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TESTING FOR *SALMONELLA* SPP. IN RELEASED PARROTS, WILD PARROTS, AND DOMESTIC FOWL IN LOWLAND PERU

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ABSTRACT: Wild animal populations face threats from pathogens from both intentionally released captive animals and domestic animals that accompany human settlements. From December 2004 through August 2005, we studied free living macaws and parrots in the Tambopata National Reserve in the Peruvian Amazon and semicaptive domestic fowl in human settlements adjacent to the reserve. In 1992–1993, large macaws (*Aras* spp.) that were serologically positive for *Salmonella* Pullorum were released into this reserve, which hosts dense populations of free-living parrots and macaws. We collected cloacal swabs from 64 birds and cultured for *Salmonella* spp. via standard laboratory methods. All 35 psittacines tested were culture negative for *Salmonella* spp., while 31% of 29 domestic fowl were culture positive. Our findings suggest that the domestic fowl that accompany human settlement in this region carry and shed *Salmonella* spp. that could threaten wild bird populations in and around the reserve.

Key words: *Ara*, conservation, macaw, parrot, pathogen pollution, reintroduction, *Salmonella* spp.

INTRODUCTION

Wildlife populations, including rare and endangered species, can suffer mass mortality and possibly extinction from disease outbreaks (Friend et al., 2001; Lips et al., 2008). Many recent outbreaks are the result of "pathogen pollution," in which pathogens are inadvertently spread to immunologically naïve populations by human activities (Daszak et al., 2000).

Release of captive raised or translocated animals is a valuable wildlife management technique. However, releases risk introducing pathogens to wild populations (Cunningham, 1996). In the early 1990s, 32 large macaws (Scarlet Macaw, *Ara macao*; Red-and-green Macaw, *A. chloropterus*; and Blue-and-yellow Macaw, *A. ararauna*) were hand-reared and released into the Tambopata National Reserve in the lowlands of southeastern Peru (Nycander et al., 1995; Brightsmith et al., 2005). Serologic testing in 1994 revealed that most of the released birds were antibody positive for *Salmonella* serotype Pullorum but the wild birds were negative (Karesh et al., 1997). *Salmonella* Pullorum is a common pathogen of poultry but has low pathogenicity in other avian species (D'Aoust and Prescott, 2007). This suggests that large populations of free-living parrots in the Tambopata National Reserve may have been exposed to *Salmonella* Pullorum. Additionally, thousands of parrots consume soil at a large river-edge cliff or "clay lick" located within 1 km of the release site (Brightsmith, 2004). These parrots feed and defecate in extremely high densities on this lick providing ample opportunity for fecal-oral transmission of *Salmonella* and other pathogens.

Released birds are not the only potential sources of pathogens faced by wild populations (Daszak et al., 2000). Each year, millions of live domestic fowl are sold and transported worldwide (USDA Foreign Agricultural Service, 2005). Some of these fowl end up in backyard flocks adjacent to natural forest and protected areas where they come in contact with wild species (Roelke-Parker et al., 1996; Chen et al., 2005; Hernandez-Divers et al., 2006). The purpose of this study was to determine if *Salmonella* spp. were present in free-living parrots in the Tambopata National Reserve and in domestic fowl in local communities adjacent to the reserve.

MATERIALS AND METHODS

Study sites

This study was conducted in and adjacent to the Tambopata National Reserve (275,000 ha), in the Department of Madre de Dios in the lowlands of southeastern Peru. The main study site was Tambopata Research Center (TRC, 13°8′17″S, 69°36′29″W, 250 m elevation, 3,200 mm of rain per year), which lies at the interface of tropical moist and subtropical wet forest (Tosi, 1960; Brightsmith, 2004). The center is surrounded by mature floodplain forest, successional floodplain forest, *Mauritia flexuosa* (Arecaceae) palm swamp, and upland forest (Foster et al., 1994). The center is a tourist lodge owned and operated by Rainforest Expeditions, which has supported parrot research since 1989 (Brightsmith, 2008). At the time of this study, the lodge was located about 300 m from a large "clay lick," a 500-m-long, 25- to 30-m high bank of exposed soil along the western bank of the upper Tambopata River, where over 1,000 psittacine birds of up to 17 species gather daily to consume soil (Brightsmith, 2004).

We also studied domestic fowl from five communities along the Tambopata River near the northern border of the Tambopata National Reserve. The communities studied were: Baltimore (12°49′59″S, 69°25′59″W), Condenado (12°52′1″S, 69°25′1″W), Sachavacayoc (12°51′0″S, 69°22′1″W), La Torre (12°46′59″S, 69°19′1″W), and Infierno (12°43′59″, 69°13′59″W). These small communities consist mainly of families with subsistence and small-scale commercial agriculture operations, who supplement their income with hunting, gold mining, timber harvest, and collection of nontimber forest products. Most families maintain a few domestic fowl (chickens [*Gallus gallus*], ducks [genera *Anus* and *Cairina*], or turkeys [*Meleagris gallopavo*]) and some also keep parrots as pets.

