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SALMONELLA ENTERICA SUBSP. *ENTERICA* IN CATTLE EGRET (*BUBULCUS IBIS*) CHICKS FROM CENTRAL TEXAS: PREVALENCE, SEROTYPES, PATHOGENICITY, AND EPIZOOTIC POTENTIAL

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ABSTRACT: Cattle Egrets have a worldwide distribution, feed in proximity to cattle and other domestic animals, and often nest in large colonies in urban woodlots. Over a 3-yr period, nestlings from five Cattle Egret colonies from Central Texas, USA, were surveyed for salmonellosis. Prevalence of infection ranged from 29% to 95%. Seventeen *Salmonella enterica* subsp. *enterica* serotypes were isolated, of which the 4,5,12:i-monophasic serotype predominated in cultures of both the digestive tract and pooled spleen and liver. Of 11 4,5,12:i-monophasic isolates phage typed, eight were determinate type 193. The 4,5,12:i-monophasic isolates were susceptible to all antibiotics tested and were highly invasive in the day-old chick infection model. Microscopic lesions were found in the livers of Cattle Egrets with systemic infections with the 4,5,12:i-monophasic serotype, suggesting that infections with this serotype may often be fatal. Twenty-nine serotypes were identified in 179 *S. enterica* subsp. *enterica* isolates from horses admitted to the Texas A&M University Veterinary Teaching Hospital in 2 yr following the Cattle Egret study. The 4,5,12:i-monophasic serotype was not isolated from horses, but 12 serotypes were isolated from both horses and Cattle Egrets. The temporal distribution of the horse cases suggested that Cattle Egrets and horses may be exposed to similar sources of *Salmonella*, but provided no evidence of transmission between these two species. Similar conclusions were drawn when Cattle Egret isolates were compared to isolates from feedlot and dairy cattle from Texas and surrounding states. Given that the Cattle Egret 4,5,12:i-monophasic serotype was highly invasive and other isolates of this serotype have been associated with food poisoning, it is likely that Cattle Egret colonies pose a health risk to humans living near them.

Key words: *Bubulcus ibis*, Cattle Egret, pathogenicity, prevalence, *Salmonella enterica* subsp. *enterica*, serotype, 4,5,12:i-monophasic.

INTRODUCTION

Salmonellosis in wild birds is a well-documented phenomenon that occurs in widely divergent gregarious species of birds, including gulls, ducks, and passerine birds such as the Cowbird (*Molothrus ater*) and House Sparrow (*Passer domesticus*; Tizard, 2004). *Salmonella enterica* subsp. *enterica* infections in birds can result in a transient colonization of the digestive tract, a carrier state, or disease. Carriers act to disseminate these bacteria and are thought to be sources of salmonellosis in livestock, pet cats and dogs, and humans (Williams et al., 1977; MacDonald and Bell, 1980; Benton et al., 1983; Coulson et al., 1983; Tauni and Österlund, 2000; Tizard, 2004). Birds that are transiently infected or carry *Salmonella* are typically infected with a variety of serotypes that reflect the organisms that they are exposed to in the wild (Quessy and Messier, 1992). In some species, such as the Pigeon, specific species of *Salmonella* appear to be host adapted (Faddoul and Fellows, 1965). Even host-adapted *Salmonella* species, however, can cause significant disease in their host species. As an example, since the 1950s specific serotypes and determinate types of *S. enterica* subsp. *enterica* caused high mortality in the House Sparrow and may be responsible for the decline of other passerine birds (Wilson and MacDonald, 1967; Prescott et al., 2000).

TABLE 1. Prevalence of *Salmonella* infections, as assessed by isolation in culture, in adult and nestling Cattle Egrets collected from five locations in Central Texas, USA (% of birds positive of those tested).

