overall edge weight between pairs was 13 TSW (range: 3-315 TSW). When contacts were defined as those 30s or longer the for the daily (24-h) networks the median network density was 16% (range: 7%-27%) and median modularity was 0.3 (range: 0.2-0.4, Table 4-9). Median network density was 4% (range: 1%-13%) for the 6-h period networks and 1% (range: 0%-4%) for the hourly networks (Table 4-9).

When contacts were defined as a MCD of 60s, a total of 433 contacts were defined (Table 4-8). Forty-four steers of the originally identified 46 were identified in the overall network. Two steers found in the original network did not have a defined contact of at least 60 s within the study period. The overall network had 217 edges with a density of 23% and modularity of 0.2 (Table 4-8). The median overall edge weight between pairs was 8 TSW (range: 6-161 TSW). For the daily (24-h) 60 s networks the median density was 1% (range: 0%-4%), 0.3% (range: 0-2%) for the 6-h period networks, and 0% (range: 0-0.6%) for the hourly networks (Table 4-9). Moderate to high community structure was detected in the daily 60s network with a median daily (24-h) modularity of 0.7 (range: 0.6-0.8, Table 4-9).

Pen 3 Network

Pen 3 contained 48 steers for the duration of the study. No steers were removed from the study. Due to tag limitations, only 42 steers were tagged with RTLS transmitter tags and six steers were not tagged. A total of 8,521,612 location (X,Y) readings were collected during the study period (Table 4-1). A median of 15,345 location readings per tag per full study day were recorded (range: 0-19,009, Table 4-2) with an average tag read rate of 9.2 readings/min. A total of 73,908 contacts were identified between dyad pairs. Forty-one of the originally tagged 42 steers were identified in the overall network. One tagged steer was not identified in any network. The overall network based off the 41 identified steers contained 787 edges (connections) between pairs of steers and yielded a network density of 96%. The median overall edge weight of contacts between dyad pairs was 100 TSW (range: 1-726, Table 4-3). The edge weights between pairs of steers showed a positive skewness of 1.9 and kurtosis of 9.2 (Figure 4-3). The daily networks had a median density of 77% (range: 73%-85%), clustering of 0.9 (range: 0.8-0.9), and modularity of 0 (range: 0-0, Table 4-4). The median daily edge weight between dyad pairs was 4 TSW (range: 0-173, Table 4-5). The 6-hour period had median density of 29% (range: 11%-72%, Table 4-4) and morning and afternoon 6-hour periods had the highest median density (Table 4-6). On an hourly timescale, the median density was 6% (range: 0%-36%, Table 4-4) with the network density trending higher in the daylight hours compared to those in the evening and overnight (Table 4-7).

For Pen 3, when contacts were defined as a MCD of 30 s, a total of 4,718 contacts were defined (Table 4-8). Forty steers were found in the overall network. One steer found in the original network did not have any contacts that were at least 30 s long during the study period.

The overall network yielded 653 edges with a density of 84% and modularity of 0 (Table 4-8). The median overall edge weight between pairs was 14 TSW (range: 3-422 TSW). When contacts were defined as those 30 s or longer for the daily (24-h) networks the median network density was 16% (range: 10%-33%), median clustering 0.2 (range: 0.1-0.4) and a median modularity of 0.3 (range: 0.2-0.4, Table 4-9). The median network density was 5% (range: 1%-14%) for the 6-h period networks, and 1% (range: 0%-5%) for the hourly networks when contacts were defined as 3 TSW or greater (Table 4-9).

When contacts were defined as a MCD of 60s, a total of 410 contacts were defined (Table 4-8). Thirty-eight steers of the originally found 41 were identified in the overall network. Three steers found in the original network did not have a defined contact of at least 60 s within the study period. The overall network based off the 38 identified steers had 169 edges with a density of 24%, clustering of 0.4 and modularity of 0.2. The median density of networks was 2% (range: 1%-4%) for the daily (24-h) 60s networks, 0.4% (range: 0-2%) for the 6-h period networks, and 0% (range: 0-0.6%) for the hourly networks (Table 4-9). Moderate to high community structure was detected with a median daily (24-h) modularity of 0.7 (range: 0.6-0.8, Table 4-9).

“Fenceline” Network

Fenceline contacts were defined contact between a steer from Pen 2 and a steer from Pen 3 within the defined fenceline zone 1 m on either side of the shared fenceline between the two pens. A total of 471,908 location (X,Y) readings were collected during the study period within the defined fenceline zone (Table 4-1). A total of 5,276 contacts were identified in the fenceline area. Of those only 788 contacts were identified as occurring across the fenceline between a steer

from Pen 2 and a steer from Pen 3. A total of 86 steers were identified in the overall fenceline contact network. Forty-six steers from Pen 2 (96% of the tagged steers) and 40 steers from Pen 3 (96% of the tagged steers) were identified in the fenceline network. The overall fenceline between pen network contained 312 edges (connections), had a network density of 17%, clustering of 0 and modularity of 0.3 (Table 4-1). The median edge weight between steers across the shared fenceline was 1 TSW (range: 1-66, Table 4-3), and the edge weights between pairs of steers across the fenceline showed a positive skewness of 13.1 and kurtosis of 234.1 (Figure 4-3). The daily (24-h), 6-h period, and hourly networks had a median density of 0% (range: 0%-0%, Table 4-4). The daily (24-h) networks had a median modularity of 0.7 (range: 0.5-0.8, Table 4-4).

There were 39 contacts identified when contacts were defined as occurring in the shared fenceline zone, containing a steer from Pen 2 and Pen 3, and having a MCD of 30s. Twenty-five steers and 21 edges (connections) were identified in this network (Table 4-8). Of the 25 identified steers, 12 were from Pen 2 and 13 were from Pen 3, yielding a network density of 13% and a modularity of 0.7 (Table 4-8). The median edge weight between pairs of steers, one from each group, was 4 (range: 3-42, Table 4-8).

Five contacts were identified when contacts were defined as occurring in the shared fenceline zone, containing a steer from Pen 2 and Pen 3, and having a MCD of 60s. Seven steers, 4 from Pen 2 and 3 from Pen 3, and 4 edges (connections) were identified in this network (Table 4-8). The density in this network was 33% with a modularity of 0.6 (Table 4-8). The median edge weight between pairs of steers, one from each group, was 7 (range: 6-15, Table 4-8).

Discussion

In this study, we presented high-resolution contact networks for two neighboring pens of feedlot steers and the shared “fenceline” network created between them. These networks provide estimates for contact networks within and between commercial U.S. feedlot pens that can be used to better inform pathogen transmission models on social contact networks for confined populations.

When examined over the full 17-day trial, the contact networks created within the two confined feedlot pens had a high network density. Variation in the within-pen contact structure patterns were masked in the overall and daily segmented networks due to this high network density. When compared to the overall and daily networks, the 6-h & hourly aggregated networks displayed more contact heterogeneity. Not surprisingly, the contact network created across the shared fenceline between the two confined pens of feedlot cattle showed less network density, even over the full study timescale. Additional work is needed to test for social affinity between pairs of steers across the fenceline.

Despite their influence on disease transmission, the contact structure in larger confined livestock populations, both within-pens and between-pens, is poorly understood. In a recently constructed model projecting the transmission dynamics and intervention applications for a FMD outbreak in a typical U.S. beef feedlot system, the beta transmission parameter for virus transmission via fence-line direct animal contact was assumed to be 25% the beta transmission parameter for the within-pen direct animal contact (Cabezas et al., 2020; Cabezas et al., 2021). Excluding fenceline direct contact increased the modeled FMD outbreak duration with slower progression through the feedlot and illustrated that the role of direct contact across fencelines

needs to be further explored (Cabazes et al., 2020). Though our observed “Fenceline” contact network was less dense than what is assumed in that model, the importance of the direct “Fenceline” contact network and its role in disease transmission is important and potential intervention applications needs to be considered.

To determine the influence of contact duration on the number of contacts created in the contact networks, the minimum contact duration (MCD) was defined as either 10 s, 30 s, or 60 s. These contact durations were chosen to differentiate between short transient contacts and longer contacts made between cattle, such as grooming events. When contacts were examined on a longer MCD, fewer within and between-pen contacts were seen. The difference in total number of contacts created at the varying MCD could have been a natural phenomenon observed in our pens of feedlot cattle, meaning that the majority of contacts were indeed truly short in nature. However, the lack of contacts at extended MCD could also have been affected by the chosen data filtering and smoothing processes. For the within-pen networks, the longer MCD also illustrated higher modularity and lower density and clustering values. The longer MCD may allow detection of structure present in longer contacts that are washed out by large numbers of transient contacts. These longer contacts may be more associated with grooming of some level of social affinity between steers. Further, the duration of contact most relevant for disease transmission would vary depending on the infectivity and route of infection for a particular pathogen and should be accounted for in a disease transmission model.

Though these networks are considered high resolution with an estimated accuracy of 90% within ± 0.5 m of individuals’ true location (Farthing et al., 2021) and an observed average tag read rate between 8.9 and 9.2 readings per minute for this study, there are still some limitations

to the system that were out of the control of the researchers. Six steers present in Pen 3 were not able to be initially tagged. Additionally, two tagged steers in Pen 2 and one tagged steer in Pen 3 were not identified in any network, though all steers were present within the pens for the entire duration of the study. These instances could contribute to some level of artificial dilution of the contact networks, though we expect this effect to be minimal. It was also noted that after the 2nd study day the system did not record any location readings, and therefore no defined contacts, for the hour of 03:00-03:59 CDT. This is likely due to a daily system rebooting and was not in the control of the researchers, though the contacts lost within this hour is likely low due to the cattle being less active during nighttime hours. Though the RTLS system used is considered high resolution in its reported individuals' location accuracy and read rate, the inherent variability in the system and the data processing decisions may have influenced the observed contact networks. It should also be noted that the adjacent pens, Pen 1 and Pen 4, also contained untagged steers which may influence the contact patterns of our observed pens.

