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SALMONELLOSIS IN A FREE-RANGING POPULATION OF JAVELINAS (*PECARI TAJACU*) IN SOUTH CENTRAL ARIZONA

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ABSTRACT: The javelina, or collared peccary (*Pecari tajacu*), is indigenous to Arizona, New Mexico, and Texas in the United States and ranges throughout Latin America. From June 2004 to April 2005, an estimated 105 javelinas died in a mortality event that occurred in Tucson, Arizona, and neighboring areas. Clinical signs observed in sick animals included emaciation, dehydration, lethargy, and diarrhea. In addition, some animals showed labored breathing and hind limb weakness. We necropsied 34 animals, and enteritis was the most frequent clinical sign, followed by colitis, pulmonary congestion, and pneumonia. The only consistent findings were isolations of *Clostridium perfringens* type A and multiple *Salmonella* serotypes. Although it is likely that these javelinas ultimately succumbed to salmonellosis, it is unclear whether other unidentified underlying factors were involved. This is the first reported case of widespread salmonellosis in free-ranging javelinas.

Key words: *Clostridium perfringens*, collared peccary, enteritis, javelina, *Pecari tajacu*, *Salmonella* spp., *Tayassu tajacu*.

INTRODUCTION

Nutrition, physiology, and reproduction of javelinas (*Pecari tajacu*) have been studied previously, but little information exists regarding diseases. Moreover, most information regarding diseases has been derived from captive animals or via serologic surveys conducted on clinically normal animals harvested during hunting seasons (Lochmiller et al., 1985; Crandell et al., 1986; Corn et al., 1987; Lord and Lord, 1991; Gruver and Guthrie, 1996; Sowls, 1997), rather than from clinically ill free-ranging populations. To date, only one disease epizootic has been reported in free-ranging javelinas. In 1989, there was an outbreak of an encephalitic disease in a southern Arizona javelina population characterized by ataxia, blindness, and myoclonus (Appel et al., 1991). A canine distemper-like virus (CDV) was isolated from the brains of three clinically affected animals. Microscopic lesions were consistent with CDV and included prominent eosinophilic intracytoplasmic neuronal inclusion bodies, neuronal necrosis, mild perivascular cuffing by mononuclear cells, and astrocytosis. In a subsequent serologic survey, over a 4-yr period (1993–1996), 58% of 364 serum samples collected from apparently healthy hunter-killed javelinas were antibody positive for CDV (Noon et al., 2003). The authors concluded that CDV was probably enzootic in southern Arizona javelina populations and that recovery postexposure was common. To our knowledge, the mortality event reported in this investigation is the only other disease outbreak studied in free-ranging javelinas. We describe a mortality event in javelinas that occurred from June 2004 to April 2005 in the greater area of Tucson, Arizona (31°47.5′–32°22.5′N, 110°40.0′–111°12.5′W).

MATERIALS AND METHODS

Local community residents reported clinically ill animals to either the Tucson Arizona Game and Fish Department (AZGFD) urban wildlife specialist or the Tucson Wildlife Center (TWC), one of the few centers licensed by the AZGFD to rehabilitate javelinas. We obtained information on the location of sick javelinas from these two sources, as well as from hunters or employees of golf courses or

state parks. Initially, animals admitted to TWC received treatment, including intravenous fluids and a variety of antibiotics. Treatment protocols for individual cases varied, and wildlife rehabilitation center medical records were not made available for all cases. In early December 2004, TWC personnel contacted the Tucson AZGFD for assistance in determining the cause of death for javelinas in the area. The AZGFD requested that the TWC euthanize ill javelinas without antibiotic treatment so that the drugs would not compromise diagnostic assays after submission of the animals to a veterinary diagnostic laboratory.

All reports of ill or dead javelinas within the greater Tucson area were included in morbidity and mortality counts. When possible, animals were necropsied and aged based upon dentition (Heffelfinger, 1997). Tissue samples from these animals were analyzed by the Arizona Veterinary Diagnostic Laboratory (AZVDL; Tucson, Arizona, USA) and the Colorado State University Veterinary Diagnostic Laboratory (CSUDL; Fort Collins, Colorado, USA) for a variety of laboratory tests.

