Short Communication

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Prevalence of *Salmonella enterica* and Shiga toxin-producing *Escherichia coli* in zoo animals from Chile

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Salmonella (*S.*) *enterica* and Shiga toxin-producing *Escherichia coli* (STEC) are foodborne pathogens. Here, we report the prevalence of *S. enterica* and STEC in feces of 316 zoo animals belonging to 61 species from Chile. *S. enterica* and STEC strains were detected in 7.5% and 4.4% of animals, respectively. All *Salmonella* isolates corresponded to the serotype Enteritidis. To the best of our knowledge, this is the first report of *S.* Enteritidis in the culpeo fox (*Lycalopex culpaeus*), black-capped capuchin (*Sapajus apella*) and Peruvian pelican (*Pelecanus thagus*) and the first STEC report in Thomson's gazelle (*Eudorcas thomsonii*).

Keywords: Chile, *Salmonella enterica*, Shiga-toxigenic *Escherichia coli*, zoo animals

The study of pathogens in captive populations is critical for implementation of programs for prevention, control and surveillance of diseases, as well as for developing public and animal health policies [9]. Several emerging pathogens in humans are classified as food-borne diseases, with certain serotypes or subgroups of pathogenic *Escherichia* (*E.*) *coli* and *Salmonella* (*S.*) *enterica* being within the most important group [9].

The causative agent of salmonellosis, *S. enterica*, produces asymptomatic and clinical infections in humans and animals, with symptoms of diarrhea, fever, vomiting, abortion, osteomyelitis and occasionally death [14]. Conversely, *E. coli* is a commensal bacterium within the large intestine of warm-blooded animals that is generally non-pathogenic toward human and other species of mammals and birds [10]. However, the presence of some virulence genes establishes a diversity of bacterial pathotypes that cause disease in their hosts which are known as pathogenic *E. coli* [16]. Among these, the Shigatoxin-producing *E. coli* (STEC) is a globally zoonotic pathogen that can cause bloody diarrhea, hemorrhagic colitis and haemolytic uremic syndrome in humans [3,10].

Both agents have been isolated from several wildlife species. *Salmonella* has been isolated from zoo animals with asymptomatic infection [9,15], and has also caused disease outbreaks with mortality [6]. In the case of STEC, reports in zoo animals suggest variable prevalence ranging from 0.1% to 50.8%, probably because of the different diagnostic methods and animal species being investigated, although asymptomatic infection is always described [11,15].

This study was conducted to determine the prevalence of *S. enterica* and STEC in zoo animals from Chile. The studied population included waterfowl and terrestrial mammals (Table 1), all of which were clinically healthy. A total of 316 fecal samples were collected through both rectal and cloacal swabbing, and these were later inoculated into Cary-Blair transport medium (Copan Diagnostics, USA). For *Salmonella* isolation, swabs were placed into buffered peptone water (Difco APT broth; Beckton, Dicknson and Company, USA) supplemented with 20 μg/mL novobiocin (Sigma, USA) and incubated for 24 h at 37°C. Aliquots of this suspension were then inoculated into modified semisolid Rappaport Vassiliadis basal medium (Oxoid, Brazil) supplemented with 20 μg/mL novobiocin and incubated for 24 or 48h at 41.5°C. Cultures were subsequently plated onto Xylose Lysine Deoxycholate agar (Difco XLD broth; Beckton, Dicknson and Company), and suspicious colonies were identified by biochemical tests and *invA* gene detection by PCR. Finally, *S. enterica* isolates were serotyped according to the Kauffman-White Scheme [8]. For isolation of STEC, swabs were placed into 5 mL buffered peptone water (Beckton, Dicknson and Company) and incubated for 24 h at 37°C.