				Source of *Salmonella* isolate			
Location	Year	Nestling or adult	*n*	Digestive tract (%)	Pooled liver/spleen (%)	Both digestive tract and pooled liver/spleen (%)	Either (not both) digestive tract or pooled liver/spleen (%)
Waco	1997	Nestlings (from tree)	29	66	7	7	66
		Nestling (ground)	5	100	100	100	100
College Station	1998	Adult	10	0	0	0	0
College Station	1998	Nestling	25	36	32	20	48
College Station	1999	Nestling	24	54	20	12	58
Eagle Lake	1999	Nestling	14	29	29	29	29
Hillsboro	1999	Nestling	21	48	33	24	57
Quinlan	1999	Nestling	22	91	77	77	95

A previous study investigating the epizootiology of spontaneous metabolic bone disease in nestling Cattle Egrets (*Bubulcus ibis*) suggested that salmonellosis may be a significant cause of mortality. In that study 20% of chicks collected from an Egret colony in Central Texas (Bryan, Texas, USA) had a subacute, severe bacterial hepatitis from which an untyped *Salmonella* was isolated (Phalen et al., 2005).

Cattle Egrets are common birds that have a worldwide distribution. They are found in most of the continental USA during the summer and in Hawaii year-round. Cattle Egrets forage in fields with cattle and other livestock and nest in dense colonies, often in urban areas (Telfair, 1993). If the prevalence of salmonellosis is high in Cattle Egrets, they may pose a health risk to livestock and humans.

The first objective of this study was to determine the prevalence of salmonellosis in Cattle Egret chicks from Cattle Egret colonies in Central Texas. Additional objectives were to determine the serotypes of the salmonellae found in the Egrets and determine their pathogenicity using the day-old chicken model. Last, the serotypes of the salmonellae identified in Cattle Egrets were compared to those found in cattle and horses within the Cattle Egrets' range to determine if the Cattle Egrets were a source of salmonellosis for livestock.

MATERIALS AND METHODS

Source of Egrets

Ten adult Cattle Egrets were collected immediately prior to the onset of nesting in Bryan, Texas, USA (30°40′N, 96°22′W) in April 1997. These birds had been shot by animal control officers in an attempt to prevent the establishment of a colony of nesting Egrets in an urban woodlot. Thirty-four Cattle Egret chicks were collected the same year from an Egret colony in Waco, Texas (31°26′N, 97°13′W). Twenty nine of these birds were collected from nests or adjacent branches and five that appeared ill were collected from the ground. An additional 106 apparently healthy nestling Cattle Egrets were collected from nests in College Station (30°40′N, 96°22′W; 1998 and 1999), Eagle Lake (29°59′N, 96°33′W; 1999), Hillsboro (32°91′N, 97°13′W; 1999), and Quinlan, TX (32°91′N, 96°14′W; 1999) during June and July (Table 1).

Salmonella isolation

Birds were euthanized by intraveneous injection of Fatal Plus (Vortech, Dearborn, Michigan, USA). Half of the right liver lobe and half of the spleen were aseptically collected, and the tissues were cultured as a pool. One-centimeter portions of the esophagus, proventriculus, duodenum, proximal jejunum, middle third of the jejunum, distal third of the jejunum, cecum, colon, and cloaca were aseptically collected, and the pools of these tissues were cultured separately. Pooled organs were minced and cultured overnight in tetrathionate broth. Tetrathionate broth was streaked onto brilliant green agar and cultured

TABLE 2. *Salmonella enterica* subsp. *enterica* serotypes used in the chicken inoculation experiment, their source, and percentage of chickens culture-positive from pooled liver and spleen 24 hr after oral inoculation.[a]

Serotype	Origin	Source	Dose[b]		
			10^3	10^4	10^5
Branderup	Waco	Liver	47	60	60
Bredeney	Waco	Liver	40	53	73
Bovis-morbificans	Waco	Liver	53	53	67
Bovis-morbificans	Hillsboro	Liver	33	53	80
Thompson	Waco	Liver	20	33	20
4,5,12:i-monophasic	Hillsboro	Liver	47	73	87
4,5,12:i-monophasic	Quinlan	Liver	60	93	100
4,5,12:i-monophasic	Quinlan	Liver	93	100	100
4,5,12:i-monophasic	College Station 1997	Liver	87	93	93
4,5,12:i-monophasic	Eagle Lake	Liver	73	100	100
Enteriditis	NA	NA	67	87	100

[a] Phosphate-buffered saline used to inoculate 15 negative control birds. *Salmonella* was not isolated from birds from this group.