Diagnostic assays performed at the AZVDL were the following: aerobic and anaerobic cultures, rotavirus via latex agglutination (Remel, Lenexa, Kansas, USA), coronavirus via enzyme-linked immunoabsorbent assay (Coronavirus Test Kit, Syracuse Bioanalytical, Ithaca, New York, USA), *Cryptosporidium* and *Giardia* via fluorescent antibody testing (FA; Merifluor, Meridean Bioscience, Cincinnati, Ohio, USA), canine distemper virus via polymerase chain reaction (PCR; DNeasy Blood and Tissue Kit, QIAGEN, Valencia, California, USA), and influenza via PCR (DNeasy Blood and Tissue Kit). To test animals for *Lawsonia intracellularis*, the AZVDL referred cases to Iowa State University Veterinary Diagnostic Laboratory (ISUVDL; Ames, Iowa, USA), where fecal DNA was extracted and PCR testing was performed following standard procedures (Lindecrona et al., 2002). Cases also were referred for carbamate and organophosphate toxicologic screens ($n=2$; California Animal Food Health and Safety Laboratory [CAHFS], Davis, California, USA), ionophores ($n=1$; Texas Veterinary Medical Diagnostic Laboratory, College Station, Texas, USA), heavy metals ($n=2$; arsenic, cadmium, copper, iron, lead, manganese, mercury, molybdenum, and zinc; CAHFS and Michigan State University, Lansing, Michigan, USA), and porcine reproductive and respiratory syndrome ($n=1$; virus isolation followed by hemagglutination; National Veterinary Services Laboratory [NVSL], Ames, Iowa, USA). In addition, *C. perfringens* cultures were referred to the University of Arizona (Songer Lab, Clostridial Enteric Disease Unit, Veterinary Science and Microbiology Department, Tucson, Arizona, USA) for genotyping following standard techniques (Meer and Songer, 1997).

Histopathology and routine aerobic (including *Campylobacter*) and anaerobic cultures were performed at CSUDL along with electron microscopy of fecal material to detect coronavirus (FA; VMRD, Inc., Pullman, Washington, USA) and rotavirus (FA; VMRD, Inc. and Rotavirus Latex Detection Kit, Wampole Laboratories, Two Research Way, Princeton, New Jersey, USA). Serum neutralization for pseudorabies virus and canine distemper virus also were performed as follows. Sera were complement-deactivated at 56 C for 30 min and tested for serum-neutralizing antibodies to canine distemper virus (CDV-Ondersteporrt) and pseudorabies virus (PRV-Aujesky's, National Veterinary Services Laboratory, Ames, Iowa, USA). Two-fold serial dilutions of serum were made in triplicate wells in 96-well microtiter plates. One hundred median tissue culture infective dose of CDV in 50 µl or PRV in 25 µl were added to duplicate columns of wells, and the plates were incubated for 1 hr at 37 C. The third column of diluted serum served as the serum control. Vero cells or PK-15 cells (1×10^4 cells/well) were added, respectively, and the plates incubated at 37 C. After 3 days, the Vero cells or after 2 days the PK-15 cells were examined for cytopathic effect by using an inverted light microscope. Serum-neutralizing antibody titers for each serum sample were the reciprocal of the highest dilution at which each respective virus was completely neutralized (Carbrey et al., 1971)

Culture of *Salmonella* was performed at both CSUDL and AZVDL by using the following methods. Culture media for *Salmonella* were obtained from Hardy Diagnostics (Santa Maria, California, USA). Tissues and fecal samples were first inoculated onto both sheep blood agar (SBA) and Tergitol 7 agar (T7) culture media and placed in a 37 C incubator for 12–24 hr. Tetrathionate broth with the addition of an iodine-potassium iodide solution also was used for the selective enrichment of *Salmonella* spp. After the broth was inoculated with the sample and the iodine-iodide mixture was added, it was also placed in a 37 C incubator. After 12–24 hr the tetrathionate broth was subcultured on Brilliant Green (BG) agar and then placed into a 37 C incubator for another 12–24 hr. The BG plates were removed, and pink colonies were identified as probable *Salmonella*. Suspect colonies from SBA, T7, and BG were inoculated into

Triple sugar iron agar, urea agar slant, Simmons citrate, and Motility Indole Ornithine agar to further identify *Salmonella* spp. suspects. Any preliminary positive *Salmonella* isolates were tested using *Salmonella* spp. antisera followed by group specific *Salmonella* O antisera (BBL, Sparks, Maryland, USA). *Salmonella* isolates were forwarded to the NVSL for serotyping, and several isolates were also sent to the Arizona Department of Health Services (ADHS; Phoenix, Arizona, USA) for pulsed-field gel electrophoresis (PFGE).

