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Enteric Human Pathogens of Wild Boar, Feral Swine, and Javelina (Order: Artiodactyla)

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ABSTRACT: Artiodactyls are even-toed ungulates that include domestic and wild ruminant herbivores (cattle, sheep, goat, deer, elk), pigs, and javelinas. There is increasing evidence that some wild ungulate species are important reservoirs of enteric human pathogens, and they may contribute to foodborne disease transmission directly through ingestion of undercooked game meat or indirectly via fecal contamination of fresh fruit and vegetable crops or agricultural water sources. To better understand zoonotic risks from feral swine and javelina in the U.S. southwestern desert, we conducted a prevalence survey of Shiga toxin-producing *Escherichia coli* (STEC) and *Salmonella* in colonic-fecal samples from animals collected through hunter-harvest or depredation. STEC O157:H7 was detected in feral swine, but not in javelina samples. *Salmonella* and non-O157 STEC serotypes of potential public health importance were found in both species. The findings underscore the importance of continued public health education efforts to protect hunters from exposure to these pathogens when handling game animals. Fresh fruit and vegetable growers should continue to prevent intrusions by wild and feral animals in the produce production environment to minimize transport of fecal-borne pathogens to crops intended for human consumption.

KEY WORDS: Arizona, *Campylobacter*, collared peccary, disease, *Escherichia coli*, feral swine, food safety, javelina, New Mexico, *Pecari tajacu*, *Salmonella*, *Sus scrofa*, wild boar, wild pigs

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INTRODUCTION

Artiodactyls are even-toed ungulates that include domestic and wild ruminant herbivores (e.g., cattle, sheep, goat, deer, elk), pigs, and javelinas (also called peccaries). Pigs and peccaries evolved separately from a common ancestor around the Eocene-early Oligocene period (Herrera et al. 2008). Pigs (*Sus scrofa*) are an "Old World" Eurasian species belonging to the family Suidae; Eurasian wild boar, domestic swine, and their crosses have since spread worldwide through intentional and unintentional introduction into non-native habitats. Expanding feral swine populations are considered a major invasive species and threat to public health, animal health, and the environment. In contrast, the collared peccary or javelina (*Pecari tajacu*) is a "New World" species belonging to the family Tayassuidae, with a geographic range from South America to the southwestern U.S. While the appearance and some behavioral characteristics of swine and javelinas are similar, they cannot interbreed.

There is increasing evidence that some wild ungulate species are important reservoirs of enteric human pathogens, and they may contribute to foodborne disease transmission directly through ingestion of undercooked game meat, and indirectly via fecal contamination of fresh fruit and vegetable crops or agricultural water sources (Langholz and Jay-Russell 2013). The enteric zoonotic bacterial foodborne pathogens of greatest concern with regard to food safety and wildlife include *Campylobacter* spp., Shiga toxin-producing *Escherichia coli*

(STEC), and *Salmonella enterica*, although *Yersinia enterocolitica*, *Y. pseudotuberculosis*, and *Listeria monocytogenes* may also be shed in the feces (Jay-Russell 2013). This paper focuses on fecal-borne bacterial zoonoses, but wild boar and feral swine are also important reservoirs of other bacterial, parasitic, and viral pathogens that may be transmitted through ingestion of contaminated game meat and/or direct contact (e.g., *Brucella*, *Cryptosporidium*, *Toxoplasma*, *Trichinella*, Hepatitis E, etc.).

In 2006, the genetically identical strain of *E. coli* O157:H7 associated with a nationwide outbreak linked to fresh bagged baby spinach was isolated from domestic cattle and free-ranging feral swine at a single ranch in the central California coast (Jay et al. 2007); *Campylobacter* spp. was also cultured from oral cavity (tonsils, buccal swabs) and colonic fecal samples taken from a subset of the feral swine trapped or hunted on the ranch (Jay-Russell et al. 2012). Since the 2006 outbreak, subsequent epidemiologic studies in the central California coast have consistently identified STEC and *Salmonella* in feral swine samples (Gorski et al. 2011, Cooley et al. 2013). Similarly, researchers in Europe and other countries have documented these foodborne pathogens in wild boar populations in Spain, Sweden, Switzerland, Portugal, Italy, Japan, and Australia (Table 1). Less is known about the role of javelina in the spread of enteric foodborne pathogens, but there is evidence that javelina may be susceptible to clinical salmonellosis (Shender et al. 2009). In

Table 1. Examples of prevalence surveys among free-ranging wild boar, feral swine, and javelina populations.