When the within-pen networks created here are compared to networks created from different pens of cattle using the same system and facility, the resulting overall networks are comparable in density and clustering metrics (Seger et al., 2023). Though the same system was used the average tag read rates seen here, 8.9 to 9.2 readings/tag/min are higher than any average read rate seen in the previous study, 2.2 to 7.4 readings/tag/min. The within-pen network density seen here are most comparable on a daily (24-h), 6-h period, and hourly timescale to previously created network with the most similar average tag read rate of 7.4 readings/tag/min (Summer 2017, Seger et al., 2023). They are also most comparable in median 6-hour and hourly density trends as well as median daily edge weight to the Summer 2017 network (Seger et al., 2023). The

within pen networks created here also showed similar trends to the previously published networks in decreasing network density and increasing community structure detection with increase MCD. It should be noted that in the “Fenceline” network we saw both an increase in network density and community structure with increasing MCD. This is likely due to the limited number of steers identified in the extended MCD networks at the fenceline.

Conclusions

In summary, our study used high temporal and spatial resolution observation data to construct contact networks for two confined pens off feedlot steers and the network created across the shared fenceline between the two pens. The resulting within-pen contact networks were homogenous when explained on longer time scales but showered increased contact heterogeneity when examined on smaller time scales. The resulting overall “Fenceline” contact network was considerably less dense when compared to the within-pen networks. These findings can be applied to better inform the construction of network-based disease transmission models for cattle within confined-space systems and additionally accounting for the direct transmission route that occurs through shared fencelines of confined feedlot cattle.

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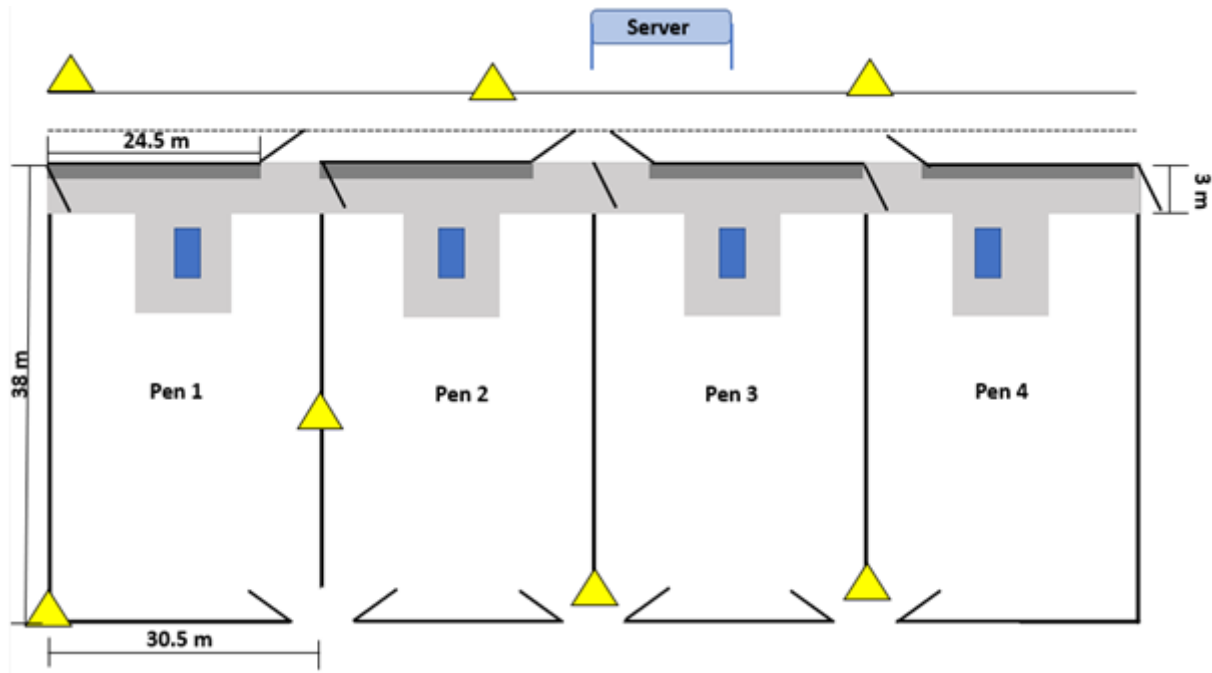
Literature Cited

1. Cabezas, A. H., M. W. Sanderson, and V. V. Volkova. 2020. A meta-population model of potential Foot-and-Mouth Disease transmission, clinical manifestation, and detection within U.S. beef feedlots. *Front. Vet. Sci.* 7:527558. <https://doi.org/10.3389/fvets.2020.527558>.
2. Cabezas, A. H., M. W. Sanderson, and V. V. Volkova. 2021. Modeling intervention scenarios during potential Foot-and-Mouth Disease outbreaks within U.S. beef feedlots. *Front. Vet. Sci.* 8:559785. <https://doi.org/10.3389/fvets.2021.559785>.
3. Dawson, D. E., T. S. Farthing, M. W. Sanderson, and C. Lanzas. 2019. Transmission of empirical dynamic contact networks is influenced by data processing decisions. *Epidemics*. 26:32-42. <https://doi.org/10.1016/j.epidem.2018.08.003>.
4. Farthing, T. S., D. E. Dawson, M. W. Sanderson, and C. Lanzas. 2020. Accounting for space and uncertainty in real-time location system-derived contact networks. *Ecol. Evol.* 10:4702-4715. <https://doi.org/10.1002/ece3.6225>.
5. Farthing, T. S., D. E. Dawson, M. W. Sanderson, H. Seger, and C. Lanzas. 2021. Combining epidemiological and ecological methods to quantify social effects on *Escherichia coli* transmission. *R. Soc. Open Sci.* 8: 210328. <https://doi.org/10.1098/rsos.210328>.

6. Paarlberg, P. L., J. G. Lee, and A. H. Seitzinger. 2002. Potential revenue impact of an outbreak of foot-and-mouth disease in the United States. *JAVMA*. 220(7):988–992. <https://doi.org/10.2460/javma.2002.220.988>.
7. Pendell, D. L., T. L. Marsh, K. H. Coble, J. L. Lusk, and S. C. Szmania. 2015. Economic assessment of FMDv releases from the national bio and agro defense facility. *PLoS ONE*. 10(6):e0129134. <https://doi.org/10.1371/journal.pone.0129134>.
8. R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>. (last accessed 10 October 2022).
9. RStudio Team. 2022. RStudio: Integrated Development Environment for R. RStudio, PBC, Boston, MA. <http://www.rstudio.com/>. (Last accessed 10 October 2022).
10. Seger, H. L., M. W. Sanderson, B. J. White, and C. Lanzas. 2023. The effect of temporal resolution and contact duration on Real-Time Location System-based contact networks for confined feedlot cattle [manuscript in preparation].
11. Schroeder, T. C., D. L. Pendell, M. W. Sanderson, and S. McReynolds. 2015. Economic impact of alternative FMD emergency vaccination strategies in the midwestern United States. *J. Agric. Appl. Econ.* 47(1):47–76. <https://doi.org/10.1017/aae.2014.5>.
12. StataCorp. 2019. Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC. (Last accessed 19 February 2023).
13. Tozer, P. R., T. L. Marsh, and E. V. Perevodchikov. 2015. Economic welfare impacts of Foot-and-Mouth Disease in the Canadian beef cattle sector. *Can. J. Agric. Econ.* 63:163-184. <https://doi.org/10.1111/cjag.12041>.

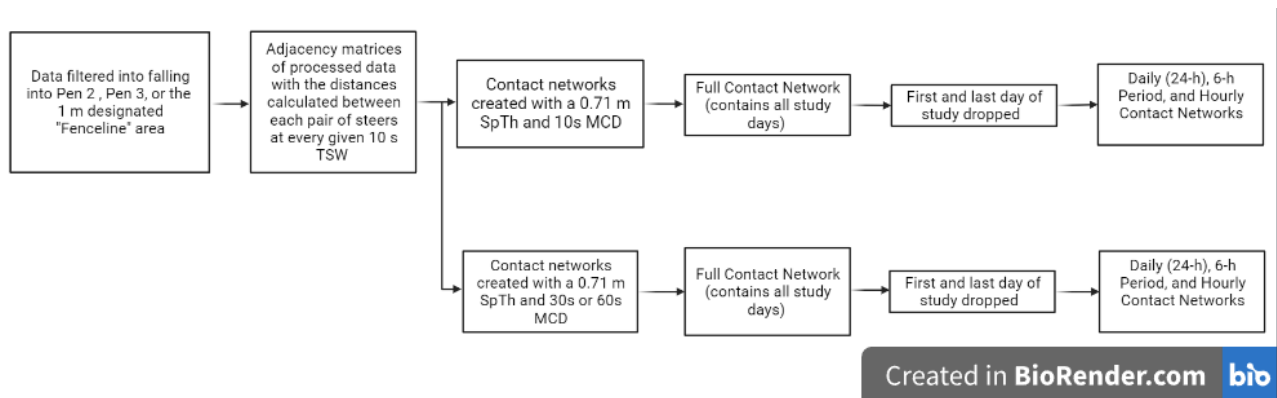
14. USDA-NASS. 2017. Overview of U.S. Livestock, Poultry, and Aquaculture Production in 2016. Fort Collins, CO: USDA-APHIS.

Figure 4-1. Map of Study Area.



Map of the study area containing four identical feedlot pens measuring 38 m by 30.5 m. Each pen contains a 24.5 m long feed bunk (dark grey), an automatic waterer (dark blue), and a concrete apron surrounding the feedbunk and waterer (light grey). Fences are outlined in solid dark lines, the feed alley is represented by the dashed line, and gates are represented as angled lines. Pens 1, 2, and 3 were equipped with the RTLS system. Receivers (yellow triangles) were positioned around the pens and hardwired to a server, which was housed in an enclosed space inside a shed. Pen 2 was housed in Pen 2; Pen 3 was housed in Pen 3. Pen 1 and 4 contained untagged steers.