Diagnostic assays varied by case according to the animals' age and gross lesions, previous diagnostic results, and financial constraints. For example, juvenile javelinas were initially tested for rotavirus and coronavirus, but when results were repeatedly negative, these assays were discontinued. Of the two animals that were tested for *L. intracellularis*, histology of one animal (case 26) revealed proliferative enteritis, but the second animal (case 33) did not have any intestinal proliferative lesions.

To investigate the normal flora and further elucidate the etiologies of this disease condition, tissues and fecal samples were collected and analyzed from nonclinical animals for comparison against those from clinically ill animals. We collected fresh liver, lung, and intestinal samples from five nonclinical hunter-killed animals during a hunting season on 19–22 February 2005 in Payson, Arizona, USA (34°15′24″N, 111°20′21″W). These samples were collected opportunistically in the field from hunters by local wildlife managers and mailed overnight to CSUDL. In addition, we obtained samples from a nuisance javelina near Tucson.

On 15 December 2004, to prevent the possible spread of the yet undiagnosed disease, the Tucson AZGFD Regional Office placed a moratorium on the relocation of javelinas, requiring their euthanasia if they would otherwise have been relocated. Therefore, this provided us the opportunity to collect samples from apparently healthy javelinas euthanized by the AZGFD, in addition to the clinically ill animals.

Nonclinical javelinas were live captured on the outskirts of Tucson on 29 March 2005, with the purpose of collecting and culturing fecal samples for *Salmonella* and *Clostridium* spp. We baited the animals with fruits and vegetables from discarded produce (predominately melons), and a distant observer released the trap door rope once the javelinas had entered the trap. We collected fecal samples from the ground after the javelinas had been held in the trap overnight; therefore, fecal samples might not have represented all animals.

To evaluate potential environmental sources of *Salmonella*, we sampled water and soil from areas where javelinas had died or were commonly observed. We collected soil with a plastic spoon at the soil surface to approximately 2 cm depth at known javelina bedding sites. Approximately 10 g of soil was submitted to CSUDL for *Salmonella* isolation. One gram of the soil was placed in buffered peptone water, which was incubated over night at 37 C; 1 ml of this solution was then inoculated into tetrathionate with 2 drops of iodine and incubated overnight at 42 C. The next day, this solution was cultured by routine methods as described above. We collected water from ornamental ponds, birdbaths, and shallow water troughs, with the greatest depth not exceeding 20 cm by submerging a 50-ml Falcon tube (Thermo Fisher Scientific, Waltham, Massachusetts, USA) approximately 10 cm below the surface at the edge of the water source (the location from where javelinas would be most likely to drink), and filling the tube completely. One milliliter of water was inoculated into tetrathionate with 2 drops of iodine and incubated overnight at 42 C. The solution was cultured by routine methods for *Salmonella* spp. after 24 hr.

RESULTS

During our study period, we received and confirmed public reports of 105 moribund and dead javelinas, as well as an additional 12 javelinas that were reported to be clinically ill but could not be located by AZGFD personnel. Several animals had been dead for many days, and if diarrhea was not detected on site, it was difficult to discern whether these animals died because of an infectious disease associated with this mortality event. Of the 105 javelinas that died, 34 were necropsied (14 males, 17 females, and three sex not recorded), and 20 javelinas were admitted to the TWC before necropsy. Half of the necropsy cases ($n=17$) were submitted by AZGFD wildlife managers or by the TWC directly to the AZVDL for necropsy. The other half were necropsied by trained AZGFD and TWC personnel. Ages ranged from 1 wk to 4–6 yr. Animals were grouped into three age classes: ≤3 mo ($n=4$), juveniles (>3 mo and <1 yr; $n=10$), and adults (≥1 yr;

TABLE 1. Histologic and microbiologic necropsy (Nx) results from 34 javelinas (*Pecari tajacu*) examined from June 2004 through April 2005 at the Arizona Veterinary Diagnostic Lab (AZ) and Colorado State University Veterinary Diagnostic Lab (CO).