[b] Number of *Salmonella* given by gavage.

overnight. Representative lactose-negative colonies were stabbed into triple sugar iron and lysine iron and tryptose slants. Culture media were purchased from Diffco Laboratories (Detroit, Michigan, USA). Colonies that fermented dextrose- but not sucrose-produced gas and H_2S were tested with the *Salmonella* poly A-I and IV agglutination test (Diffco).

Histopathology

Sections of liver were fixed in 10% neutral buffered formalin. Ten cases from which a salmonella serotype was isolated from pooled liver and spleen were randomly chosen. Tissues from these birds were paraffin-embedded and 4-μm sections were cut, stained with hematoxylin and eosin, and examined microscopically for evidence of changes associated with bacterial infection.

Salmonella characterization

Seven to 10 isolates from each Egret colony that grew from the pooled liver and spleen samples were randomly selected and sent to the National Veterinary Services Laboratories (Ames, Iowa, USA) for serotyping (Ewing, 1986). Two 4,5,12:i-monophasic isolates from each colony were also randomly selected and submitted to the same laboratory for phage typing (Ward et al., 1987). Antibiotic resistance testing to ampicillin, cephalothin, chloramphenicol, gentamicin, kanamycin, streptomycin, tetracycline, trimethoprim/sulfasoxazole, amoxicillin clavulanic acid, ciprofloxacin, and cefotamine was done on five 4,5,12:i-monophasic isolates randomly selected from the three sources with the highest prevalence of this isolate (Becton Dickenson, Sparks, Maryland, USA). Resistance tests were performed by agar disk diffusion according to protocols and guidelines of the National Committee for Clinical Laboratory Standards.

Pathogenicity studies in day-old chickens

Eleven *Salmonella* isolates from the Cattle Egrets (Table 2) and a *S. enterica* subsp. *enteriditis* isolate were grown from a single colony overnight in nutrient broth. The *Salmonella* isolates were selected so that one 4,5,12:i-monophasic isolate originated from each colony. The other serotypes represented isolates from pooled liver and spleen samples. The *S. enterica* subsp. *enteriditis* was kindly provided by Billy Hargis (Texas A&M University, College Station, Texas, USA). Bacteria were pelleted, washed, and resuspended in phosphate buffered saline (PBS; pH 7.2). White-leghorn chickens were hatched from eggs acquired from a local commercial hatchery (Hy-Line International, Bryan, Texas, USA). Using a random numbers table, 15 chicks were assigned to each treatment group. Treatment groups were separately housed in a Peter-Simme poultry battery in a climate-controlled building with continuous fluorescent lighting. Chicken scratch and water were provided ad libitum. Chicks 18–24 hr old were gavaged with a metal feeding needle and a 1-ml syringe with 100 μl PBS containing 10^2, 10^3, or 10^4 colony-forming units (CFU) of

TABLE 3. *Salmonella enterica* subsp. *enterica* serotypes and the number of times they were isolated from horses hospitalized at the Texas A&M Veterinary Teaching Hospital 1 May 2002–31 April 2004 (n=179).

Serotype	No. isolates	Serotype	No. isolates	Serotype	No. isolates
Newport	39	Mississippi	4[a]	Cerro	1
Newington	28	Rubislaw	4	Drypool	1
Oranienburg	19[a]	Java	3	Havana	1
Anatum	14[a]	Mbandaka	3	Kentucky	1
Infantis	11[a]	Muenchen	3[a]	Kiambu	1
Typhimurium	11[a]	Typhimurium var. Copenhagen	3	Montevideo	1
Branderup	8[a]	Meleagridis	2	Muenster	1
Bredeney	5[a]	Thompson	2[a]	Saint-Paul	1
Give	4[a]	Panama	2	Worthington	1
Javiana	4[a]	Carrau	1[a]		

[a] These serotypes were also isolated from Cattle Egrets.

each serotype (Guard-Petter et al., 1996). The last group of 15 birds was sham inoculated with 100 µl PBS.