Figure 4-2. Flowchart of Data Processing and Contact Network Creation.



Flowchart of data processing, adjacency matrix and contact network creation.

Table 4-1. Overall Network Metrics.

	Pen 2	Pen 3	“Fenceline”
Total Location (X,Y) Readings	9,265,677	8,521,612	471,908
Location Readings/Study Day	545,040	501,271	27,759
Average Tag Location Read Rate/Min	8.9	9.2	-
Defined Contacts	83,683	73,908	788
Contacts/Study Day	4,923	4,348	46
Steers Tagged	48	42	90
Steers Identified in Network	46	41	86
Edge Count	1,035	787	312
Density	100%	96%	17%
Clustering Coefficient	1	1	0
Modularity (no. of communities)	0 (2)	0 (2)	0.3 (7)

An overview of the overall (full) network parameters for each study. The full network contained locations from all 17 study days. Tag read rate is defined as the average location readings (X,Y) by each individual tag per minute of the study. Contacts are defined as two steers who were within a 0.71 m Spatial Threshold (SpTh) of each other within any given 10 second (10s) Temporal Sampling Window (TSW). Pen 2 and Pen 3 contacts were defined as contacts occurring between steers from that same pen. “Fenceline” contacts were defined as contacts occurring within 1 m of the shared fenceline and occurring between one steer from each Pen. Nodes in the overall network are defined as individual steers that had at least one defined contact within the study period. Edges in the overall network are the connection between two individual steers.

Table 4-2. Summary of Tag Readings Per Study Day.

	Pen 2	Pen 3
Min	3,424	0
5 th Percentile	5,031	5,691
25 th Percentile	12,355	8,592
Median	14,104	15,345
Mean	12,848	13,292
75 th Percentile	15,243	16,567
95 th Percentile	16,589	17,675
Max	18,733	19,009

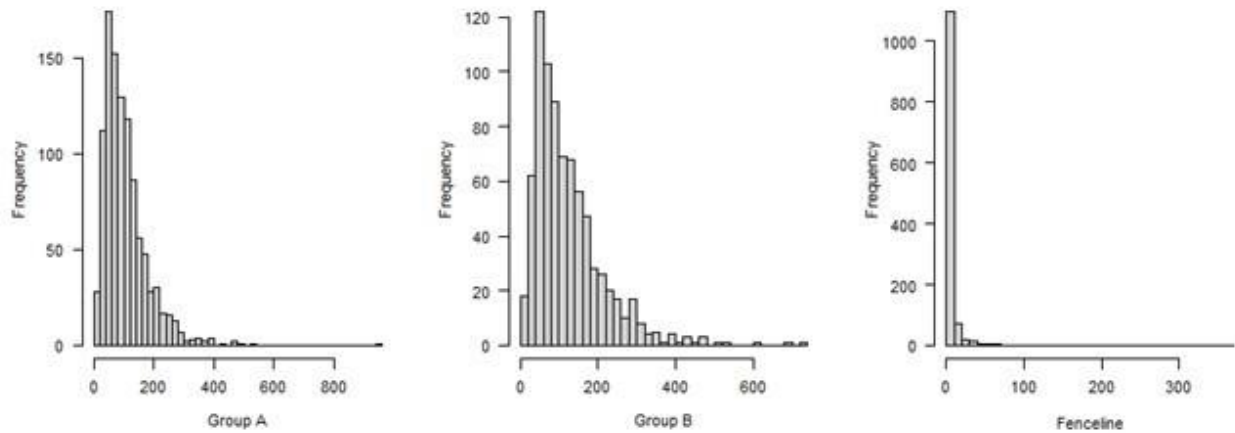
Summary statistics of individual tag readings for each study day. The first and last day of each study are excluded, as they were not full days. A reading is defined as an individual location (X,Y) reading identified in the system by that individual tag for each day.

Table 4-3. Summary of Overall Edge Weight.

	Pen 2	Pen 3	“Fenceline”
Min	9	1	1
5 th Percentile	27	28	1
25 th Percentile	54	60	1
Median	88	100	1
Mean	106	124	3
75 th Percentile	136	162	3
95 th Percentile	248	295	11
Max	960	726	66

Summary statistics for edge weight for each study. Edge weight is defined as the cumulative 10 s temporal sampling windows (TSW) that a pair of steers were in defined as being in contact for the duration of the study. Contacts are defined as two steers who were within a 0.71 m Spatial Threshold (SpTh) of each other within any given 10 s Temporal Sampling Window (TSW). Pen 2 and Pen 3 contacts were defined as contacts occurring between steers from that same pen. “Fenceline” contacts were defined as contacts occurring within 1 m of the shared fenceline and occurring between one steer from Pen 2 and one steer from Pen 3.

Figure 4-3. Histograms of Total Edge Weight.



Histograms demonstrating the total edge weight for the entire study each dyad pair for each group: Pen 2, Pen 3, “Fenceline”. Edge weight is defined as the cumulative 10 second (10s) temporal sampling windows (TSW) that a pair of steers were in defined as being in contact for the duration of the study. Contacts are defined as two steers who were within a 0.71 m Spatial Threshold (SpTh) of each other within any given 10s Temporal Sampling Window (TSW). Pen 2 and Pen 3 contacts were defined as contacts occurring between steers from that same pen. “Fenceline” contacts were defined as contacts occurring within 1 m of the shared fenceline and occurring between one steer from each Group. Frequency is the number of dyad pairs that fell within that edge weight range.

Table 4-4. Daily, 6-hour Period, and Hourly Contact Network Metrics.

		Pen 2	Pen 3	“Fenceline”
Daily (24-h)	Median Edge Count (Range)	891 (759-979)	661 (627-736)	25 (8-71)
	Median Density (Range, %)	86% (73-95)	77% (73-85)	0% (0-0)
	Median Clustering Coefficient (Range)	0.9 (0.7-1)	0.9 (0.8-0.9)	0 (0-0)
	Median Modularity (Range)	0 (0-0)	0 (0-0)	0.7 (0.5-0.8)
	Median No. of Communities (Range)	2 (2-3)	2 (2-3)	7 (3-11)
6-h Period	Median Edge Count (Range)	315 (133-773)	250 (97-621)	6 (0-28)
	Median Density (Range, %)	30% (13-75)	29% (11-72)	0% (0-0)
	Median Clustering Coefficient (Range)	0.4 (0.3-0.8)	0.4 (0.2-0.8)	0 (0-0)
Hourly	Median Edge Count (Range)	74 (0-355)	55 (0-314)	1 (0-12)
	Median Density (Range, %)	7% (0-34)	6% (0-36)	0% (0-0)
	Median Clustering Coefficient (Range)	0.3 (0-0.6)	0.3 (0-0.5)	0 (0-0)

Daily (24-h), 6-h period, and hourly network metrics. The first and last days of the study are not included in this analysis. The 6-h period networks were defined in Central Daylight time from 00:00-05:59 (night), 06:00-11:59 (morning), 12:00-17:59 (afternoon), and 18:00-23:59 (evening). Contacts are defined as two steers who were within a 0.71 m Spatial Threshold (SpTh) of each other within any given 10 second (10s) Temporal Sampling Window (TSW). Pen

2 and Pen 3 contacts were defined as contacts occurring between steers from that same pen.

“Fenceline” contacts were defined as contacts occurring within 1 m of the shared fenceline and occurring between one steer from each group.

Table 4-5. Summary of Median Daily Edge Weight.

	Pen 2	Pen 3
Min	0	0
5 th Percentile	0	0
25 th Percentile	1	1
Median	4	4
Mean	7	8
75 th Percentile	8	9
95 th Percentile	26	27
Max	144	173

Summary statistics for edge weight for each year by study day. Edge weight is defined as the cumulative 10 second (10s) temporal sampling windows (TSW) that a pair of steers were in defined as being in contact for that day. The first and last days of each study were excluded, due to being incomplete days. A contact is defined as two steers having location (X,Y) readings within 0.71 m of each other within any given 10s TSW. Median values are reported.

Table 4-6. Median Density 6-hour Periods.

Period	Hours	Pen 2	Pen 3
Night	00:00-05:59	20%	16%
Morning	06:00-11:59	56%	66%
Afternoon	12:00-17:59	46%	44%
Evening	18:00-23:59	26%	19%

The median density for all 6-hour periods in each study. The first and last day of each study are not included in this analysis. The 6-h period networks were defined in Central Daylight time from 00:00-05:59 (night), 06:00-11:59 (morning), 12:00-17:59 (afternoon), and 18:00-23:59 (evening). Contacts are defined as two steers who were within a 0.71 m Spatial Threshold (SpTh) of each other within any given 10 second (10s) Temporal Sampling Window (TSW).

Table 4-7. Median Density by Hour of Day.

	Pen 2	Pen 3
00:00-00:59	6%	5%
01:00-01:59	5%	4%
02:00-02:59	5%	4%
03:00-03:59	0%	0%
04:00-04:59	5%	4%
05:00-05:59	7%	6%
06:00-06:59	18%	20%
07:00-07:59	21%	26%
08:00-08:59	13%	28%
09:00-09:59	11%	29%
10:00-10:59	11%	12%
11:00-11:59	11%	11%
12:00-12:59	8%	9%
13:00-13:59	10%	10%
14:00-14:59	8%	8%
15:00-15:59	10%	10%
16:00-16:59	10%	12%
17:00-17:59	9%	9%
18:00-18:59	7%	5%
19:00-19:59	6%	5%
20:00-20:59	6%	5%
21:00-21:59	5%	4%
22:00-22:59	6%	5%
23:00-23:59	6%	4%

The median density for individual hourly periods in each study. The first and last day of each study are not included in this analysis. Contacts are defined as two steers in that study who were within a 0.71 m Spatial Threshold (SpTh) of each other within any given 10 second (10s) Temporal Sampling Window (TSW). Density is the number of edges (connections) present out of all edges possible.

Table 4-8. Overall Metrics for 30 and 60 s Minimum Contact Duration (MCD) Networks.