Case no.	Accession no.	Lab	Nx date	Location	Age[a]	Sex	Diar[b]	Histo[c]	Cultures[d]			Other tests[e]
									Salm	Clost	E. coli	
1	04-3320	AZ	1 June 2004	Benson	Juv	M	Y	WNL	N	ND	N	CV, RV
2	04-4816	AZ	22 July 2004	Tucson	Juv	F	N	E	N	ND	N	
3	04-4869	AZ	23 July 2004	Tucson	Juv	F	Y	C, P	P	ND	N	CY, GI
4	04-5500	AZ	13 August 2004	Tucson	≤3 mo	Unk	Y	E	P	ND	P	CV, RV, CY, GI
5	04-6012	AZ	30 August 2004	Tucson	Juv	F	Y	WNL	N	ND	P	CDV
6	04-6013	AZ	30 August 2004	Tucson	Ad	M	N	WNL	N	ND	N	CDV
7	04-6557	AZ	16 September 2004	Marana	Ad	F	Y	E, PC	N	ND	N	
8	04-6914	AZ	29 September 2004	Marana	Juv	Unk	N	E, P	N	ND	N	CY, GI, CDV
9	04-6915	AZ	29 September 2004	Marana	Ad	M	N	WNL	P	ND	N	
10	04-6916	AZ	29 September 2004	Marana	Ad	F	Y	C	P	ND	N	
11	04-7006	AZ	1 October 2004	Tucson	Ad	M	Y	E	N	ND	N	
12	04-7631	AZ	26 October 2004	Unk	≤3 mo	M	N	E, PC	N	P	N	
13	04-8216	AZ	22 November 2004	Tucson	Unk	Unk	N	WNL	N	ND	N	Tox
14	04-8333	AZ	29 November 2004	Tucson	Unk	F	N	WNL	N	ND	N	CDV, Tox
15	045-35698[f]	CO	10 December 2004	Tucson	Ad	F	Y	E, C, PC	P	P	N	CDV, EM, PR
16	045-35698[f]	CO	10 December 2004	Tucson	Ad	F	Y	E, PC	P	N	N	CDV, EM, PR
17	045-R04641[g]	CO	7 December 2004	Tucson	Ad	M	Y	WNL	N	P	N	CV, RV
18	045-R04641[g]	CO	21 December 2004	Tucson	Juv	M	Y	E, C, PC	N	N	N	CV, RV
19	045-R04641[g]	CO	30 December 2004	Tucson	Ad	M	n/a	PC	ND	ND	ND	
20	045-R04641[g]	CO	30 December 2004	Tucson	Juv	M	Y	PC, P	N	P	N	
21	045-R04641[g]	CO	30 December 2004	Tucson	≤3 mo	M	Y	PC	N	N	N	CV, RV
22	045-R04725	CO	4 January 2005	Tucson	Juv	M	N	C, PC, P	N	P	P	CV, RV
23	045-R05456	CO	9 February 2005	Tucson	≤3 mo	F	Y	P	P	N	P	CV, RV, CM, CY, GI
24	045-R05508	CO	10 February 2005	Tucson	Ad	F	Y	E, PC	P	P	P	CV, RV, CM, CY
25	045-47834	CO	7 February 2005	Tucson	Juv	M	Y	E, C, PC	P	P	P	
26	05-1019	AZ	21 February 2005	Unk	Ad	F	Y	E	P	N	P	LI, Tox
27	045-49964	CO	24 February 2005	Tucson	Ad	M	Y	E, C	P	N	N	
28	045-50661	CO	24 February 2005	Tucson	Unk	F	N	PC	P	P	N	
29	045-56421	CO	22 March 2005	Sahuarita	Juv	M	Y	E	P	P	N	
30	05-1859	AZ	25 March 2005	Sahuarita	Ad	F	N	WNL	N	P	N	PRRS, IV
31	045-60322	CO	4 April 2005	Sahuarita	Juv	F	N	C	P	N	N	VI

TABLE 1. Continued.

Case no.	Accession no.	Lab	Nx date	Location	Age[a]	Sex	Diar[b]	Histo[c]	Cultures[d]			Other tests[e]
									Salm	Clost	E. coli	
32	045-60321	CO	4 April 2005	Sahuarita	Ad	F	Y	E, C	P	N	N	
33	05-2133	AZ	7 April 2005	Sahuarita	Ad	F	Y	WNL	P	P	P	LI, Tox
34	045-62775	CO	13 April 2005	Sahuarita	Ad	F	N	PC	N	P	N	

[a] Age class, where Juv = juvenile = <1 yr; Ad = adult = >1 yr; Unk = unknown.

[b] Diarrhea, where Y = yes and N = no.

[c] Histology results, where E = enteritis; C = colitis; PC = pulmonary congestion; P = pneumonia; WNL = within normal limits.