Twenty-four hours after inoculation, chickens were killed by CO_2 asphyxiation. Spleens and livers from each bird were aseptically collected, minced, and incubated overnight in tetrathionate broth. After 24 hr incubation, each tube of tetrathionate broth was streaked onto a brilliant green agar plate and incubated 24 hr. The presence of lactose-negative colonies was considered proof of *Salmonella* invasion of either the liver or spleen. To verify that lactose-negative bacterial isolates were *Salmonella*, representative isolates were randomly selected and tested with the *Salmonella* poly A-I and IV agglutination test. The experiment was repeated using six of the original isolates and with *S. enterica* subsp. *enteriditis* as a positive control and PBS as the negative control (Table 2). The percentage of chicks that were culture-positive was compared between isolates and within dosages using the chi-square test of independence (Feinstein, 2002). Differences were considered significant at $P \leq 0.05$. Isolates were considered more invasive than other isolates if a significant difference was noted on for two or more challenge dosages.

Permits and ethics approvals

Cattle Egrets were collected under permit MB027977-0 issued by the United States Fish and Wildlife Service and permit SPR-0493-605 issued by Texas Parks and Wildlife. The method of Cattle Egret euthanasia and day-old chick infection trials were approved by the Texas A&M University Laboratory Animal Care Committee, Animal Use Protocol no. 8-225.

Salmonella serotypes and temporal distribution of *Salmonella* isolated from horses at Texas A&M University

All horses that present to the Veterinary Teaching Hospital, College of Veterinary Medicine, Texas A&M University, College Station, Texas, USA, with diarrhea or develop more than a transient diarrhea while hospitalized are cultured for *Salmonella*. These horses were from an 80.5-km radius of College Station, and it was expected that the majority fed in pastures used by Cattle Egrets from the College Station colony. All isolates were submitted to the National Veterinary Services Laboratories for serotyping. Serotypes isolated between May 1, 2000, and April 30, 2003, were cataloged and compared to the *Salmonella* serotypes isolated from the Egrets (Table 3). *Salmonella* serotypes isolated from the Egrets were also compared to *Salmonella* isolates from feedlot and dairy cattle from Texas and adjoining states (Dargatz et al., 2003; Edrington et al., 2004; Callaway et al., 2005).

RESULTS

Observations at Egret colonies

The Waco colony, which consisted of approximately 1,000 pairs of nesting Egrets was 300 m from the nearest residential buildings. There were scattered dead birds under the nest trees and approximately a dozen live nestlings on the ground. The College Station colony (1998 and 1999) and the Eagle Lake colony surrounded a pond, and most nests

were in trees that had water at their base. Dead nestlings were not observed, but alligators were present in these ponds, and it was assumed that dead birds had been eaten. The College Station colony was at least 1 km from residential buildings and contained approximately 2,000 pairs of nesting birds. A single home was immediately adjacent to the Eagle Lake colony, which also nested in trees over water. This was the smallest colony with 200–300 nesting pairs. Dead nestlings were not observed, but again alligators were present in the pond. There were many dead nestling Egrets on the ground at the Hillsboro and Quinlan colonies. The Egret colony abutted several residential buildings in Hillsboro, and some nests were in trees in backyards of these residences. Several residences were within 50 m of the Quinlan colony, and children were observed playing close to the edge of the colony. Both colonies contained approximately 2,000 nesting pairs.

Prevalence of salmonellosis and serotypes in Cattle Egrets

Salmonella was not isolated from the adult Egrets collected in 1997. *Salmonella* was isolated from the digestive tract and pooled liver and spleen samples from the five nestling Cattle Egrets collected from the ground at the Waco colony. The prevalence of *Salmonella*-positive digestive tract cultures from nestlings collected from trees ranged from 29% (Eagle Lake) to 91% (Quinlan). The prevalence of *Salmonella*-positive cultures of the pooled spleen and liver for these birds ranged from 7% (Eagle Lake) to 77% (Quinlan). The overall prevalence of infection in these birds, as determined by positive culture on digestive or pooled spleen and liver, ranged from 29% (Eagle Lake) to 95% (Quinlan; Table 1).