Common name	Country	No. tested	No. positive (%)	Reference
<i>Campylobacter</i> spp.				
Wild boar	Sweden	66	8 (12%)	Wahlstrom et al. 2003
Feral swine	USA (CA)	30	12 (30%)	Jay-Russell et al. 2012
Wild boar	Spain	287	188 (65.5%)	Diaz-Sanchez et al. 2010
Wild boar	Japan	121	53 (43.8%)	Sasaki et al. 2013
Wild boar	Spain	126	49 (38.9%)	Carbonero et al. 2014
Wild boar	Spain	150	18 (12.0%)	Navarro-Gonzalez et al. 2014
<i>Escherichia coli</i> O157:H7				
Wild boar	Sweden	68	1 (1.5%)	Wahlstrom et al. 2003
Feral swine	USA (CA)	87	13 (14.9%)	Jay et al. 2007
Wild boar	Spain	212	7 (3.3%)	Sanchez et al. 2010
Wild boar	Spain	262	1 (0.4%)	Mora et al. 2012
Wild boar	Spain	301	0 (0.0%)	Diaz-Sanchez et al. 2013
Wild boar	Japan	121	0 (0.0%)	Sasaki et al. 2013
Wild boar	Spain	117	4 (3.4%)	Navarro-Gonzalez et al. 2015
Feral swine	USA (CA)	240	12 (5.0%)	Gordus, unpubl.
Feral swine	USA (CA)	306	11 (3.6%)	Jay-Russell, unpubl.
Feral swine	USA (AZ, NM)	53	1 (1.9%)	This study
Javelina	USA (AZ)	13	0 (0.0%)	This study
<i>Salmonella</i> spp.				
Wild boar	Sweden	66	0 (0.0%)	Wahlstrom et al. 2003
Wild boar	Australia	217	3 (1.4%)	Eglezos et al. 2008
Javelina	USA (AZ)	34	16 (47.1%)	Shender et al. 2009
Wild boar	Switzerland	153	19 (12.4%)	Wacheck et al. 2010
Feral swine	USA (NC)	161	8 (5.0%)	Thakur et al. 2011
Feral swine	USA (CA)	41	10 (2.4%)	Gorski et al. 2011
Wild boar	Portugal	77	17 (22.1%)	Vieira-Pinto et al. 2011
Feral swine	Australia	543	223 (41.1%)	Cowled et al. 2012
Wild boar	Japan	121	9 (7.4%)	Sasaki et al. 2013
Wild boar	Spain	333	4 (1.2%)	Diaz-Sanchez et al. 2013
Wild boar	Italy	1313	326 (24.8%)	Chiari et al. 2013
Wild boar	Italy	499	54 (10.8%)	Zottola et al. 2013
Feral swine	USA (AZ, NM)	53	15 (28.3%)	This study
Javelina	USA (AZ)	13	2 (15.4%)	This study

Brazil, captive peccaries were PCR positive for *Salmonella*, but free-ranging animals were negative (Real 2010). Leptospirosis and rabies are other zoonoses that have been documented in javelina and could represent a public health risk for hunters (CDC 1986, Corn 1987).

To better understand zoonotic risks from feral swine and javelina in the U.S. southwestern desert, we conducted a prevalence survey of STEC and *Salmonella* occurrence in colonic-fecal samples from animals collected

through hunter-harvest or depredation. The study was initiated after lettuce and spinach growers in a major produce production region of southern Arizona (Yuma) reported an increasing number of javelina intrusions into their vegetable fields resulting in crop loss and food safety concerns (Figure 1). Because of the extreme heat and lack of water in southern Arizona, feral swine are usually found in other areas of the southwest.

METHODS

Sample Collection

U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Wildlife Services' employees in Arizona and New Mexico collected colon and fecal samples from feral swine harvested during routine activities. University of California, Davis provided them with kits and instructions for collecting the samples. Wildlife Services staff were instructed to tie off a 6-inch area of the colon with sterilized string. They then removed that section of the colon, keeping the contents intact, and placed it in a sterile zip-lock bag sealed in a second clean bag. Samples were shipped overnight to UC Davis in a cooler with ice at 4°C (not frozen) and processed within 48 hours of sample collection.