[d] Microbiologic cultures, where Salm = *Salmonella* spp. isolated; Clos = *Clostridium* spp.; P = positive; N = negative; ND = no cultures done.

[e] Other tests that were performed and were negative, where CV = Coronavirus; RV = Rotavirus; PR = Pseudorabies; CDV = distemper virus; CM = *Campylobacter*; CY = *Cryptosporidium*; GI = *Giardia*; LI = *Lawsonia intracellularis*; IV = influenza virus; PRRS = porcine reproductive and respiratory syndrome; EM = electron microscopy for virus identification; Tox = multiple toxicology diagnostics, detailed by case number in text.

[f] These cases were submitted together and therefore have the same accession number.

[g] These cases were submitted together and therefore have the same accession number.

$n=17$) (Table 1). Age was not reported for three animals.

Pathology

Twenty-one of 34 animals (62%) had evidence of diarrhea based on antemortem observations, watery colonic contents, and liquid feces on their perineum (Table 1). Of these 21 cases, 14 (67%) had enteritis ($n=8$), colitis ($n=2$), or both ($n=4$) upon histologic examination. Of the remaining 13 animals without diarrhea upon gross examination, two had histologic lesions of enteritis and two of colitis (Table 1). Enteritis was characterized by an increase in lymphocytes, plasma cells, and macrophages within the lamina propria of the small intestine (Fig. 1). Small intestinal crypts often had hyperplastic epithelium, were dilated, and contained neutrophils. In several cases, the intestinal villi were shortened or necrotic and covered by a fibrinous pseudomembrane containing fibrin, degenerate inflammatory cells, and a mixed population of bacteria. Vessels and lymphatics in the lamina propria occasionally contained fibrin thrombi and inflammatory cells. Segments of small intestine in a few cases had extensive hemorrhage in the mucosa and active lymphoid follicles.

Colitis was characterized by edema within the mucosa with increased numbers of lymphocytes, plasma cells, and neutrophils in the lamina propria. Similar to small intestine, crypts were dilated and contained cellular debris, mucus, and/or neutrophils. In one animal (case 15), sections of large intestine had a necrotizing colitis with small crypt abscesses containing numerous bacteria surrounded by inflammation with hemorrhage present within the lamina propria.

Four animals (cases 2, 26, 29, and 32) had proliferative enteritis, with crypt distention, hyperplasia of the deeper portions of the crypts, and increased numbers of mononuclear inflammatory cells in the deeper portions of the lamina propria. Mitotic figures were common