Capture

To determine if *Salmonella* spp. were present in the wild parrots, we captured birds at the clay lick adjacent to TRC using a blind surrounded by five snare traps. The traps consisted of a long pole with two thin branches covered with nylon nooses at the top. When parrots perched on these branches their feet became ensnared in the nooses. When a parrot became entrapped, the pole was lowered to the ground and the bird removed for sampling.

To determine if *Salmonella* spp. were present in the macaw population around TRC, we sampled both chicks and adult birds. We sampled chicks from Scarlet and Red-and-green Macaw nests. We accessed the nests using single rope climbing techniques (Perry and Williams, 1981). Chicks were placed in a bucket and lowered to the ground for sampling. Chicks were all sampled at 2–3 wk of age. We also sampled adult macaws that had been hand-raised and released at Tambopata Research Center during 1992–1993. To do this, we captured macaws by hand as they foraged in the lodge (Brightsmith et al., 2005).

To determine if *Salmonella* was present in domestic fowl (chickens, ducks, and turkeys) in the communities along the border of the Tambopata National Reserve, we located small farms with backyard domestic fowl, obtained permission from farm owners, and captured domestic fowl by hand. The small farms sampled contained a total of about 170 domestic birds, of which about 20% were sampled. The number sampled was limited by the ability to quickly capture the free-ranging animals and the willingness of the owners to have their animals pursued and handled. All Psittaciformes and domestic fowl were manually restrained for cloacal swab collection. All samples were collected from August to December 2005.

Analyses

Upon collection, all cloacal swabs were placed in Stuart transport medium (Becton Dickinson [BD], Franklin Lakes, New Jersey, USA) and selenite broth (BD), and refrigerated at 4–8 C for up to 3 days until the samples were transported to the laboratory for analysis. Presence of *Salmonella* spp. was determined by culture in the Laboratory of Microbiology and Immunology of the Faculad de Veterinaria y Zootecnia of the Universidad Peruana Cayetano Heredia. Samples were pre-enriched in peptone water (Merck, Whitehouse Station, New Jersey, USA) for 24 hr and enriched in selenite cystine broth (BD) and tetrathionate broth (Merck) for 24 hr then plated on xylose–lysine–desoxycholate agar (DIFCO, BD) and *Salmonella–Shigella* agar (DIFCO) and incubated at 37 C for 24 hr. Presumptive positive growths (dark bacterial colonies) were grown on tryptic soy agar (Merck) and isolates from them were inoculated on Kligler triple sugar iron agar (TSI, Merck) and lysine-decarboxylase agar (LDA, DIFCO) and incubated at 42 C for 24 hr. Positive isolation of *Salmonella* spp. was determined by the slope reverting to red, indicating a lactose-negative reaction, a yellow butt indicator of a glucose-positive reaction, a purple reaction throughout the LDA, and a blackening of the Kligler's agar superimposed on the previous reactions due to the production

of hydrogen sulfide (H_2S), as the characteristic reactions caused by most *Salmonella* spp. species in Kligler's agar (Vadillo et al., 2002).

RESULTS

Cloacal swabs from 35 individual psittacine birds from the area around TRC were cultured for *Salmonella* spp. These included four Scarlet Macaws and one Red-and-green Macaw that were hand raised and released in 1993–1994 (Karesh et al., 1997). One of these Scarlet Macaws was sampled twice (3 mo apart). In addition, we cultured cloacal swabs from five chicks from four different nests of wild Scarlet Macaws and 25 wild adult psittacines captured at the clay lick, including one Scarlet Macaw, three Blue-and-yellow Macaws (*Ara ararauna*), one Red-bellied Macaw (*Orthopsittaca manilata*), eight Blue-headed Parrot (*Pionus menstruus*), 11 Mealy Parrots (*Amazona farinosa*), and one Yellow-crowned Parrot (*A. ochrocephala*). None of these birds cultured positive for *Salmonella*. Of the domestic fowl from the local communities, 31% (95% CI=13–49%, n=29) cultured positive for *Salmonella* spp. Of the chickens, 27% (95% CI=7–47%, n = 22) were positive, and of the ducks 43% (95% CI = 3–93%, n=7) were positive (Table 1).

TABLE 1. Testing for *Salmonella* spp. in released macaws and wild parrots in the Tambopata National Reserve, Peru and domestic fowl in human settlements adjacent to the reserve. Species sampled are included in the Methods section.