Javelina samples were collected during the 2012 hunting season in Arizona. Sampling kits were shipped directly to hunters on request, or available for pick-up at Arizona Game and Fish Department regional offices. Hunters were instructed to collect and ship samples as described above.

Laboratory Methods

Ten grams of feces were placed into tryptic soy broth (TSB) (BD Diagnostics, Sparks, MD) for simultaneous STEC and *Salmonella* pre-enrichment. For detection of STEC, immunomagnetic separation (IMS) using Dynal beads (Invitrogen, Grand Island, NY) was performed on TSB enrichment broths with the automated Dynal BeadRetriever (Invitrogen) followed by selective plating as described previously (Cooley et al. 2013). Up to 6 suspect colonies per positive plate were characterized by PCR (*stx1*, *stx2*, *eaeA*, *hlyA*, *fliC* and *rfbE*) and confirmed isolates were stored on a cryogenic media (TSB with 15% glycerol, Fisher Scientific, Pittsburgh, PA) at -80°C. Selected isolates were submitted to the Pennsylvania State University *E. coli* Reference Laboratory, University Park, PA, for O-serotyping.

Figure 1. Javelina (*Pecari tajacu*) foraging on lettuce in southern Arizona.

Table 2. Detection of Shiga toxin-producing *Escherichia coli* (STEC) and *Salmonella* spp. in javelina and feral swine colonic-fecal samples collected in Arizona and New Mexico, 2012-2014.

Species	Total Tested	STEC O157	Non-O157 STEC	<i>Salmonella</i>
ARIZONA				
<i>Pecari tajacu</i>	13	0 (0%)	1 (7.7%)	2 (15.4%)
<i>Sus scrofa</i>	34	0 (0%)	3 (8.8%)	7 (20.6%)
NEW MEXICO				
<i>Sus scrofa</i>	19	1 (5.3%)	3 (15.8%)	8 (42.1%)

To detect *Salmonella*, TSB enrichment broth was added to Rappaport-Vassiliadis (RVS) (BD Diagnostics, Sparks, MD) followed by selective plating as described previously (Gorski et al. 2011). Up to 6 suspect colonies per positive plate were confirmed by PCR (*invA*) and stored on a cryogenic media at -80°C. Selected isolates were submitted to the USDA APHIS National Veterinary Services Reference Laboratory, Ames, IA, for serotyping.

RESULTS AND DISCUSSION

Overall, a total of 13 javelina and 53 feral swine (34 from Arizona and 19 from New Mexico) colonic-fecal samples were collected (Table 2). STEC O157:H7, the serotype of most public health significance, was isolated from 1 (5.3%) feral swine sample collected in New Mexico. Isolates belonging to non-O157 STEC serotypes were detected in both javelina and feral swine samples in Arizona and New Mexico (Table 2). Three feral swine were positive for non-O157 STEC serotypes (O103, O111) considered among the “top six” serogroups associated with human disease as defined by the Centers for Disease Control and Prevention (CDC 2006). All STEC isolates encoded 2 or more virulence factor genes suggesting potential human clinical importance (Table 3).

Salmonella spp. was cultured from 2/13 (15.4%) javelina and 7/34 (20.6%) feral swine samples collected in Arizona, and 8/19 (42.1%) feral swine collected in New

Mexico (Table 2). There were 12 different *Salmonella* serovars identified among 20 isolates analyzed (Table 4). Feral swine #18985 collected in August 2013 in New Mexico was dually infected with STEC O157 and *Salmonella* (*S. Oranienburg* and *S. Poona*).

The findings are comparable with previous studies documenting feral swine and wild boar as reservoirs of STEC and *Salmonella* (Table 1). In the Central California Coast, another major produce production region, STEC O157:H7 was found in ~4-15% of fecal samples in 3 separate studies as shown in Table 1 (Jay et al. 2007, Gordus et al. unpubl. data, Jay-Russell et al. unpubl. data).

In contrast, *Salmonella* was detected at much lower rates in feral swine in other parts of the country compared with our survey in the southwestern desert (Thakur et al. 2011, Gorski et al. 2011). However, a study of *Salmonella* prevalence in coyotes during 2010-2011 in southern Arizona and southern California revealed ~30% positive fecal samples collected near produce fields (Jay-Russell et al. 2014). Of note, differences in prevalence may also be attributed to variations in sample collection and culture methodologies. Interestingly, Shender et al. (2009) investigated a putative outbreak of salmonellosis in 2004-2005 involving an estimated 105 javelina deaths in Tucson, AZ. Multiple serovars were isolated from necropsy tissues, including *S. Oranienburg* from small and large intestinal samples tested during their investigation. We also isolated *S. Oranienburg* from a javelina sample collected in Yuma County in 2012, but were not aware of any gastroenteritis-like illnesses or deaths in the javelina population at that time.