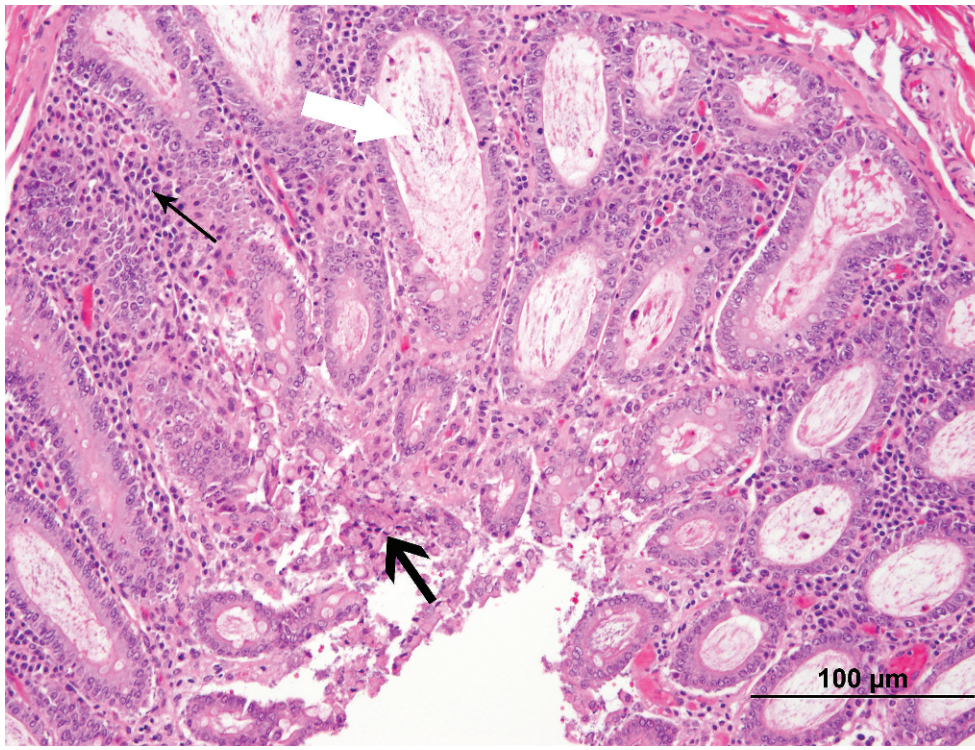

FIGURE 1. Photomicrograph of large intestine of javelina (*Pecari tajacu*) with enteritis. Changes include dilated crypts containing excess mucus (thick white arrow), superficial mucosal erosion (thick black arrow), and an infiltration of inflammatory cells between the crypts and above the submucosa (thin black arrow). H&E stain.

within the depths of the crypts. The lesions in these javelinas were compatible with proliferative enteritis of domestic swine. In one of the four cases (case 32), there was a large crypt within a lymphoid follicle impacted with neutrophils and exudate forming a cyst, similar to diverticulitis in primates and humans. Two cases (26 and 33) were tested for *L. intracellularis* and were negative.

Pneumonia was found in five of 34 animals (15%) and was characterized by fibrin, inflammatory cells, macrophages, and syncytial cells in the alveoli. One animal (case 22) admitted to TWC was placed on an oxygen mask and had copious amounts of blood oozing from its mouth and nose. Upon necropsy, the lungs of this animal were severely congested and edematous. On histologic examination the pulmonary lesions were consistent of a severe fibrinopurulent pneumonitis, possibly caused by a bacteremia secondary to the necrotizing colitis in this animal. Thirteen animals had histologic lesions of pulmonary congestion and edema, but they lacked inflammatory changes consistent with pneumonia.

Toxicology

Tissues were negative for the following toxins: carbamates and organophosphates in liver and stomach content (cases 13 and 14), and ethylene glycol and strychnine in stomach content (case 13). Heavy metal screens were performed on liver tissues from cases 13 and 33. All metals were within normal limits compared with standard porcine values (Puls, 1988). Ionophore values (laidlomycin, lasalocid, monensin, narasin, and salinomycin) in case 13 ranged from 3 ppm to 7 ppm and can

TABLE 2. Serotypes and sources of javelina (*Pecari tajacu*) and environmental *Salmonella* spp.

Case[a]	Source[b]	Subspecies[c]	Serogroup	Serotypes[d]
3	LU	*enterica* (I)	D	NT
4	SI	unknown	No group	NT
9	LI, LIV	unknown	No group	NT
10	LI, LIV	*arizonae* (IIIa)	No group	NT
15	SI	*enterica* (I)	E	Anatum
16	SI	*enterica* (I)	E	Anatum
23	LI, SI	*enterica* (I)	C2	Muenchen
24	LI, SI	*enterica* (I)	C2	Muenchen
25	LI, LU, SI	*enterica* (I)	D1	Panama
		arizonae (IIIa)	No group	IIIa 44z4, z32: -
26[e]	LI, LIV, SI, SP	unknown	No group	NT
27	SI	*enterica* (I)	C1	Montevideo
28	SI	*enterica* (I)	B	Typhimurium
29	LIV, ST	*arizonae* (IIIa)	No group	IIIa 18:g, z51: -
31	LI, SI	*enterica* (I)	C1	Oranienburg
32	LI, SI	*enterica* (I)	C1	Oranienburg
33	LIV, SI, SP	*enterica* (I)	C	NT
Env1[f]	Soil	*enterica* (I)	E	Anatum
Env2[g]	Soil	*enterica* (I)	D1	Panama
Env3[h]	Fountain water	*enterica* (I)	D1	Panama

[a] The final three samples in this column were isolates from environmental sources at private residences.

[b] Source from which *Salmonella* was isolated, where LIV = liver; LU = lung; SI = small intestine; LI = large intestine; SP = spleen; ST = stomach.

[c] *Salmonella* subspecies (if known) are designated both by names and by Roman numerals.

[d] Isolates were identified by National Veterinary Services Laboratory (NVSL), Ames, Iowa, USA; Isolates designated as NT were not serotyped at NVSL.

[e] Serotyping of this isolate was unsuccessful due to *Proteus* overgrowth at NVSL.

[f] Soil source was the javelina wallow associated with cases 15 and 16.

[g] No javelinas were necropsied from this site; this site was not geographically approximate to case 25 (other Panama isolate).