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Table 1. Detection of *Salmonella* (*S.*) *enterica* and Shigatoxin-producing *Escherichia* (*E.*) *coli* (STEC) in zoo animals from Chile

Host		Number samples (sampling fraction*)	Number of positive samples	
Order	Species		*S. enterica*	STEC (detected genes)
Carnivora	*Canis lupus*	4 (27%)	0	0
	Chrysocyonbrachyurus	10 (83%)	0	0
	Galictis cuja	1 (50%)	0	0
	Genetta genetta	5 (83%)	0	0
	Mephitis mephitis	7 (100%)	0	0
	Panthera leo	1 (20%)	1	0
	Panthera tigris	2 (66%)	0	0
	Procyon lotor	5 (100%)	0	0
	Pseudalopex culpaeus	7 (100%)	1	0
	Puma concolor	1 (50%)	0	0
	Suricata suricata	8 (100%)	0	0
Primate	*Alouatta caraya*	5 (83%)	0	0
	Ateles geoffroyi	4 (100%)	0	0
	Cebus apella	10 (100%)	1	0
	Colobus guereza	2 (50%)	0	0
	Lagothrix lagotricha	1 (100%)	0	0
	Lemur catta	2 (33%)	0	0
	Papio hamadryas	7 (78%)	0	0
	Symphalangussyndactylus	2 (100%)	0	0
Artiodactyla	*Cervus elaphus*	4 (100%)	3	0
	Dama dama	12 (100%)	12	7 (4 *stx1*; 3 *stx1* + *stx2*)
	Eudorcas thomsonii	14 (100%)	0	2 (*stx1*)
	Lama glama	5 (100%)	0	1 (*stx1* + *stx2*)
	Lama guanicoe	3 (100%)	0	1 (*stx1* + *stx2*)
	Ovis ammon aries	9 (50%)	2	0
	Ovis aries	11 (100%)	3	0
	Ovis orientalis musimon	21 (100%)	0	3 (*stx1* + *stx2*)
	Pudu pudu	1 (13%)	0	0
	Sus scrofa	12 (100%)	0	0
	Tragelaphus angasii	3 (100%)	0	0
	Tragelaphus spekii	1 (100%)	0	0
	Vicugna pacos	15 (100%)	0	0
Pelecaniformes	*Pelecanus onocrotalus*	2 (100%)	0	0
	Pelecanusthagus	7 (100%)	1	0
Charadriiformes	*Larus dominicanus*	4 (100%)	0	0
Anseniformes	*Aix galericulata*	2 (25%)	0	0
	Aix sponsa	8 (100%)	0	0
	Anas acuta	1 (100%)	0	0
	Anas bahamensis	3 (30%)	0	0
	Anas castanea	3 (30%)	0	0
	Anas falcata	1 (50%)	0	0
	Anas georgica	3 (16%)	0	0
	Anas rhynchotis	2 (67%)	0	0
	Anas sibilatrix	2 (20%)	0	0
	Anser indicus	2 (100%)	0	0
	Aythya australis	9 (69%)	0	0
	Aythya marila	5 (100%)	0	0
	Branta leucopsis	4 (100%)	0	0

Table 1. Continued

Host		Number samples (sampling fraction*)	Number of positive samples	
Order	Species		*S. enterica*	STEC (detected genes)
	Branta ruficollis	4 (100%)	0	0
	Callonetta leucophrys	4 (100%)	0	0
	Chloephaga picta	6 (100%)	0	0
	Chloephaga poliocephala	2 (100%)	0	0
	Chloephaga rubidiceps	4 (100%)	0	0
	Chroicocephalus maculipennis	1 (100%)	0	0
	Coscoroba coscoroba	13 (100%)	0	0
	Cygnus atratus	1 (50%)	0	0
	Cygnus melancoryphus	9 (100%)	0	0
	Cygnus olor	4 (100%)	0	0
	Dendrocygna bicolor	1 (34%)	0	0
	Netta peposaca	3 (43%)	0	0
	Netta rufina	5 (71%)	0	0
	Tadorna cana	6 (100%)	0	0
Total		316	24	14

*Sampling fraction refers to the percentage of sampled animals from the total population belonging to each species.