	Sampled	Positive (n)	Positive (%)	95% CI
Released macaws	5	0	0	NA
Scarlet Macaw chicks	5	0	0	NA
Wild adult parrots	25	0	0	NA
Total parrots	35	0	0	NA
Poultry	22	6	27	7–47%
Ducks	7	3	43	3–93%
Total fowl	29	9	31	13–9%

DISCUSSION

None of the hand-raised macaws tested were culture positive for *Salmonella* spp., suggesting that the population of released macaws may not be currently shedding *Salmonella* spp. None of the wild birds trapped at the clay lick tested positive for *Salmonella* spp., suggesting that shedding of *Salmonella* spp. is not common among the thousands of birds that use this clay lick (Brightsmith, 2004). However, additional testing in this region is needed as only 24 of the >1,000 birds that use this site could be sampled due to the difficulty of trapping wild psittacines. In addition, hold time, transport time, storage conditions, and media type may have affected our ability to recovery *Salmonella* spp. and the methods used only detect individuals that are shedding bacteria at the time of sample collection. It is possible that some of these birds were infected and were shedding *Salmonella* spp. intermittently.

Given that the released macaws, including those antibody positive for *Salmonella* Pullorum, have used the clay lick for many years, we were concerned that they may have infected other birds with *Salmonella* spp., leading to endemic salmonellosis among these wild parrot populations. Among wild birds, salmonellosis outbreaks can occur via fecal-oral transmission when large numbers of birds congregate (Tizard, 2004). *Salmonella* spp. can remain viable for up to 200 days in soil, and some birds can become subclinical carriers and intermittently shed bacteria (Gerlach, 1994). Mortalities of wild parrots due to salmonellosis have not been reported, but antibodies against *S.* Pullorum have been found in wild and captive Blue-fronted Amazons (*Amazona aestiva*) in Bolivia (Deem et al., 2005). In addition, *Salmonella* spp. are known to infect a variety of psittaciformes in captivity (Panigrahy et al., 1979; Shima and Osborn, 1989) and outbreaks of different intensity have been reported in aviaries (Shima and Osborn, 1989; Ward et al., 2003).

The risk of introducing pathogens to native populations is cited as the major

reason to avoid reintroduction of psittacine birds (Derrickson and Snyder, 1992; Snyder et al., 1996). In 1994, the majority of the hand-raised macaws tested at TRC (61% of 13) had antibody titers suggestive of exposure to *Salmonella* Pullorum (Karesh et al., 1997), while none of the eight wild macaws tested had positive antibody titers. This suggests exposure of the released birds to *Salmonella* Pullorum, a known poultry pathogen, during the rearing process. However, the epidemiological implications of these antibody findings are ambiguous. Positive antibody titers do not necessarily imply ongoing *Salmonella* spp. infection because in some cases antibodies can still be detected long after the bacteria has cleared and fecal shedding has ceased (Gast, 1997). As *Salmonella* spp. infections are often transitory, it is possible that the released birds were exposed to the pathogen during hand rearing and cleared the bacteria from their system before release into the wild. In addition, lack of known sensitivity and specificity of *Salmonella* Pullorum serologic testing in wild birds results in unknown validity (D'Aoust and Prescott, 2007).

In the communities adjacent to the Tambopata National Reserve, over 30% of the backyard domestic fowl were culture positive for *Salmonella* spp. These results confirm that the domestic fowl that accompany human settlement carry pathogens that could pose a risk to wildlife populations (Daszak et al., 2000; Hernandez-Divers et al., 2006). This finding highlights the need for additional investigation regarding other pathogens these domestic fowl may harbor. The risk of pathogen spread is exacerbated as people in these communities also keep wildlife as pets (Deem et al., 2005). During this study, we observed pet Cobalt-winged Parakeets (*Brotogeris cyanoptera*) and a Spix's Guan (*Penelope jacquacu*) kept alongside domestic fowl. Because pets are often allowed to roam freely and frequently escape, pathogens could be spread from domestic fowl to wild native bird populations via free-roaming pets.

The relationships between human and ecosystem health are complex. Many pathogens introduced to frontier areas can spread from domestic animals that accompany human settlement (Roelke-Parker et al., 1996; Hernandez-Divers et al., 2006). In areas where newly arriving domestic animals and their pathogens are channeled through a few commercial markets before being dispersed to rural communities, improved sanitary controls at markets could reduce the risk of pathogen pollution for millions of hectares of rural and wild lands. Improved markets coupled with education to improve the sanitary conditions of backyard fowl could provide multiple benefits, including improved human health through reduced exposure to zoonotic diseases, improved welfare of local people through reduced livestock loss to disease, and protection of wildlife from the introduction of new or emerging pathogens. As a result, conservation practitioners should collaborate with agricultural extension agents and public health officials to encourage local authorities to increase biosecurity and health monitoring in live animal markets and educate animal owners about good sanitary practices. Additional attention to live animal markets may also provide other benefits such as reduced illegal wild animal trade, improved control of zoonotic diseases, and identification and control of new and emerging diseases (Karesh et al., 2005). Given the economic cost and health implications of diseases linked directly to the live animal trade like SARS and avian influenza (Bell et al., 2004; Kilpatrick et al., 2006), increased disease surveillance of live animal markets should be cost effective and provide multiple benefits at the local, national, and global levels.

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