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Isolation of *Salmonella* spp. in cattle egrets (*Bubulcus ibis*) from Fernando de Noronha Archipelago, Brazil

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ABSTRACT

The growth of the population of cattle egrets (*Bubulcus ibis*) in the archipelago of Fernando de Noronha constitutes a threat to public health and biological diversity because of their competition with and predation on native species and the possibility of transmission of pathogens to human beings, livestock and native wildlife. The aim here was to search for, isolate and identify serovars of *Salmonella* in clinically healthy local cattle egrets. Cloacal swabs were obtained from 456 clinically healthy cattle egrets of both sexes and a variety of ages. The swabs were divided into 51 pools. Six of these (11.7%) presented four serovars of *Salmonella enterica* subspecies *enterica*: *Salmonella* serovar Typhimurium; *Salmonella* serovar Newport; *Salmonella* serovar Duisburg; and *Salmonella* serovar Zega. One sample was identified as *S. enterica* subspecies *enterica* O16:y:-. Results in this study suggest that cattle egrets may be reservoirs of this agent on Fernando de Noronha and represent a risk to public health and biological diversity.

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Introduction

Exotic invasive species are animals and/or plants that have been introduced into a place where they did not previously exist naturally. They are one of the biggest threats to the environment, causing enormous harm to biodiversity and to natural ecosystems. Furthermore, they may present risks to human health, given that they may transmit diseases to endemic species and cause ecological imbalances.[1,2]

Fernando de Noronha is an archipelago composed by 21 oceanic islands with 26 km^2 of total area that are part of the Brazilian state of Pernambuco. They are located in the equatorial zone of the South Atlantic (3°50′28.9″ S, 32°24′39.4″ W), at a distance of 545 km northeastwards from Recife, the state capital of Pernambuco. The main island has a resident population of approximately 2930 inhabitants and is designated as a Marine National Park and an Environmental Protection Area.[3,4]

Among the exotic invasive species seen in the archipelago of Fernando de Noronha, cattle egrets (*Bubulcus ibis*) have caused emerging problems.[2] These include competition for resources and predation of endemic species; potential for transmission of diseases to the human population and production animals; and potential for collisions with aircraft.[2,5,6]

Cattle egrets belong to the family Ardeidae, order Pelecaniformes. They are insectivorous and are commonly observed foraging in open field areas, close to cattle, and feeding off insects that disturb the cattle.[7] The first reports of occurrences of this species on Fernando de Noronha date from the 1980s, and there has been exponential growth of the population since then.[2]

Among the pathogens transmitted by wild birds, *Salmonella* spp. has taken on an important role and has even been indicated as a threat to wildlife conservation in general terms.[8,9] *Salmonella* belongs to the family Enterobacteriaceae; it colonizes the intestines of reptiles, birds and mammals and has great importance in relation to both human and animal pathology.[9,10] Some serovars like Typhimurium and Newport are commonly associated to food poisoning or enteric infection outbreaks worldwide, being a great threat to public health.[8–10,24,28,30,32,34]

Presence of this genus has been reported in several bird species and groups, such as domestic poultry,[11,12] Psittaciformes,[13,14] ratites,[15,16] raptors,[1] Passeriformes[18,19] and Ardeidae.[8,10,19–23] In cattle egrets, *Salmonella* has previously been isolated only in the United States.[8,19,23,24]

Considering the scarcity of studies on isolation of this enterobacteria in cattle egrets, the aim here was to search for, isolate and identify serovars of *Salmonella* in clinically healthy birds in the archipelago of Fernando de Noronha, Pernambuco, Brazil.

Material and methods

Study area

The covered area of the composting unit of waste treatment station in the main island of the archipelago of Fernando de Noronha was used as the site for catching cattle egrets.

Capture, physical restraint, clinical examination and collection of biological material

Between December 2007 and July 2008, 456 cattle egrets (*B. ibis*) of both sexes and various ages were caught. The birds were attracted into the covered area of the composting plant by the presence of prey (larvae and insects). They were contained there by closing the doors and were physically restrained by using nets and gloves suitable for the procedure. All the birds were subjected to clinical examination at the time of capture.[25] After they had been confirmed as clinically healthy, sterile swabs (CB Products, Corumbataí, São Paulo, Brasil) were introduced into the cloaca of each bird. Those swabs were subsequently kept under refrigeration and sent to the mainland in Styrofoam boxes containing recyclable ice, no more than 48 h afterwards, for laboratory procedures to be performed.

Laboratory procedures

The laboratory analyses relating to isolation and identification were performed in the Infectious-Contagious Diseases Laboratory of the Department of Veterinary Medicine of the Universidade Federal Rural de Pernambuco (UFRPE). The swabs were divided into pools of not more than 10 swabs, in accordance with the order of capture of the cattle egrets, thus totaling 51 pools. To isolate the agent, the classical scheme for investigating *Salmonella* recommended by the National Program for Poultry-rearing Health (PNSA) was used. This involved use of 1% buffered peptone water (Oxoid®, Cambridge, England) as a pre-enrichment medium and tetrathionate (Oxoid®, Cambridge, England) and Rappaport-Vassiliadis (Acumedia®, Lansing, Miami, USA) as enrichment broths, with subsequent seeding into brilliant green (Biobrás Diagnósticos®, Montes Claros, Minas Gerais, Brasil) and xylose lysine deoxycholate (XLD) (Oxoid®, Cambridge, England) selective agars. Colonies that were characteristic for *Salmonella* were crushed into the screening medium TSI (triple sugar iron agar) (Oxoid®, Cambridge, England), which enabled presumptive identification of the genus.[26,27]

Simplified biochemical profiling was used, consisting of characterization of the isolates according to their capacity for decarboxylation of lysine, use of citrate as the sole source of carbon, mobility and production of hydrogen sulfide (H_2S) and indole in the following media: lysine iron agar (LIA) (Biobrás Diagnósticos®, Montes Claros, Minas Gerais, Brasil), Simmons' citrate agar (Oxoid®, Cambridge, England), urea broth, methyl red broth (Oxoid®, Cambridge, England), Voges Proskauer (VP) (Biobrás Diagnósticos®, Montes Claros, Minas Gerais, Brasil) and sulfide indole motility (SIM) agar (Biobrás Diagnósticos®, Montes Claros, Minas Gerais, Brasil).[28]

After biochemical profile had been confirmed, the samples were sent to the National Reference Laboratory for Enterobacterial Infections (LRNEB) of Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro. At this location, antigen characterization was performed based on serological classification of Kauffmann-White and Le Minor, with representation in accordance with the criteria of Grimont and Weill.[29] All the antisera used in the rapid seroagglutination tests were produced by LRNEB.[11,26]

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