Limitations of this study include a relatively small sample size and lack of detailed ecological information including proximity to other wildlife and domestic animals. Future studies should assess temporal and spatial risk factors for foodborne pathogen incidence in free-ranging javelina and feral swine populations, the potential influence of livestock-wildlife interactions, and the effect of human interventions to mitigate damage from these species.

Table 3. Shiga toxin-producing *Escherichia coli* (STEC) isolates cultured from free-ranging javelina (*Pecari tajacu*) and feral swine (*Sus scrofa*) in the southwestern desert, 2012-2014.

Species	Field Animal ID	Sampling Date	State	County	O Serogroup	Virulence Factors			
						<i>stx1</i>	<i>stx2</i>	<i>eaeA</i>	<i>hlyA</i>
<i>Pecari tajacu</i>	4-1	02/24/12	AZ	Pinal	136	+	-	+	+
<i>Sus scrofa</i>	PN07	05/30/13	NM	San Miguel	111	+	-	+	+
<i>Sus scrofa</i>	PN08	05/30/13	NM	San Miguel	136	+	-	+	+
<i>Sus scrofa</i>	61113	06/11/13	NM	San Miguel	103	+	-	+	+
<i>Sus scrofa</i>	18985	08/14/13	NM	Otero	157	-	+	+	+
<i>Sus scrofa</i>	46	01/16/14	AZ	Mohave	103	+	+	+	+
<i>Sus scrofa</i>	61	04/15/14	AZ	Mohave	22	+	-	-	+

Table 4. *Salmonella* isolates cultured from free-ranging javelina (*Pecari tajacu*) and feral swine (*Sus scrofa*) in the southwestern desert, 2012-2014.

Species	Field Animal ID	Sampling Date	State	County	Serovars
<i>Pecari tajacu</i>	5-1	02/25/12	AZ	Gila	Aba
<i>Pecari tajacu</i>	9-1	09/16/12	AZ	Yuma	San Diego, Oranienberg*
<i>Sus scrofa</i>	P001	04/04/13	AZ	Mohave	Typhimurium
<i>Sus scrofa</i>	P002	04/05/13	AZ	Mohave	Typhimurium
<i>Sus scrofa</i>	PN08	05/30/13	NM	San Miguel	3,10:e,h:-
<i>Sus scrofa</i>	PN13	06/13/13	NM	Otero-Mescalero	Newport
<i>Sus scrofa</i>	PN14	06/13/13	NM	Otero-Mescalero	III_61:l,v:1,5,7
<i>Sus scrofa</i>	18985	08/14/13	NM	Otero	Oranienburg, Poona*
<i>Sus scrofa</i>	18923	08/28/13	NM	San Miguel	Bonn, Oranienburg*
<i>Sus scrofa</i>	PN25	09/13/13	NM	Quay	Muenchen
<i>Sus scrofa</i>	18934	11/23/13	NM	San Miguel	Rough O
<i>Sus scrofa</i>	20087	01/08/14	NM	Otero	Muenchen
<i>Sus scrofa</i>	56	01/22/14	AZ	Mohave	Houston
<i>Sus scrofa</i>	3004	03/16/14	AZ	Mohave	Typhimurium
<i>Sus scrofa</i>	3010	03/19/14	AZ	Mohave	Newport

* multiple serovars from individual sample

CONCLUSIONS

Javelina and feral swine populations may serve as reservoirs of STEC and *Salmonella* in the southwestern desert, and therefore pose a public health risk due to direct contact with fecal material, ingestion of undercooked game meat, or indirect transmission to raw vegetable crops or irrigation water. The findings underscore the importance of continued public health education efforts to protect hunters from exposure to these pathogens when handling game animals. Fresh fruit and vegetable growers should continue to prevent intrusions by wild and feral animals in the produce production environment to minimize transport of fecal-borne pathogens to crops intended for human consumption (Jay and Wiscomb 2008, Massei et al. 2011).

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