

# Genomic sequencing of STEC isolates from feral pigs and outdoor-raised pigs in high-risk contact areas in California

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## Background

Although a majority of American swine production occurs inside confinement buildings, the US is currently experiencing a resurgence of outdoor-based (i.e., pasture-raised) pig operations, due to growing consumer demand for sustainably-produced foods, including humanely-raised meat. A challenge in raising pigs outdoors is the increased risk of domestic pigs interfacing with wildlife, like feral pigs. California has one of the largest and widest distributions of feral pig populations, which continues to expand. While still primarily considered a niche market, outdoor-raised pig operations (OPO) are numerous in CA, providing an opportunity for the widespread transmission of diseases throughout the state, as each pig could serve as a link in the spread of pathogens between wildlife, livestock and humans. Both domestic and feral pigs are asymptomatic reservoirs for zoonotic and foodborne pathogens. For instance, feral pigs were implicated in the 2006 nationwide *E. coli* O157:H7 outbreak in spinach. Several studies have identified feral pigs in CA as carriers of *E. coli* O157:H7, with prevalence ranging from 3.6% to 14.9%. A recent CA study found a prevalence of 5.6% Shiga toxin-producing *Escherichia coli* (STEC), with a range per farm of 0-25%, in pigs raised outdoors on small-scale farms, including serogroups, which are on the CDC's list of top seven STEC of public health concern. These two parallel trends of an expanding feral pig population and an increase in OPO, create a potential risk for zoonotic diseases exposure and transmission, which could negatively impact both public and livestock health, and agricultural economy. The objective of this study was to characterize STEC isolates from fecal samples from outdoor-raised pigs and feral pigs collected in a cross-sectional study and use shotgun whole genome sequencing (WGS) to assess the relatedness of strains between domestic outdoor-raised pigs living near feral pig habitats in high-risk areas of interface in California.

## Materials and Methods

A cross-sectional study targeting high-risk counties with known interaction between feral and outdoor-raised pigs was conducted. Fecal samples (17 farms total), from domestic pigs (149

samples) and feral pigs (43 feral samples) were collected, cultured for *E. coli* O157:H7 and STEC. Presumptive positive isolates were confirmed by PCR. A subset of STEC isolates were sent to the UC Davis Genome Center for whole genomic sequencing (23 isolates from domestic pigs and 2 from feral pigs). STEC isolates were serotyped and a phylogenetic tree was used to assess genetic relatedness between feral and domestic pigs.

## **Results**

Farm STEC prevalence ranged from 0% to 83.3%. County-level STEC prevalence for feral pigs ranged from 0% to 25.0% and 0% to 57.14% for domestic outdoor-raised pigs. Overall, 35 out of the total 192 samples (18.23%) were positive for STEC, with five samples collected from feral pigs (11.63%; 5/43) and 30 samples gathered from domestic pigs (20.13%; 30/149). Several serotypes with public health implications were identified (O157:H7; O26:H11 and O103:H11). Heatmaps of virulence and antimicrobial resistance genes show clustering by county and farm, with multi-drug resistant genes present in outdoor-raised pigs.

## **Conclusions**

As the number of outdoor-raised swine continues to grow, evaluating factors implicated in transmission of foodborne pathogens between these two swine populations will help identify mitigation strategies to reduce the food safety risks in OPO. Similarity of STEC isolates can be used as a biological indicator to track possible transmission of diseases between feral and outdoor-raised swine.

## **Funding**

This project was supported by the Agriculture and Food Research Initiative grant no. 2019-67011-29609 / project accession no. 1019249 from the USDA National Institute of Food and Agriculture and a CFAH grant.