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Zoonotic Risks from Amphibians and Reptiles

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Abstract: Captive amphibians and reptiles are well-documented sources of human salmonellosis through direct contact with contaminated feces or fomites. However, the relative significance of wild cold-blooded vertebrates as hosts/reservoirs of zoonotic enteric pathogens in their natural habitat is not known. Wild amphibian and reptile populations are present in the leafy green produce production environment in the central California coast, and there are reports from growers that frogs and other species sometimes intrude into produce fields. These intrusions may result in destruction of the crop and economic losses, due to food safety and quality concerns. Environmental groups have also noted potential conflicts between conservation efforts and food safety practices that result in removal or damage to aquatic habitat (e.g., farm ponds, adjacent wetland areas). To address these concerns, we conducted a survey of foodborne pathogen prevalence in common amphibian and reptile populations during the 2011 produce growing season in the central California coast. Preliminary results indicate that *Salmonella* prevalence was higher among wild-caught reptile (33%) compared with amphibian (4%) taxa. In contrast, all samples were negative for *E. coli* O157. Wildlife damage management in fresh produce production fields is challenging, due to the diversity of potential hosts/reservoirs of foodborne pathogens and a limited number of mitigation strategies. Professionals working in vertebrate pest control could play an important role in assisting fresh produce growers with implementation of co-management approaches that promote public health and conservation goals in the central coast agricultural landscape.

Key Words: amphibians, California, *E. coli* O157, food safety, reptiles, *Salmonella*, zoonoses

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INTRODUCTION

Non-venomous wild amphibians and reptiles are not generally considered major vertebrate pests (Hygnstrom et al. 1994). In contrast, exotic pets such as turtles, snakes, lizards, and frogs have been linked to numerous human *Salmonella* infections, especially among children (Austin and Wilkins 1998, Mermin et al. 2004). Following unprecedented numbers of turtle-associated salmonellosis cases, the United States instituted a federal ban in 1975 on the sale of pet turtles with shell lengths less than 4 inches (Harris et al. 2010). The ban resulted in a significant reduction in reported salmonellosis outbreaks linked to pet turtles, but multi-state outbreaks re-emerged in 2006 possibly due to the increased popularity of exotic pets (CDC 2008, 2012). Additionally, the Centers for Disease Control and Prevention recently linked 241 *Salmonella typhimurium* cases in 42 states to contact with infected pet African dwarf frogs (*Hymenochirus boettgeri*) from a single breeding facility in Madera County, California (CDC 2011).

There are limited surveys of other zoonotic enteric pathogens such as *E. coli* O157 and *Campylobacter* in cold-blooded vertebrates (Tu et al. 2004, Dipineto et al. 2010). Gray et al. (2007) reported preliminary evidence that American bullfrog (*Rana catesbeiana*) metamorphs can be experimentally infected with *Escherichia coli* O157:H7. Rarely, amphibians and reptiles have been associated with parasitic encephalitis due to accidental exposure to *Angiostrongylus cantonensis* (rat lungworm) following consumption of raw, whole animals (Panackel et al. 2006, Tsai et al. 2011). Snakes and other reptiles may also shed pentostomid eggs and if ingested by humans can lead to visceral larval migrans (Pantchev and Tappe 2011). Amphibians and reptiles do not appear to be significant reservoirs of viral zoonoses but may serve as a vertebrate host for some mosquito-borne arboviral diseases (Ariel 2011, Graham et al. 2012). Table 1 summarizes potential zoonotic diseases that have been associated with amphibians and reptiles.

PRODUCE FOOD SAFETY AND WILDLIFE

The body of knowledge concerning potential wildlife reservoirs of foodborne pathogens is growing, especially for large fauna (deer, elk, feral pigs), smaller mammals (coyotes, rodents), and wild avian and insect (fly) species

Table 1. Examples of potential zoonoses associated with amphibians and reptiles.

Organism		Transmission	Disease	Reference
Bacteria	*Salmonella* spp.	Fecal-oral, ingestion of contaminated food/water, wound infection	Gastroenteritis, invasive infection (septicemia, osteomyelitis, peritonitis, meningitis)	Austin and Wilkins 1998; Mermin et al. 2004
	Campylobacter fetus subsp. *fetus*	Fecal-oral, ingestion of contaminated food/water	Opportunistic gastroenteritis or invasive disease in immunocompromised people	Tu et al. 2004
	E. coli O157	Fecal-oral, ingestion of contaminated food/water	Hemorrhagic colitis, hemolytic uremic syndrome	Dipineto et al. 2010
	Atypical Mycobacterium spp.	Broken skin contact with contaminated aquarium or animal	Localized skin infection ("fish tank granuloma")	Ippen and Swart 1996
Viruses	Arboviruses (Eastern equine encephalitis virus, West Nile virus)	Mosquito-borne (possible amphibian/reptile vertebrate host)	Aseptic meningitis, encephalitis	Ariel 2011; Graham et al. 2012
Parasites	*Angiostrongylus cantonensis* (rat lungworm)	Ingestion of uncooked paratenic host (frog, lizard)	Eosinophilic meningitis, encephalitis	Panackel et al. 2006; Tsai et al. 2011
	Armillifer spp.	Ingestion of eggs found in reptile feces	Pentostomiasis (visceral larval migrans)	Pantchev and Tappe 2011

in pre-harvest contamination of fresh produce (Talley et al. 2009, Jay et al. 2007, Cooley et al. 2007). However, among the major groups of wildlife that may be present in the produce production environment, wild amphibians and reptiles are the least studied. Following a salmonellosis outbreak linked to unpasteurized orange juice from a Florida citrus-processing facility in 1995, investigators isolated *Salmonella enterica* from tree frogs and toads captured outside the processing facility, which suggested these animals could serve as a source of environmental contamination (Parish 1998).