Seventeen *Salmonella* serotypes were identified from the 65 samples submitted for serotyping. The most common serotype isolated was 4,5,12:i-monophasic, representing 35.4% of all isolates submitted for serotyping. This serotype was identified from all Egret colonies, but was the only serotype isolated from the Quinlan colony. Multiple serotypes were isolated from all of the remaining colonies. The second most common serotype was Bredeney (10.7%), which was isolated only from the Waco colony. The third-most common was the 4,5:i-monophasic (4.6%), which was identified only in the Hillsboro colony. The remaining serotypes were identified only once or twice. Examining the serotypes isolated from the liver and spleen pooled samples and the digestive samples separately identified seven serotypes from the 31 pooled liver and spleen isolates submitted for serotyping. The 4,5,12:i-monophasic serotype was most commonly identified (82%). Fourteen serotypes were identified from the 34 isolates from pooled digestive tract cultures. The most commonly identified serotype was again 4,5,12:i-monophasic (32.4%). Eleven of these serotypes were isolated only from the digestive tract (Table 4).

Characterization of *S. enterica* subsp. *enterica* serotype 4,5,12:i-monophasic isolates

All five of the 4,5,12:i-monophasic isolates for which antibiotic susceptibility testing was done were susceptible to ampicillin, cephalothin, chloramphenicol, gentamicin, kanamycin, streptomycin, tetracycline, trimethoprim/sulfasoxazole, amoxicillin/clavulanic acid, ciprofloxacin, and cefotaxmine. Eleven 4,5,12:i-monophasic isolates were submitted to the National Veterinary Services Laboratory for phage typing. Two isolates from the Waco colony were determinate type (DT) 191, and one isolate from the College Station colony (1999) was untypable. The remaining eight isolates, including two from the College Station Colony (1998), one from the College Station colony (1999), one from the Quinlan Colony, and two from the Eagle Lake and Hillsboro colonies were DT 193.

TABLE 4. Distribution of serotypes of *S. enterica* subsp. *enterica* isolates from Cattle Egrets in Central Texas, USA, submitted for serotyping.

	No. isolates								
Serotype	Total	Digestive	Liver/ Spleen	Waco (1997)	College Station (1998)	College Station (1999)	Eagle Lake (1999)	Hillsborough (1999)	Quinlan (1999)
4,5,12:i-monophasic	36	11	25	2	3	5	7	5	14
Bredeney[a,b]	7	5	2	7	—	—	—	—	—
4,12,i-monophasic	3	3	—	—	—	—	—	3	—
Anatum[a,b]	2	2	—	—	—	—	—	2	—
Bovis-morbificans	2	1	1	1	—	—	—	1	—
Ibadan	2	2	—	—	2	—	—	—	—
Mississippi[a]	2	2	—	—	—	2	—	—	
Oranienburg[a]	2	2	—	—	2	—	—	—	—
Branderup[a,b]	1	—	1	—	1	—	—	—	—
Carrau[a,b]	1	1	—	—	—	—	—	1	—
Give[a,b]	1	1	—	—	1	—	—	—	—
Infantis[a,b]	1	1	—	—	—	1	—	—	—
Javiana[a]	1	1	—	—	—	1	—	—	—
Muenchen[a,b]	1	1	—	—	—	1	—	—	—
Poona	1	1	—	—	—	—	—	1	—
Thompson[a,b]	1	—	1	—	1	—	—	—	—
Typhimurium[a,b]	1	—	1	—	—	—	—	1	—
Total	65	34	31	10	10	10	7	14	14

[a] Serotype also isolated from horses with diarrhea.

[b] Serotype also isolated from feedlot or dairy cattle (Dargatz et al., 2003; Edrington et al., 2004; Callaway et al., 2005).

Day-old chicken pathogenicity trial

To determine the pathogenicity of 11 isolates (5 serotypes) obtained from Cattle Egret chicks, 15 1-day-old chickens were orally dosed with 10^3, 10^4, and 10^5 CFU of each isolate, and combined liver and spleen were cultured 24 hr after dosing. *Salmonella enterica* subsp. *enteriditis* was used as control invasive *Salmonella* species. Four of the five 4,5,12:i-monophasic isolates were significantly more invasive than the Branderup, Bredeney, both Bovis-morbificans, and Thompson isolates. The fifth 4,5,12:i-monophasic isolate was significantly more invasive than the Thompson isolate. The control isolate, *S. enterica* subsp. *enteriditis*, was significantly more invasive than the Branderup, one Bovis-morbificans, and the one Thompson isolates. Three isolates (Bovis-morbificans, Branderup, Thompson) did not increase their invasiveness with one or more increases in challenge dose. This experiment was repeated with these isolates, and the results were essentially identical (data not shown).