MCD (seconds)	Pen 2		Pen 3		“Fenceline”	
	30	60	30	60	30	60
Defined Contacts	5,235	433	4,718	410	39	5
Contacts/Study Day	308	25	278	24	2	0.3
Nodes	46	44	40	38	25	7
Edge Count	850	217	653	169	21	4
Density	82%	23%	84%	24%	13%	33%
Median Edge Weight (Range)	13 (3-315)	8 (6-161)	14 (3-422)	12 (6-223)	4 (3-42)	7 (6-15)
Clustering Coefficient	0.9	0.3	0.9	0.4	0	0
Modularity	0 (3)	0.2 (5)	0 (2)	0.2 (4)	0.7 (6)	0.6 (3)

An overview of the overall (full) network parameters for each study at 30 s and 60 s minimum contact duration (MCD). Contacts are defined as two steers who were within a 0.71 m Spatial Threshold (SpTh) of each other within any given 10 second (10s) Temporal Sampling Window (TSW) for a minimum of 30 second (30s) or 60 second (60s). Pen 2 and Pen 3 contacts were defined as contacts occurring between steers from that same pen. “Fenceline” contacts were defined as contacts occurring within 1 m of the shared fenceline and occurring between one steer from each Group. Nodes in the overall network are defined as individual steers that had at least one defined contact of that MCD within the study period. Edges in the overall network are the connection between two individual steers that met the MCD criteria.

Table 4-9. Daily, 6-hour Period, and Hourly Network Metrics for 30 and 60 s Minimum Contact Duration (MCD) Networks.

	MCD (seconds)	Pen 2		Pen 3	
		30	60	30	60
Daily (24 h)	Median Edge Count (Range)	167 (73-281)	13 (0-34)	128 (76-255)	12 (5-29)
	Median Density (Range, %)	16% (7-27)	1% (0-4)	16% (10-33)	2% (1-4)
	Median Clustering Coefficient (Range)	0.3 (0.1-0.4)	0 (0-0.5)	0.2 (0.1-0.4)	0 (0-0.2)
	Median Modularity (Range)	0.3 (0.2-0.4)	0.7 (0.6-0.8)	0.3 (0.2-0.4)	0.7 (0.6-0.8)
	Median No. of Communities (Range)	4 (3-7)	6 (4-8)	4 (3-6)	6 (3-9)
6-h Period	Median Edge Count (Range)	40 (15-131)	3 (0-19)	36 (8-108)	3 (0-11)
	Median Density (Range, %)	4% (1-13)	0.3% (0-2)	5% (1-14)	0.4% (0-2)
	Median Clustering Coefficient (Range)	0.1 (0-0.1)	0 (0-0.6)	0.2 (0-0.4)	0 (0-0.3)
	Median Edge Count (Range)	7 (0-39)	0 (0-6)	6 (0-38)	0 (0-4)
Hourly	Median Density (Range, %)	1% (0-4)	0% (0-0.6)	1% (0-5)	0% (0-0.6)
	Median Clustering Coefficient (Range)	0 (0-1)	0 (0-0)	0 (0-1)	0 (0-0.8)
	Median Edge Count (Range)	7 (0-39)	0 (0-6)	6 (0-38)	0 (0-4)

Daily (24-h), 6-h period, and hourly network metrics for contacts meetings a 30 second (30s) or

60 second (60s) minimum contact duration (MCD) threshold. The first and last days of each

study are not included in this analysis. The 6-h period networks were defined in Central Daylight

time from 00:00-05:59 (night), 06:00-11:59 (morning), 12:00-17:59 (afternoon), and 18:00-23:59

(evening). Contacts are defined as two steers in that study who were within a 0.71 m Spatial Threshold (SpTh) of each other within any given 10 second (10s) Temporal Sampling Window (TSW) that lasted for either 30s or 60s.

Chapter 5 - An Agent-based model approach for transmission of Shiga toxin-producing *Escherichia coli* in feedlot cattle

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Abstract

Shiga toxin-producing *Escherichia coli* (STEC) are zoonotic enteric pathogens known to cause illness in humans and have been associated with healthy cattle and their environment, though their transmission dynamics in cattle are poorly understood. The objective of this study was to create an *Escherichia coli* transmission model reflective of empirical data from a real-life transmission experiment highlighting both direct steer-to-steer contact and indirect steer-to-pen environment transmission routes using NetLogo 6.2. The direct transmission route was modeled based off a percentage of the average CFU/400cm² of the samples collected off the hides of the steers in the study, and the indirect transmission route was modeled based off a weekly average of the CFU/g of soil based off three pen areas (front, middle, rear). Colonization period within the model was based on a weighted average of the total study days positive for individual fecal samples taken from the empirical data. Median pen-level prevalence of *E. coli* colonization

ranged from 2.9% to 75% based on different levels of *E. coli* transmitted by direct and indirect contact routes. In general, as the percentage of average CFU transmitted per direct and indirect contact increases, so does the pen-level prevalence, with a greater increase seen with an increase in direct contact than indirect contact. Though these preliminary results suggest that direct animal-to-animal contact may play an important role for pen-level prevalence for *E. coli* colonization compared to indirect contact with pen environment, additional work is needed to determine the influence of these contact parameters on the modeled pen-level prevalence.

Introduction

Shiga toxin-producing *Escherichia coli* (STEC) are zoonotic enteric pathogens that cause severe illnesses in humans including hemorrhagic colitis and hemolytic uremic syndrome (Boyce et al., 1995). A primary reservoir for STEC are healthy cattle and their environment (Rasmussen and Casey, 2001), with food and water contaminated by STEC-containing cattle feces being the most frequent sources of exposure and subsequent infection for humans (Armstrong et al., 1996; Rasmussen and Casey, 2001). Though STEC are pathogens of human importance with a significance in the beef cattle industry, transmission dynamics of STEC in feedlot settings are not fully understood (Van Donkersgoed et al., 2001; Turner et al., 2003; Vosough Ahmadi et al., 2006). Our current understand of STEC transmission dynamics is largely dependent on longitudinal studies of naturally occurring STEC infections in cattle, though these naturally occurring infections the temporal initiation of infection as well as the infection source is unknown.

Computational models are important tools that allow the study of complicated and dynamic systems. Agent-based models (ABM) use a flexible modeling framework that captures

emergent phenomena and provides a natural description of the systems being modeled (Robins et al., 2015). Modeling provides a cost-effective alternative for incorporating multiple sources of data to study real system behavior. This approach is beneficial for studying STEC transmission in cattle for many reasons. First, isolation of STEC in laboratory settings requires use of selective media and additional characterization of serotypes and virulence factors (Parsons et al., 2016). Additionally, though cattle may persistently harbor STEC they do not develop any clinical signs of colonization (Menge, 2020). STEC research also must be carried out in Biosafety Level 2 (BSL-2) facilities, precluding performance of inoculation studies in commercial sized operations, and limiting research to observational studies. Use of inoculation studies allows control of the initially inoculated individuals and prospective description of the progressing outbreak. However, the requirement of conducting these studies in BSL-2 facilities limits their external validity to real production settings. Collection of data in production settings can only be done observationally, capturing data during a production outbreak event, but leaving the source and timing of initial infection unknown. Specific data on transmission of *E. coli* in a livestock production setting requires the use of a non-pathogenic surrogate to allow use of a known inoculation time and source and subsequent longitudinal sampling (Seger et al., 2022). Using a modeling approach allows researchers to surrogate data to study STEC transmission dynamics in a less intensive way while still gaining insights about the natural phenomena.

The objective of this study was to create a transmission model reflective of empirical data from a real-life nonpathogenic *E. coli* transmission experiment highlighting both direct contact and indirect pen environment transmission routes of a surrogate non-pathogenic *E. coli* strain using NetLogo 6.2.

Materials and Methods

All procedures performed in studies involving animals were in accordance with the ethical standards of Kansas State University College of Veterinary Medicine, IACUC #4065.

Study Population

In May of 2019, seventy Angus/Angus-cross steers were purchased through commercial channels for use in a certified nonpathogenic *Escherichia coli* inoculation transmission experiment (Seger et al., 2022). The steers were housed in an outdoor pen at a commercial feedlot (Pen 2, Figure 5-1). All steers received ear tags for visual identification, and a Real-Time Location System (RTLS) ear tag placed centrally in the left ear (Smartbow GmbH, Weibern, Austria). Two steers were removed from the study due to chronic lameness, one on day 35 and one on day 56. Due to limited RTLS location tag readings, two steers and their corresponding *E. coli* data were also removed from the study.

Model Construction

Model Description

A discrete time, stochastic, agent-based model based on the explicit contact network between steers and between steers and environmental areas was created and implemented using Netlogo 6.2.

Purpose

The model was created to simulate *E. coli* colonization in feedlot steers and contamination to their pen environment and subsequent transmission through direct steer-to-steer contact and indirect steer-to-pen environment contact.

Model Inputs

Escherichia coli Data

Inoculation, sampling, and quantification of the certified nonpathogenic *Escherichia coli* strain (O28:H43) can be found in Seger et al., 2022. Briefly, oral inoculations of a Nalidixic acid and Rifampicin resistant certified nonpathogenic *E. coli* strain were done for five consecutive days in five randomly selected steers in a pen of seventy. Samples were taken on both an individual steer level and pen environment level weekly for nine weeks. Fecal, hide, and oral swab samples were taken from each steer weekly. Pen surface soil samples in 25 pen locations (Figure 5-2), five feed samples, and three water samples were taken each study week. Spiral plating methods on selective media were used to determine concentration of the certified nonpathogenic *E. coli* strain in each sample. If samples were negative via spiral plating methods, 6 h enrichments at 37°C were done for detection of the inoculated *E. coli*. For the model, quantification and enrichment data from the hide samples was used as a proxy for direct contact exposure and transmission between steers. The average $\log_{10}\text{CFU}/400\text{ cm}^2$ concentration was taken across all hide samples. Exposure dose of 1%, 5%, and 10% of the average hide concentration was modeled in separate runs as the exposure dose per direct contact between steers to examine the effect of variable direct exposure.