Aliquots of this suspension were then plated into MacConkey medium (Beckton, Dicknson and Company) and incubated for 24 h at 37°C. Next, 10 lactose positive suspicious colonies from each sample were analyzed by PCR for detection of *stx1*, *stx2* and *eae* genes, as previously described [16]. The STEC reference strains C600J (*stx1*) and C600W (*stx2*) and the Enteropathogenic strain 2348/69 (*eae*) were used as positive controls [16]. Finally, strains were analyzed by biochemical tests to confirm their identity.

A χ^2 test was performed to identify statistical associations between order, gender and age of sampled animals using the InfoStat (ver. 2010) software..

From the sampled population, *S. enterica* strains were isolated from 24 animals (7.5%) (Table 1), all of which belonged to the serotype Enteritidis. In this group, the order Artiodactyla had the highest abundance ($p < 0.05$), with a prevalence of 18%. In contrast, previous studies have reported less than 10% prevalence of Artiodactyla [2,5,12], suggesting epidemiological variability between populations and reinforcing the need for ecological studies and selection of preventive measures for specific scenarios.

To the best of our knowledge, this is the first report of *S.* Enteritidis detected in the culpeo fox (*Lycalopex culpaeus*), black-capped capuchin (*Sapajus apella*) and Peruvian pelican (*Pelecanus thagus*). Additionally, this is the first description of STEC in Thomson's gazelle (*Eudorcas thomsonii*).

S. enterica is a priority pathogen for establishing surveillance programs in wild ruminants from Europe [4]. These animals represent sanitary risks for transmission of pathogens with costly control programs in humans and production animals [4]. Although several reports of *Salmonella* in artiodactyls have suggested that it causes an asymptomatic infection, mortality has also been described and could have ecological relevance in certain populations [5,6].

In this study, all *S. enterica* isolates corresponded to the serotype Enteritidis, which is the most frequent *Salmonella* serotype isolated from humans. Despite wild birds being considered reservoir hosts of *Salmonella* in Chile [13], no infected birds were observed in the present study, suggesting that the sanitary condition of zoo birds does not necessarily represent the infectious status of free-range populations for this pathogen. Moreover, these findings indicate that mammals from the zoo do not share transmission routes with zoo birds. For this reason, future genotypic analyses of bacteria must be more informative of their source in zoo facilities.

In the case of pathogenic *E. coli*, STEC strains were detected in 4.4% of samples (Table 1), all of which belonged to the order Artiodactyla ($p < 0.05$). Positive samples resulted in amplification of the *stx1* and *stx2* genes (individually or simultaneously), but not the *eae* sequence (Table 1). Among artiodactyls, the prevalence of STEC was 12.6%, which is lower than that reported in previous studies (20–50%) [10,11]. However, target populations differ between studies.

Within Artiodactyla, a mammal order that includes all even-toed hoofed animals, 30.6% of animals were positive for at least one pathogen, having isolation rates of 18.0% and 12.6% for *S. enterica* and STEC, respectively. This was the most epidemiologically relevant order ($p < 0.05$) for detection

of these enteric pathogens. The sex and age of animals were not associated ($p > 0.05$) with bacterial detection.

Artiodactyla usually carry bacteria in their gastrointestinal tract with no symptoms [1], which is supported by the results of this study. Moreover, they have been subjected to greater exposure to enterobacteria than other confined animals investigated to date. Because their pens are distributed in several sectors of the zoo, geographically associated contamination might be irrelevant. Moreover, their food is prepared under strict hygiene standards and their water is properly chlorinated. Therefore, other transmission routes or risk factors likely explain its infection status. Such potential routes include confinement conditions, quality and storage of raw foods, access of synanthropic wild animals (rodents, birds), contact with other captive animals, and direct feeding by visitors [7], although none of the animals from the petting zoo were positive. The isolation of bacteria from these potential carriers and the genotypic characterization of isolates would provide definitive evidence of transmission through such routes. Regardless of the source of infection, the detection of these zoonotic enterobacteria suggest a potential risk of transmission between workers and zoo animals, highlighting the need for awareness of risks and improvement of hygienic procedures, staff training and advertisements targeting visitors to optimize both recreational and educational activities of the zoo.

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Conflict of Interest

There is no conflict of interest.

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