Histopathology

To determine if the 4,5,12,:i-monophasic isolates were causing hepatic disease, sections from 10 livers from Cattle Egrets that were positive on combined spleen and liver cultures were examined histologically. Four liver samples from birds that were culture-negative were used as negative controls. All liver sections from birds that were culture-positive had lesions consistent with those of a bacterial hepatitis. Lesions were multifocal and were typically moderate. Most were granulomatous and consisted of a central necrotic core surrounded by multinucleated giant cells, histiocytes, and, to a lesser extent, lymphocytes and plasma cells. Some granulomas were surrounded by a thin, fibrous capsule (Fig. 1). Acute lesions were less common. These contained focal areas of necrosis, but either did not have an

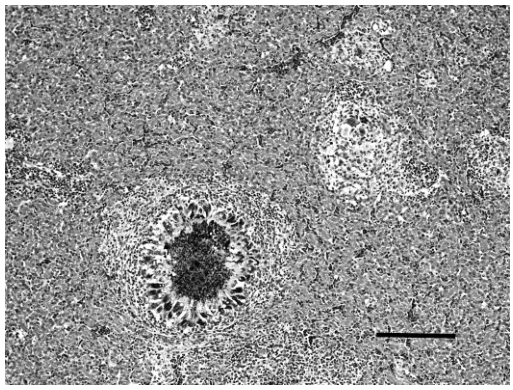

FIGURE 1. Hematoxylin and eosin–stained section of liver of a Cattle Egret from which a 4,5,12:i-monoformans serotype of *S. enterica* subsp. *enterica* was isolated, demonstrating a chronic (bottom left) and an acute (upper right) focus of necrosis. Bar = 200 μm.

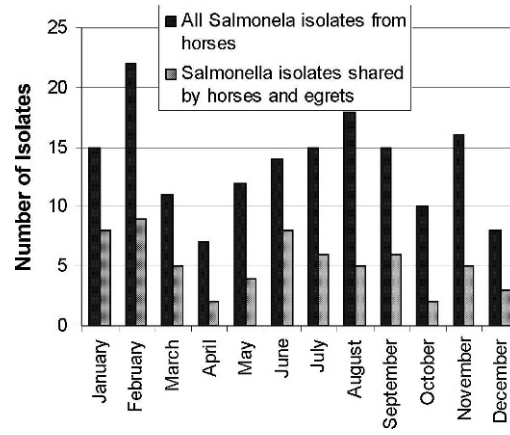

FIGURE 2. Seasonal distribution of *Salmonella* isolates from culture-positive horses at the Texas A&M University Veterinary Teaching Hospital, 31 April 2000–1 May 2002.

associated inflammatory response or the inflammatory response was heterophilic. Inflammatory lesions were not seen in sections of liver from birds that were culture-negative.

Salmonella isolates from horses

A total of 179 horses were culture positive for *S. enterica* subsp. *enterica* during the period April 31, 2000, to May 1, 2002, at the Texas A&M University Veterinary Teaching Hospital. A total of 29 serotypes were found in these 179 horses. Of these, 12 serotypes were also found in Cattle Egrets (Table 3). Forty-three percent of all horse isolates were serotypes found in Cattle Egrets, and 30.2% of the isolates serotyped from Cattle Egrets were serotypes also isolated from horses. The month with the most cases of salmonellosis caused by all serotypes was February; the next highest month was January, followed by July (Fig. 2). A rise in the number of *Salmonella* cases began in May, peaked in August, and declined again in September and October. *Salmonella* cases caused by isolates also found in Cattle Egrets followed a similar trend, only they peaked in June and declined or remained low in July, August, and September, a period when recently fledged Cattle Egrets would most likely contact horses. A second peak occurred in February, a month that Cattle Egrets would not be present in the horses' environment.