As a proxy for indirect transmission between the steers and their pen environment the quantification and enrichment data from the pen soil surface samples was used. The pen was divided into three areas (front, middle, rear, Figure 5-2). A weekly average $\log_{10}\text{CFU/g}$ concentration for all samples within each pen area were calculated. Indirect exposure dose of

1%, 5%, and 10% of the pen area and week specific averages were modeled in separate runs as the indirect exposure dose for the corresponding pen area.

An average for the number of study days that fecal samples were positive from the empirical study (Seger et al., 2022, Study 2-B) was used to determine the colonization period for this steers within the model.

Point Location Data

All steers received two ear tags for visual identification, as well as a RTLS ear tag placed centrally in the left ear (Smartbow GmbH, Weibers, Austria). Data processing decisions and contact network creation can be found in Chapter 3. Briefly, raw location data were filtered and smoothed to represent individuals' average location (X,Y) at 10 second temporal sampling windows (TSW) over the course of the study period. Direct contact matrices between each pair of steers were created by aggregating daily total 10 s TSW on a daily (24-h) basis.

Environmental contact between each individual steer and each pen area (front, middle, rear) was estimated by first determining the raw location (X,Y) data readings per steer by study day by pen area. The proportion of the number of location readings per steer per pen area per day was then converted to a proportion of TSW per day. Due to the weekly sampling interval for the *E. coli* data, weekly proportions were then calculated and applied on an individual basis. If an individual steer did not have any location readings for a particular week, the previous week in which that steer had location readings was applied.

Entities, Globals, and Scales

Agents are defined as cattle or individual pen area (front, middle, rear) and represent individual steers in a commercial feedlot pen and three pen environmental areas that make up the

entire pen area. A visual representation of model setup can be seen in Figure 5-3. Each time step in the model represents 1 day, and the model is set up to run for 63 steps, the duration of time for which we have empirical data. For each day of the model, an explicit direct contact matrix between steers and an indirect contact matrix between steers and each pen area are incorporated into the model with contact defined in total 10 s TSW for that day. For all direct contacts that occurred between pairs of steers within the model a CFU amount based off 1%, 5%, or 10% of the average $\log_{10}\text{CFU}/400\text{ cm}^2$ for all hide samples collected in the study was applied. For all indirect contacts that occurred between individual steers and the pen environment areas (front, middle, and rear) a CFU amount based off 1%, 5%, or 10% of the average $\log_{10}\text{CFU}/\text{g}$ of soil samples collected in that area for that study week was applied. Values change weekly in the model.

Model Setup and Functions

To set up the model an initial contact matrix representing the first day of the study was imported with links created between pairs of steers and between individual steers and the three pen environment areas. Global variables including the CFU per contact with the front, middle, and rear of the pen are updated accordingly. Links are then set-up based on the representative contact matrix between pairs of steers and to individual steers with the pen environment areas. Transmission of *E. coli* occurs between steers and to individual steers in the pen environment area. Five steers are initially set as colonized in the model, subsequent colonization events of *E. coli* in the model are based off the probability of colonization (P_{col}) from daily accumulated CFU's from direct and indirect contacts for a particular steer. Probability of colonization was

calculated using a re-arranged form of the Hill-1 dose-response equation presented in Brouwer et al., 2017:

$$P_{col} = \frac{1}{1 + K_{50}/CFU_{cum}}$$

where K_{50} is equal to the median infection dose of the population and CFU_{cum} is the total CFU's accumulated over the day from both direct and indirect contacts. Whether or not colonization occurred was determined by drawing a random value from a uniform distribution between 0 and 1. This value was then assessed whether it fell below (colonized) or above (not colonized) the value of P_{col} .

After the initial colonization period for an individual steer, subsequent colonization events are half as likely based on the same equation, but colonization was determined by drawing a random value from a uniform distribution between 0 and 2 resulting in a probability of colonization of 50% of the initial colonization. The colonization period for each steer within the model is determined based off a average of the total duration in study days of shedding positives from the fecal samples collected from the empirical data (Seger et al., 2022, Study 2-B) The last step for each day in the model is to assign cattle color based on colonization status, red for infectious and white for non-infectious.

Observation

The pen-level prevalence of infected individuals was tracked at each time step. Running totals for initial colonization and total colonization (initial and recolonizations) are also kept. Pen-level prevalence within the model was then compared to a 2-week rolling average of the positive fecal samples detected in the empirical study (Seger et al., 2022, Study 2-B).

Sensitivity Analysis

Due to the stochastic nature of the model, a local sensitivity analysis varying the amount (1, 5, or 10%) of CFU per direct or indirect contact was analyzed. A thousand runs were simulated for each amount combination for CFU per direct and indirect contact.

Results

The study being modeled is Study 2-B from Seger et al., 2022. The study and rearing period simulated in the model ran for 63 days. Two steers removed were removed from study due to lameness, one on day 35 and one on day 56. The *E. coli* and location data for these two steers were kept in the model until their removal from the study period. The *E. coli* and location data from two steers were removed entirely from the model due to poor tag quality. Three additional steers had missing location data for one to four weeks of the study period. The weeks for which these 3 steers were missing pen location area from were filled with average pen location proportions from the previous week. The average concentration for hide samples collected in the study was 97.6 CFU/400cm². Colonization period within the model was based off the weighted average for study days in which fecal samples from the empirical data were positive. This weighted average was 15.5 study days.

Twelve soil samples per study week fell within the designated front pen area. There were 6 study weeks when all 12 soil samples in the front pen area were positive (Table 5-1). The average concentration for soil samples collected in the front area ranged from 50 CFU/g (Week 9) to 2,313 CFU/g (Week 8). There were eight weekly soil samples collected in the designated middle pen area, with all 8 samples being positives for 7 study weeks. The average concentration for samples collected in the middle pen area ranged from 37.8 CFU/g (Week 5) to 2,626.7

CFU/g (Week 8). Five weekly soil samples taken in the rear pen area with 6 study weeks having all 5 soil samples positive. The average concentration for samples collected in the rear pen area ranged from 0 CFU/g (Week 6) to 2,360 CFU/g (Week 2).

Table 5-2 represents summary statistics for the modeled final pen-level prevalence of *E. coli* colonization at the end of the modeled rearing period of all thousand iterations run for each combination of three levels average CFU per contact type (1, 5, 10%). As the percentage of average CFU transmitted per direct and indirect contact increases, so does the pen-level prevalence. For example, at the colonization period of 15.5, the median prevalence of *E. coli* in the pen ranged from 2.9 to 10.3% when direct contact was 1% and ranged from 73.5 to 75% when direct contact was 10%. Similarly, the median pen-level prevalence ranged from 2.9% to 73.5% when indirect contact was modeled at 1% and ranged from 10.3% to 75% when direct contact was set to 10%. This can also be seen in Figure 5.3, where the pen-level prevalence of fifty iterations of each combination of 1, 5, and 10% of both direct and indirect transmission amounts are plotted over the study period. When both direct and indirect transmission routes are set to 1% the pen-level prevalence is low, with a pen-level prevalence at the end of the study period (Day 63) around 10-20%. When both direct and indirect transmission routes are set to 5 or 10% there is a rise in pen-level prevalence for the entire study period, and the 5% modeled exposure dose for both direct and indirect contact most closely reflects the plotted 2-week rolling average of fecal samples positive collected in the empirical study (Seger et al., 2022, Study 2-B).

Discussion and Conclusions

In this study, pen-level prevalence results for *Escherichia coli* colonization within a pen of feedlot cattle using Netlogo 6.2 was presented. The model contained both a direct and indirect

transmission component reflective of direct contacts made between pairs of steers in the model and contacts individual steers had with three pen environment areas. A previous agent-based model created by Dawson et. al., investigated the behavioral drivers of seasonal STEC patterns in a pasture of grazing cattle using both a direct and indirect (graze-based, water) transmission routes (2018). The model presented here highlighted *E. coli* transmission dynamics in a commercial U.S. feedlot setting and has the advantage of being tightly coupled to observed data.

Direct transmission of STEC is generally thought to occur via the fecal-oral route, either through social interactions (e.g., grooming events) or from incidental contacts that due to proximity can transfer feces between hides (McGee et al., 2004). It has also been suggested that STEC transmission can occur between cattle in close proximity via aerosols (Besser et al., 2001). Based on the best knowledge of direct transmission of STEC between cattle the *E. coli* concentration data found on the hides of the steers was used as the value for the direct contact transmission pathway in the model.

When the modeled exposure dose was set as 5% of the average hide concentration for direct contact and 5% of the average weekly pen soil surface concentration for indirect contact it most closely matched a 2-week rolling average of the fecal samples collected from the steers positive by enumeration or enrichment methods (Seger et al., 2022, Study 2-B). Increasing the indirect environmental exposure from 1 to 10% of the weekly concentration for each TSW of contact had little impact on modeled pen-level prevalence within categories for direct contact exposure (Table 5-2). Based on these preliminary results within the bounds of exposures modeled, it is suggestive that direct contact between animals is more important for pen-level prevalence for *E. coli* colonization than indirect contact with pen environment.

There are multiple inherent limitations in the model presented here, first being the variability in the quantification and detection methods of the non-pathogenic *E. coli* strain. Briefly, there was variability seen between study weeks in the fecal shedding of the steers and the limit of detection for the enumeration methods used for the study were $1.0 \log_{10}\text{CFU}/400 \text{ cm}^2$ in hide samples and $2.0 \log_{10}\text{CFU}/\text{g}$ in feces. Though enrichment methods were used to detect the presence of the inoculated strain below the detection limit of the enumeration methods. Another limitation is that samples from the steers and their pen environment were collected on a weekly basis, thus limiting our understanding of individual colonization status of the status to a week-by-week basis, whereas the model is on a day-by-day basis for colonization status. Additional comments on this variability in detection can be found in Seger et al., 2022. Along with this variability in detection and quantification, there is also variability in the number of pen samples that were collected in each of the 3 pen areas, with the front pen area having approximately 1.5 and 2.5 times the number of samples collected each study week compared to the middle and rear pen areas, respectively. The number of samples collected in each pen area were determined prior to the start of the initial inoculation study and were determined based on expected cattle density within different areas of the pen. At this time, hide sample concentrations cannot be modeled to reflect an individual steer's hide exposure to all other steers within the pen and there is a gap in knowledge surrounding the true exposure level for CFU of *E. coli* cumulated by both contact with the pen area contacts and direct contact with hides of other steers within the pen.