[h] No javelinas were necropsied from this site; case 14 was possibly from this vicinity but no *Salmonella* was isolated in this animal.

be considered negligible as compared to feed levels reported to cause toxicity in domestic swine (Plumlee et al., 1995). Pathologic lesions (i.e., myocardial and skeletal muscle damage) typically associated with ionophore toxicity in swine (Van Vleet et al., 1983) were not observed in this animal.

Microbiology

Electron microscopy scans for viral agents performed in two animals were negative. Pseudorabies ($n=2$), canine distemper virus ($n=6$), porcine reproductive and respiratory syndrome ($n=1$), influenza ($n=1$), rotavirus ($n=8$), and coronavirus ($n=8$) were not detected. Animals also were negative for *Cryptosporidium* ($n=5$), *Giardia* ($n=3$), *Campylobacter* ($n=2$), and *L. intracellularis* ($n=2$).

Salmonella spp. were isolated in 16 of 34 (47%) animals necropsied and were isolated in more than one tissue type in 10 animals. *Salmonella* spp. were isolated from the small intestine ($n=12$), large intestine ($n=8$), liver ($n=5$), lung ($n=2$), spleen ($n=2$), and stomach ($n=1$). Serotypes of 14 (67%) of 21 isolates were identified and included *Salmonella* serotype Panama, *Salmonella* serotype Montevideo, *Salmonella* serotype Muenchen, *Salmonella* serotype Oranienburg, *Salmonella* serotype Typhimurium, and *Salmonella* serotype Anatum (Table 2). Nine isolates were submitted for PFGE profiles, and all were distinct with the

FIGURE 2. A group of javelinas (*Pecari tajacu*) in a wallow. Dark staining of soil around wallow is diarrhea.

exception of *Salmonella* Oranienburg and *Salmonella* Panama. The two *Salmonella* Oranienburg isolates were obtained from herd-mates, and the *Salmonella* Panama isolates were from soil and water samples (Table 2). *Clostridium perfringens* was isolated in 12 of 20 animals (60%), and two cases had *C. perfringens* from more than one sample source. *Clostridium perfringens* was found in the small intestine ($n=8$), feces ($n=4$), large intestine ($n=2$), and mesenteric lymph node ($n=1$). In eight cases, *C. perfringens* was genotyped as type A. In eight of the necropsy cases, *Escherichia coli* was isolated from the lung ($n=4$), liver ($n=4$), small intestine ($n=2$), large intestine ($n=1$), and spleen ($n=1$).

Healthy animal samples

Fresh lung, small intestine, and large intestine were submitted for aerobic and anaerobic culture and histopathologic examination from five apparently healthy javelinas that were killed by hunters near Payson, Arizona, USA, in mid-February. All tissues were histologically normal, *C. perfringens* was not isolated in any animals, and *Salmonella* sp. was isolated from the small intestine of only one; this isolate was mistakenly not serotyped. Four fecal samples were collected from nonclinical live-trapped javelinas. *Salmonella* spp. were not isolated from any of these samples; however, *C. perfringens* type A was isolated from two. Necropsy of a euthanized apparently healthy javelina revealed no histologic lesions, and neither *Clostridium* sp. nor *Salmonella* spp. was isolated. Isolations of *E. coli* were made from the liver and lung of this animal.

Environmental sampling

In March 2005, we collected 14 water samples and eight soil samples from nine residences. *Salmonella* serotype Panama was isolated from water collected from a birdbath and soil collected from a javelina bedding area. S*almonella* serotype Anatum was isolated from a soil sample from a third residence. This particular sample was taken from a wallow containing dried feces where seven javelinas had died over a 1-wk period in December 2004 (Fig. 2). *Salmonella* serotype Anatum was isolated from javelinas necropsied from this location.

DISCUSSION

Of the 34 necropsy cases, 23 (68%) had *Salmonella* spp., *C. perfringens*, or *E. coli*

isolated, or a combination of these organisms. Of these 23, 14 had enteritis, colitis, or both. This is the first reported outbreak of a diarrheal disease in a free-ranging javelina population, although there are few isolated reports of enteritis in javelinas. Sipos et al. (2003) isolated *Clostridium perfringens* type E from the stomach, small intestine, and large intestine from a javelina with acute enteritis. Disseminated ulcers were seen in the stomach and the colonic mucosa on postmortem examination. The mucosa was covered by a severe diphtheritic membrane and the mesenteric lymph nodes were moderately enlarged (Sipos et al., 2003). Sowls (1997) reported that three captive and two wild javelinas had gross lesions suggestive of "some type of enteritis." *Salmonella enterica* serotype Muenchen was isolated from the two wild animals; one animal had hemorrhagic enteritis and pulmonary congestion, and the other animal had enteritis and an impacted colon. Of the three captive animals, the first had an acute diffuse enteritis, the second a necrotic hemorrhagic enteritis, and the third an acute necrotic enterocolitis with diarrhea.