We conducted a survey of *Salmonella* and *E. coli* O157:H7 prevalence in common amphibian and reptile species captured near fresh produce production fields, wetland areas, and surrounding rangeland during the 2011 production period in the central California coast, to better understand the potential zoonotic risk from these animals in the context of produce food safety.

MATERIALS AND METHODS

Private properties with ponds or wetland areas in Monterey and Santa Cruz counties were enrolled confidentially. Field sampling was conducted from March to October 2011. Appropriate permits from wildlife agencies in California were obtained to live-capture and release unlisted species of amphibians and reptiles. The protocol was approved by the UC Davis Institutional Animal Use and Care Committee.

For each farm property enrolled, we first assessed the amphibian and reptile population and their habitat by visual observation. Based on findings from these visual surveys, and to minimize harm to the animals, we decided to use a combination of passive trapping and hand-catching the animals. Coverboards and PVC pipe passive traps were set strategically at measured distances from produce fields, riparian corridors, holding ponds, stock ponds, and wetlands by using a range finder (Nikon Prostaff 550; Nikon Inc., Melville, NY). Coverboards and PVC pipes take advantage of the natural tendency of amphibians and reptiles to seek refuge; unlike active traps, the traps do not need to be checked daily because the animals are free to enter and exit. Coverboards were left at the farms throughout the duration of the study and checked at least monthly. We used hand-grabs and hand-nets to catch terrestrial animals such as snakes, toads, and adult frogs around natural objects. Lizards were caught using a "noose" constructed by connecting fishing line to a solid pole. Aquatic animals such as tadpoles and frogs were also captured by using seine and dip nets during both day and night surveys.

Cloacal swabs were collected and placed in liquid Stuart transport media (Copan Diagnostic Inc., Murrieta, CA). The swabs were inserted in the cloacae and rolled against the inner wall several times. A second swab, "ventral swab," was taken by rolling the swab from the cloaca to the neck. The animal was then placed gently into a sterile stand-up whirlpak bag containing 250-500 ml of phosphate buffered saline (PBS) for 10 minutes and allowed to defecate. Animals too small to be swabbed (<2 cm) were pooled in groups of up to 20 and placed in the PBS "bath." After processing, animals were released at the location where they were captured. Samples were kept in a cooler on ice during transport, stored in a refrigerator (4°C) upon arrival at the laboratory, and processed within 24 hours after sampling.

Samples were pre-enriched as described previously (Cooley et al. 2007, Gorski et al. 2011). After pre-enrichment, *E. coli* O157 and *Salmonella* were recovered by immunomagnetic separation (IMS) followed by selective plating. Suspect colonies were confirmed by biochemical profiles and/or polymerase chain reaction (PCR).

PRELIMINARY RESULTS

A total of 10 farms comprising 44 sites (4 conventional and 3 organic produce; 2 wetland preserves; 1 cattle ranch) in California were enrolled in the study. We collected and tested 463 individual animals from March - October 2011. All samples were negative for *E. coli* O157. *Salmonella* was cultured from 4 (1%) of 331 frogs, 1 (5%) of 20 toads, 0 of 5 newts, 0 of 6 salamanders, 23 (59%) of 39 snakes, and 9 (15%) of 59 lizards. The *Salmonella* prevalence was

Table 2. Damage prevention and control methods for amphibians and reptiles[a].

Method	Approach	Limitations
Exclusion	Fencing; buried mesh hardware cloth	Labor intensive; not effective for climbing species (e.g., tree frogs)
Habitat modification	Vegetation removal on pond shoreline; removal of boards and debris providing cover	Vegetation removal may interfere with on-farm runoff and erosion control
Frightening	Not applicable	
Repellents/toxicants/fumigants	None registered for most species	Snake repellents not consistently effective
Trapping/capture	Funnel traps, buckets, glue boards	Not desirable due to capture of non-target species

[a]Adapted from Hygnstrom et al. (1994)

higher among reptile (33%) compared with amphibian (4%) species. Additional analyses are underway to analyze correlations between pathogen prevalence and genotypes, ecological data, and antibiotic resistance.

DISCUSSION

We confirmed that common species of wild amphibians and reptiles in the central California coast may shed *Salmonella*, but *E. coli* O157 was not found in these populations during the study period. A higher *Salmonella* prevalence in wild-caught reptiles compared with amphibians was similarly documented during surveys in Spain and Pennsylvania (Briones et al. 2004, Chambers and Hulse 2006). Additional studies are needed to better understand the ecology and epidemiology of *Salmonella* in wild reptile and amphibian populations in other regions. Moreover, characterization of the *Salmonella* isolates by serotyping and genotyping may reveal insights into their zoonotic potential.

The findings from this study emphasize the need to continue to follow food safety practices, especially those relating to pre-season and pre-harvest environmental assessments, to identify and mitigate potential food safety risks during fresh produce production. Wildlife damage management in fresh produce production fields is challenging, due to the diversity of potential hosts/reservoirs of foodborne pathogens and the limited availability of control strategies to prevent animal intrusions, particularly for herpetofauna (Table 2). There is an opportunity for professionals working in vertebrate pest control to play an important role in assisting fresh produce growers with implementation of co-management approaches that promote public health and conservation goals in the central coast agricultural landscape.

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