Though the contact networks used as model inputs are considered high resolution with an estimated accuracy of 90% within $\pm 0.5 \text{ m}$ of individuals' true location (Farthing et al., 2021)

they are still static in nature and aggregated to a daily (24-h) timescale. Using dynamic networks is the preferred method for simulating disease transmission, both within and between populations (Duncan et al., 2012; Vernon and Keeling, 2009). Though there are limitations in Netlogo's capacity to implement this type of network.

Additional work is needed to determine the influence of the direct and indirect contact parameters on the modeled pen-level prevalence, as well as determining the R-naught calculated from the modeled *E. coli* to compare to published R-naught values of *E. coli* O157:H7 and additional STEC serotypes.

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Literature Cited

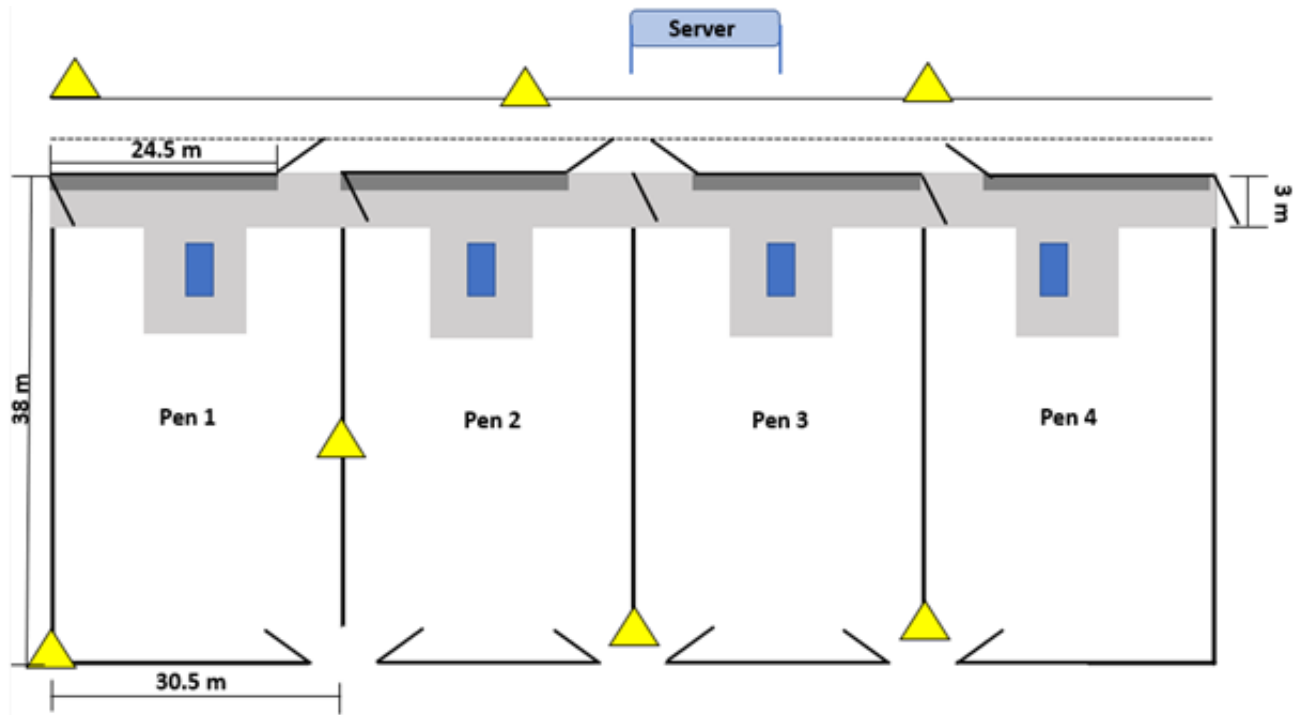
1. Armstrong, G. L., J. Hollingsworth, and J. G. Morris. 1996. Emerging foodborne pathogens: *Escherichia coli* O157:H7 as a model of entry of a new pathogen into the food supply of the developed world. *Epidemiol. Rev.* 18:29-51.
<https://doi.org/10.1093/oxfordjournals.epirev.a017914>.

2. Besser, T. E., B. L. Richards, D. H. Rice, and D. D. Hancock. 2001. *Escherichia coli* O157:H7 infection of calves: infectious dose and direct contact transmission. *Epidemiol. Infect.* 127:555-560. <https://doi.org/10.1017/S095026880100615X>.
3. Boyce, T. G., D. L. Swerdlow, and P. M. Griffin. 1995. *Escherichia coli* O157:H7 and the hemolytic-uremic syndrome. *N. Engl. J. Med.* 333: 364-368. <https://doi.org/10.1056/NEJM199508103330608>.
4. Brouwer, A. F., M. H. Weir, M. C. Eisenberg, R. Meza, and J. N. S. Eisenberg. 2017. Dose-response relationships for environmentally mediated infectious disease transmission models. *PLoS Comput. Biol.* 13:e1005481. <https://doi.org/10.1371/journal.pcbi.1005481>.
5. Dawson, D. E., J. H. Keung, M. G. Napoles, M. R. Vella, S. Chen, M. W. Sanderson, and C. Lanzas. 2018. Investigating behavioral drivers of seasonal Shiga-Toxigenic *Escherichia coli* (STEC) patterns in grazing cattle using an agent-based model. *PLoS One.* 13(10): e0205418. <https://doi.org/10.1371/journal.pone.0205418>.
6. Duncan, A.J., Gunn, G.J., Lewis, F.I., Umstatter, C., Humphry, R.W., 2012. The influence of empirical contact networks on modeling diseases in cattle. *Epidemics.* 4:117-123. <https://dx.doi.org/10.1016/j.epidem.2012.04.003>.
7. Farthing, T.S., Dawson, D.E., Sanderson, M.W., Seger, H., Lanzas, C., 2021. Combining epidemiological and ecological methods to quantify social effects on *Escherichia coli* transmission. *R. Soc. Open Sci.* 8: 210328. <https://doi.org/10.1098/rsos.210328>.
8. McGee, P., L. Scott, J. J. Sheridan, B. Earley, and N. Leonard. 2004. Horizontal transmission of *Escherichia coli* O157:H7 during cattle housing. *J. Food. Prot.* 67: 2651-2656. <https://doi.org/10.4315/0362-028x-67.12.2651>.

9. Menge, C. 2020. The role of *Escherichia coli* Shiga Toxins in STEC Colonization of Cattle. *Toxins*. 12:607. <https://doi.org/10.3390/toxins12090607>.
10. Parsons, B. D., N. Zelyas, B. M. Berenger, and L. Chui. 2016. Detection, Characterization, and Typing of Shiga Toxin-Producing *Escherichia coli*. *Front. Microbiol.* 7:478. <https://doi.org/10.3389/fmicb.2016.00478>.
11. Rasmussen, M. A., and T. A. Casey. 2001. Environmental and food safety aspects of *Escherichia coli* O157:H7 infections in cattle. *Crit. Rev. Microbiol.* 27: 57-73. <https://doi.org/10.1080/20014091096701>.
12. Robins, J., S. Bogen, A. Francis, A. Westhoek, A. Kanarek, S. Lenhart, and S. Eda. 2015. Agent-based model for Johne's disease dynamics in a dairy herd. *Vet. Res.* 46-68. <https://doi.org/10.1186/s13576-015-0195-y>.
13. Seger, H. L., M. W. Sanderson, and T. G. Nagaraja. 2022. Nonpathogenic *Escherichia coli* strains as a surrogate for Shiga toxin-producing *E. coli*: Fecal shedding dynamics and pen environmental contamination in weaned calves and feedlot steers. *App. Ani. Sci.* 38: 305-316. <https://doi.org/10.15232/aas.2021-02260>.
14. Turner, J., M. Begon, R. G. Bowers, and N. P. French. 2003. A model appropriate to the transmission of a human food-borne pathogen in a multigroup managed herd. *Prev Vet Med.* 57:175-198. [https://doi.org/doi:10.1016/s0167-5877\(03\)00006-0](https://doi.org/doi:10.1016/s0167-5877(03)00006-0).
15. Van Donkersgoed, J., J. Berg, A. Potter, D. Hancock, T. Besser, D. Rice, J. LeJeune, and S. Klashinsky. 2001. Environmental sources and transmission of *Escherichia coli* O157 in feedlot cattle. *Can Vet J.* 42:714-720.