The findings from our study suggest that many of these animals succumbed to an enteritis induced by *Salmonella* spp. and that in some instances, the organism had probably spread hematogenously to the lungs. In several cases, *C. perfringens* and *E. coli* also may have contributed to the enteritis; however, both of these organisms are commonly found as part of the gastrointestinal flora in apparently healthy animals of many domestic species and therefore their presence may not necessarily be related to disease (Hornitsky et al., 2002; Kalender et al., 2005; Feder et al., 2007). In addition, *C. perfringens* was often isolated in conjunction with *Salmonella* spp. Finally, animals carrying *Salmonella* spp. may shed organisms intermittently (Sanchez et al., 2002); therefore, it is possible that more javelinas in this outbreak may have actually had *Salmonella* spp. than the 47% detected.

One puzzling aspect of this outbreak is that many serotypes of *Salmonella* were isolated, and rarely did any two animals have the same serotype unless they came from the same herd. The javelinas examined in this investigation originated from the urban fringe, and it is possible that some of the *Salmonella* serotypes came from feeding on discarded fruits and vegetables. In some instances, residents purposely discard this material to attract javelinas to their yards. Also, javelinas commonly feed on scattered birdseed from feeders, which is often mixed with bird feces, and could be another potential source of *Salmonella*.

Through the normal rooting behavior of javelinas, constant exposure to *Salmonella* is to be expected. Therefore, we think it is noteworthy that javelinas were potentially succumbing to salmonellosis in this mortality event. Although the histologic lesions indicated involvement of an enteric factor and *Salmonella* was a frequent finding, we think that there may have been some undiagnosed underlying factor(s), either infectious, environmental, or both that caused these javelinas to become more susceptible to salmonellosis. For example, four individuals had lesions consistent with proliferative ileitis as described in domestic swine; however, the primary causative agent, *L. intracellularis*, was not identified.

This study demonstrates the difficulty in diagnosing disease outbreaks in free-ranging wildlife. The lack of knowledge of the normal flora in many wildlife species makes it difficult to determine whether organisms detected are responsible for the clinical symptoms being observed. This point was recently illustrated in a study where 500 fecal samples were examined from white-tailed deer for the presence of *Salmonella*; only five samples were found to be positive (1%), and there were four different *S. enterica* serotypes identified (Renter et al., 2006). In addition, diseased wild animals often die unnoticed and by the time the carcasses are found, they have been partially scavenged and are in

an advanced state of autolysis. In our case, we confirmed public reports of 105 moribund and dead javelinas in the Greater Tucson area during our study period; however, the time lapse between the animals' death and collection of the carcasses in many cases precluded us from performing any diagnostic evaluations. We suspect that the pathogens identified in our necropsied javelinas probably contributed toward the deaths of those javelinas not necropsied; however, we cannot accurately specify the magnitude of this mortality event. Once an outbreak becomes publicized, there is heightened awareness among local citizens; therefore, morbidities and mortalities are more likely to be found and reported in a timely manner, thereby facilitating disease diagnosis. It is therefore crucial for wildlife agencies to have a well-organized public reporting system established.

We recommend that agencies have a pre-established hotline reporting number that can be activated when necessary and a media announcement template available for rapid dissemination by agency public information officers. This announcement should include the hotline number and should be easily modified to specify which animals are affected by the mortality event, the geographic region where the event is occurring, a description of how affected animals may look, and any public health concerns (i.e., zoonotic diseases) if known. In addition, we recommend wildlife agencies have a well-developed contact system with licensed wildlife rehabilitation centers, as well as a simple database to enter public reporting details (e.g., animal location, date, public member contact number, etc.) and agency responses. The above-mentioned suggestions are minimal steps which can be used as part of a plan to promote and expedite public reporting of morbidity/mortality events, hasten wildlife agency responses, facilitate disease diagnosis, and initiate appropriate management actions if applicable.

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