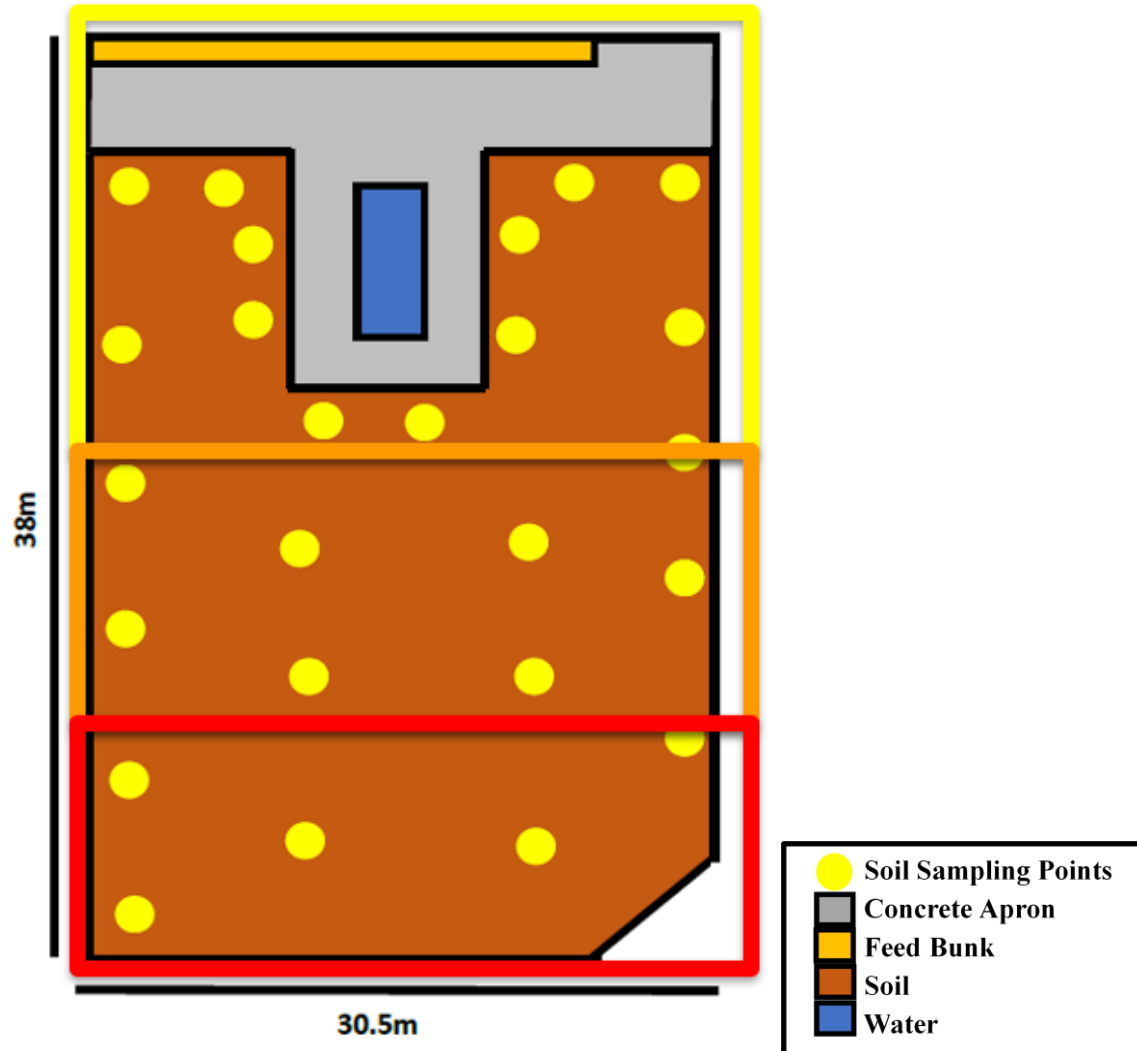
16. Vernon, M.C., Keeling, M.J., 2009. Representing the UK's cattle herd as static and dynamic networks. *Proc. R. Soc. B: Biol. Sci.* 276:469-476.
<https://doi.org/10.1098/rspb.2008.1009>.
17. Vosough Ahmadi, B, A. G. J. Velthuis, H. Hogeveen, and R. B. M. Huirne. 2006. Simulating *Escherichia coli* O157:H7 transmission to assess effectiveness of interventions in Dutch dairy-beef slaughterhouses. *Prev Vet Med.* 77:15-30.
<https://doi.org/doi:10.1016/j.prevetmed.2006.05.011>.

Figure 5-1. Map of Study Area.



Map of the study area containing four identical feedlot pens measuring 38 m by 30.5 m. Each pen contains a 24.5 m long feed bunk (dark grey), an automatic waterer (dark blue), and a concrete apron surrounding the feedbunk and waterer (light grey). Fences are outlined in solid dark lines, the feed alley is represented by the dashed line, and gates are represented as angled lines. Pens 1, 2, and 3 were equipped with the RTLS system. Receivers (yellow triangles) were positioned around the pens and hardwired to a server, which was housed in an enclosed space inside a shed. Steers from Study 2-A were housed in Pen 2 (Seger et al., 2022).

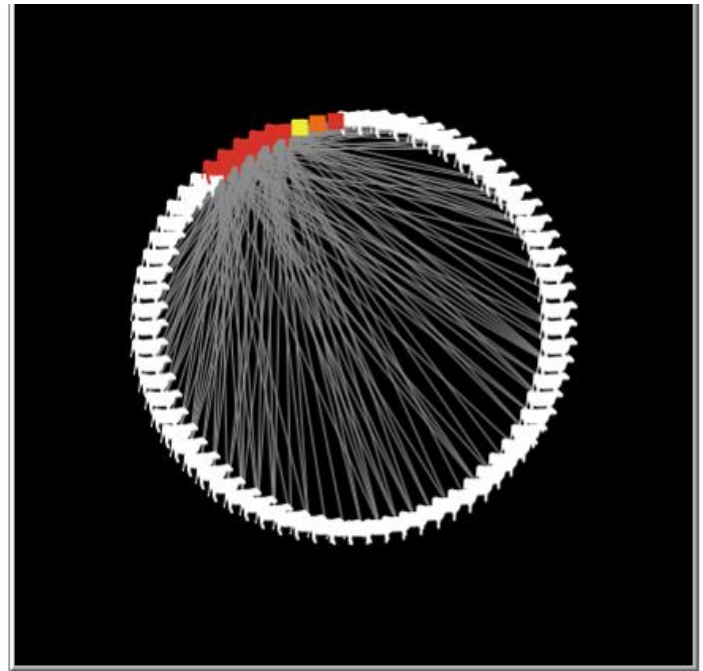
Figure 5-2. Map of Pen Surface Soil Sampling and Designated Pen Areas.



Pen map for Study 2-B outlining the areas of the feed bunk, concrete apron, water tank, and the 25 locations around the pen that weekly pen surface soil samples were collected from (Seger et al., 2022). The front area of the pen is outlined in the yellow square. The middle area of the pen is outlined in the orange square. The rear area of the pen is outlined in the red square.

Figure 5-3. Netlogo Model Setup.

setup		go	
Initial Inputs			
Rearing_period	Colonized_t0		
63	5		
Model Parameters			
cfupcd	K50		
4.7	10000		



Netlogo 6.2. model interface. Individual agents within the model are represented in a circular layout as either uncolonized cattle (white), colonized cattle (red), or three pen areas (colored squares). Connections between agents are shown in grey lines. Model inputs of rearing period and number of cattle colonized at time 0 are included as well as model parameters of Colony Forming Units (CFU) per direct contact (cfupcd) which is based on 1, 5, or 10% of the total study average CFU/400cm² taken from the hide samples from Seger et al. Study 2-B (2022). K₅₀ is equal to the median infection dose of cumulative CFUs for the population.

Table 5-1. Percent Positive and Average CFU/g of Collected Pen Surface Samples Per Pen Area.

Front Pen Area (n=12)			Middle Pen Area (n=8)		Rear Pen Area (n=5)	
Study Day	Samples Positive (%)	Average CFU/g	Samples Positive (%)	Average CFU/g	Samples Positive (%)	Average CFU/g
7	11 (92%)	576.7	8 (100%)	1,850.0	4 (80%)	1,810.2
14	11 (92%)	1,050.3	8 (100%)	1,174.2	5 (100%)	2,360.0
21	12 (100%)	1,828.8	8 (100%)	1,404.1	5 (100%)	511.2
28	9 (75%)	716.7	8 (100%)	2,475.0	5 (100%)	2,170.0
35	12 (100%)	37.8	6 (75%)	37.8	3 (60%)	30.4
42	12 (100%)	173.4	2 (25%)	69.5	0 (0%)	0.0
49	12 (100%)	50.0	8 (100%)	50.0	5 (100%)	50.0
56	12 (100%)	2,313.0	8 (100%)	2,646.7	5 (100%)	1,330.4
63	12 (100%)	50.0	8 (100%)	50.0	5 (100%)	50.0

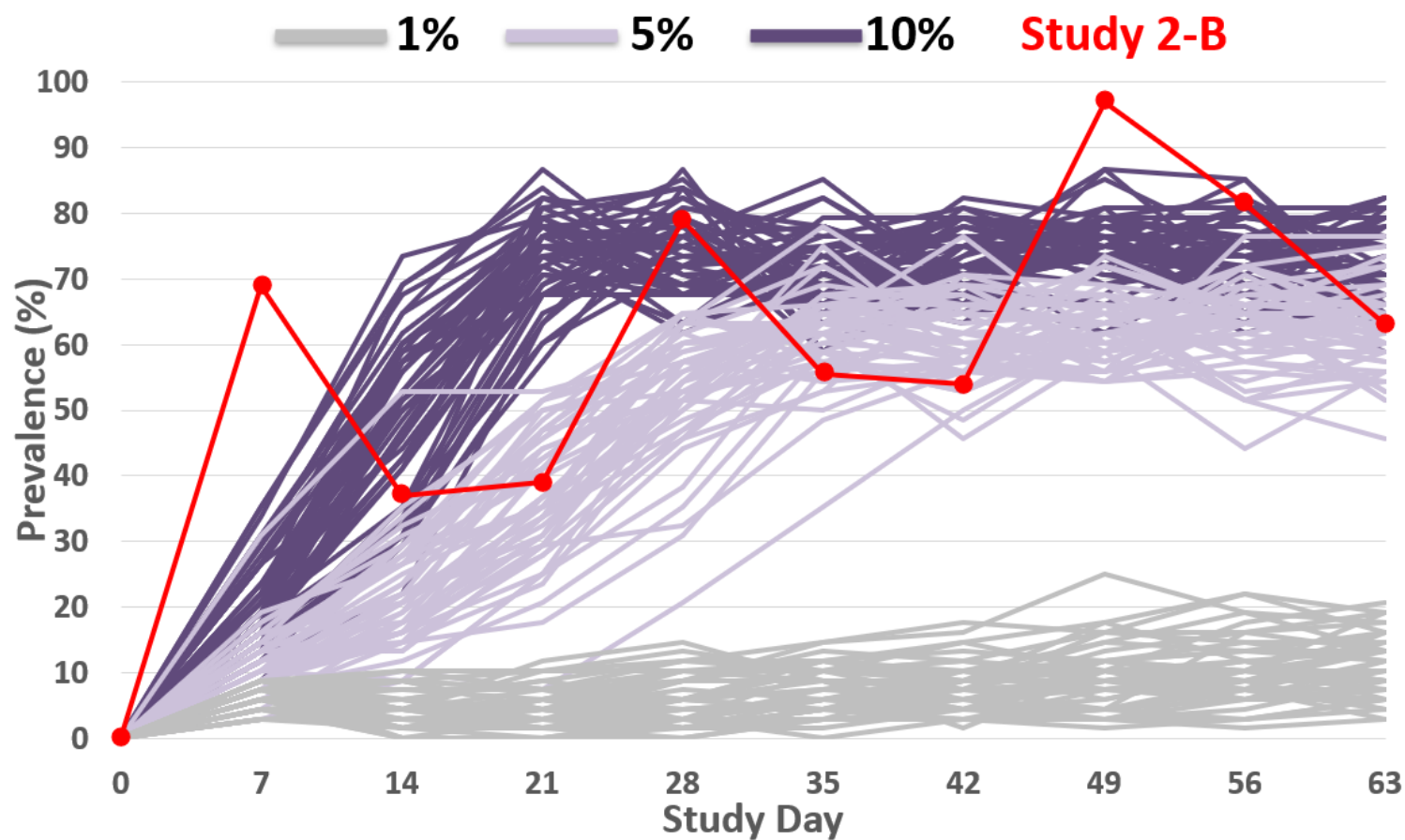
Total number positive (%) and average colony forming units (CFU)/g of soil in the environmental pen samples day and designated pen area. Positive samples are defined as those positive by either enumerable or enrichment methods described in Seger et al., 2022.

Table 5-2. Summary of Modeled Pen-level Prevalence of Colonized Steers Based Off Colonization Period and Percent of Average CFU Transmitted Per Contact Type (Direct and Indirect).

Colonization Period	% of Average CFU per contact		Pen-Level Prevalence (%)					
	Direct	Indirect	Min	25 th	Median	Mean	75 th	Max
15.5	1	1	0.0	1.5	2.9	3.2	4.4	13.2
	1	5	0.0	7.4	10.3	11.2	14.7	30.9
	1	10	0.0	7.4	10.3	11.1	14.7	30.9
	5	1	47.1	58.8	63.2	62.6	66.2	79.4
	5	5	42.6	57.4	61.8	62.3	66.2	79.4
	5	10	44.1	58.8	61.8	62.2	66.2	79.4
	10	1	55.9	70.6	73.5	73.9	77.9	91.2
	10	5	57.4	70.6	73.5	73.8	77.9	88.2
	10	10	58.8	70.6	75.0	74.0	77.9	88.2

Summary statistics for pen-level prevalence of colonized steers based on the end of the rearing period (63 days) the three levels of both direct and indirect transmission. Colonization period was defined as a weighted average based off the time in days individual fecal samples were positive in Study 2-B from Seger et al., 2022. Direct transmission was modeled as 1, 5, or 10% of the average of the total study *E. coli* concentration in CFU/400cm² from the collected hide samples. Indirect transmission was modeled as 1, 5, or 10% of the weekly average pen surface soil samples collected in each of the three pen areas (front, middle, and rear). Values were applied for 7 days of the model to represent the previous week from which they were collected. One thousand iterations of the model were collected for each parameter combination.

Figure 5-4. Line graph of modeled pen-level prevalence of colonized steers at 1, 5, and 10% for direct and indirect transmission per study day compared to 2-week rolling average for fecal samples from Seger et al., 2022 (Study 2-B).



Line graph showing the pen-level prevalence for steers colonized for *E. coli* within the model at 1, 5, and 10% for direct and indirect transmission routes by study day. Direct transmission was modeled as 1, 5, or 10% of the average of the total study *E. coli* concentration in CFU/400cm² from the collected hide samples. Indirect transmission was modeled as 1, 5, or 10% of the weekly average pen surface soil samples collected in each of the three pen areas (front, middle, and rear). Values were applied for 7 days of the model to represent the previous week from which they were collected. The first fifty iterations of the model that were ran are plotted for each 1 (grey), 5 (light purple), and 10% (dark purple). Overlayed in the red line is the 2-week rolling average for fecal samples positive by either enumeration or enrichment methods collected from the individual steers in Seger et al. (2022, Study 2-B).

Chapter 6 - Dissertation conclusions

The objectives of the work described in this document were to describe the use of social network analysis (SNA) in cattle and highlight the data gaps in understanding individual and group characteristics of feedlot cattle, describe the shedding and within pen transmission of a certified nonpathogenic *Escherichia coli* in feedlot cattle as a surrogate for modeling transmission of Shiga toxin-producing *E. coli* (STEC), use remote, real-time location system (RTLS) to create and describe within and between pen contact networks of feedlot cattle to inform disease transmission modeling, and examine the influence of transmission routes on pen-level prevalence of *E. coli* colonization using an agent-based model.

Social Network Analysis is a multidisciplinary tool that aims to characterize the social structure of a network of individuals and their connections. SNA applications specific to the cattle industry include the modeling of networks based on trade and cattle movements, modeling the transmission of infectious diseases between farms, and modeling herd dynamics to better understand the social behavior of dairy cattle in groups. The use of SNA on networks of cattle movement has shown influential "hubs" that can help direct the use of surveillance and disease interventions more precisely. It has been observed that all individuals in various herds of dairy cow are connected to one another on a group level, either directly or indirectly. Yet, there is evidence of preferential associations developing between dairy cows on an individual basis depending on traits like lactation frequency, breed, gregariousness, and milk output. Within a network, these favored social grouping connections cause non-linear interactions between dairy cows. It is noted that there is a gap in the literature describing the group and individual

characteristics of beef cattle, specifically in feedlot settings. By better understanding the network and behavior of feedlot cattle there can be a better understanding of production-type differences in network structure and metrics as well as providing information to create more robust disease transmission models for within disease transmission within-pens and between-pens of feedlot cattle.

Three verified non-pathogenic *E. coli* strains (O19:H+, O101:H10, and O28:H43) were found to establish and colonize in the gastrointestinal tract of weaned Holstein calves, shed in the feces, and spread to their pen environment. The fecal shedding established in the calves was variable between calves and between sampling days. Next, it was established that one verified non-pathogenic *E. coli* strain, O28:H43, was able to colonize, shed in the feces, spread to the pen environment, and transmit between steers in two inoculation trials. There was variability in week-to-week detection of the inoculated strain, this could be due to individual variability in shedding concentration. Overall, these studies showed the use of a non-pathogenic *E. coli* strain with a known temporal onset and source as a surrogate to help understand enteric pathogen host-to-host and host-to-environment transmission.

Contact network analysis was used to quantify contacts within pens of feedlot cattle across multiple years at varying timescales (24-h, 6-h, and h) to better inform the construction of network-based disease transmission models for cattle within confined-housing systems. The cattle observed in these studies were confined within a limited pen space and the resulting contact networks on a longer timescale (overall, 24-h) yielded high network density, with the majority of potential connections possible between pairs of steers present over each study period. We also describe the influence of RTLS read rate (average readings per minute) and extended

minimum contact duration (MCD) (10 seconds (10s), 30 seconds (30s), and 60 seconds (60s)) to reflect different contact types (i.e., transient vs. grooming events) on the realized description of contact networks of feedlot cattle. Though the same system was used for all three years, there was variation in the observed tag read rate between each year, resulting in variation in the observed contact networks. In all three studies, there was a larger number of contacts found at the 10s MCD, compared to the 30s and 60s MCD. This highlights the importance for choosing a MCD that reflects the assumed amount of contact needed for transmission of a particular disease.

Additionally, contact network analysis to quantify contacts within two pens of feedlot cattle and across a shared fenceline at varying timescales (24-h, 6-h, and h) to better inform the construction of network-based disease transmission models for cattle within confined-space systems with barriers to transmission. Similar to the within pen networks in Chapter 3, the within pen networks created here resulted in high network density and variation in within-pen contact structure patterns was masked at longer timescales (overall, 24-h). The contact network created across the shared fenceline resulted in lower network density, even at a longer timescale, but still highlighted the importance of modeling contacts that occur between pens in disease transmission models of confined livestock populations.

An agent-based model was then created using the nonpathogenic *E. coli* shedding and transmission data presented in Chapter 2 (Study 2-B) and the contact networks created in Chapter 3 (2019). The objective of the model was to examine the influence of direct steer-to-steer and indirect steer-to-pen environment transmission routes on the pen-level prevalence of *E. coli* colonization. Direct transmission was modeled based off a percentage of the average colony forming unit (CFU)/400cm² of the samples collected off the hides of the steers in the study, and

the indirect transmission was modeled based on a weekly average of the CFU/g of soil based off three pen areas (front, middle, rear). Colonization period within the model was based off the weighted average time in which fecal samples from the empirical study (Seger et al., 2022, Study 2-B) were positive. It is suggestive based off the bounds of exposures being modeled, that direct contact between animals is more important for pen-level prevalence for *E. coli* colonization than indirect contact with pen environment., though further research is necessary to fully understand the impact of the modeled contact parameters on the prevalence of *E. coli* colonization at the pen-level.

The research in this dissertation highlights the importance of a nonpathogenic surrogate for *E. coli* transmission in feedlot cattle, the use of RTLS for quantifying and describing the contact networks of feedlot cattle both within-pens and between-pens, and the use of agent-based modeling as a form of studying the transmission routes of *E. coli* transmission in feedlot cattle. These findings can be applied to better inform the construction of network-based disease transmission models for cattle within confined-space systems both within-pens and direct transmission across barriers, such as fencelines. Additionally, these findings can be used to both help understand enteric pathogen transmission in commercial U.S. feedlot systems and model STEC transmission to explore effective control options in real world situations.