Comparison of *Salmonella* isolates from Cattle Egrets and cattle

Thirty-seven serotypes of *S. enterica* subsp. *enterica* are reported to have been isolated from feedlot and dairy cattle from Texas and surrounding states. Of these, only nine serotypes were also isolated from Cattle Egrets. The 4,5,12: i-monophasic serotype was not isolated from cattle in these studies. The second most common serotype from Cattle Egrets, Bredeney, was isolated from cattle only once (Dargatz et al., 2003; Edrington et al., 2004; Callaway et al., 2005).

DISCUSSION

A previous study revealed that 20% of Cattle Egret chicks found in a Central Texas colony had severe hepatitis from which an untyped *Salmonella* was isolated (Phalen et al., 2005). It was the initial objective of this study to determine if this was a limited observation or if *Salmonella* infection and disease was a common

phenomenon in Cattle Egret chicks in Central Texas. This report demonstrates that colonization of the digestive tract with *S. enterica* subsp. *enterica* can be expected in some Egret chicks from most or possibly all Egret colonies in this geographic area year after year. Salmonellosis was not identified in a small sampling of adult Cattle Egrets, suggesting that salmonellosis in Cattle Egrets occurs predominately in young birds as it does in other species of birds (Cizek et al., 1994). In the Cattle Egret chicks, the prevalence of salmonellosis and the probability that infection will result in bacteremia varied considerably and appeared to relate to the serotype of *S. enterica* subsp. *enterica* present in the colony.

Salmonellosis in birds can be the result of a temporary colonization of the digestive tract by environmental salmonellae, infection from host-adapted strains that may or may not be pathogenic to the host, or exposure to highly pathogenic strains (Tizard, 2004). Two of these scenarios appear to be occurring in the Cattle Egret colonies that were studied. Seventeen serotypes of *S. enterica* subsp. *enterica* were identified in the Egrets; 11 of these were found only in the digestive tract and were isolated only one or a few times. This is consistent with the model of transient digestive colonization from environmental organisms. It also appears that Cattle Egret chicks are frequently exposed to a *S. enterica* subsp. *enterica* serotype 4,5,12:i-monophasic that has the ability to colonize their digestive tract and is highly invasive, resulting in bacteremia and disease. The serotype 4,5,12:i-monophasic serotype was isolated from all the colonies and was the predominate isolate from the cultured pooled liver and spleens. The most common determinate type of this serotype was DT 193. It was found in subsequent years in one colony and in three of the four colonies that were tested only once, suggesting that this determinant type is highly pathogenic to Cattle Egret chicks and that it may be originating from a specific environmental source.

Previous studies have shown that *Salmonella* infections can cause high mortality and act as a key threatening process in certain species of birds (Wilson and MacDonald, 1967; Prescott et al., 2000). The impact of infection with serotype 4,5,12:i-monophasic on the survival of the Cattle Egret nestlings appears to be significant. Based on the number of dead chicks on the ground, chick mortality was highest in the Hillsboro and Quinlan colonies, which also had the highest prevalence of infection with this serotype. Also, a moderate hepatitis was found histologically in birds that were bacteremic with this serotype. The observed hepatitis was not as severe as that reported in a previous study (Phalen et al., 2005), and it was not clear if the observed lesions were sufficient to cause chick mortality in every case. However, these lesions were significant and would have been expected to at least cause morbidity, impacting birds in a critical stage of their development. If the majority of infections were to result in death, then the Quinlan colony would have experienced a minimum of 77% chick mortality the year this colony was sampled.

We used the day-old chick model to estimate the invasive potential and potential pathogenicity of 11 *Salmonella* isolates for a species other than the Cattle Egret. Results paralleled the findings in the Egret chicks. *Salmonella* isolates that were more likely to cause bacteriemia in Cattle Egrets were more invasive in the day-old chick model. The most invasive was the 4,5,12:i-monophasic serotype, and it was as invasive or more invasive than the *S. enterica* subsp. *enteriditis* control, suggesting this organism could cause disease in poultry and possibly mammals. An unexpected finding in this study was that higher doses of serotype Thompson did not result in increased invasiveness, and higher doses of Bovis-morbificans caused only a minimal increase in invasiveness. The experiment was repeated to confirm

this phenomenon, and the results were the same. Although the cause of this phenomenon is unknown, it may be that these are poorly invasive *Salmonella* serotypes.

Another objective of this study was to determine the potential health hazards that Cattle Egrets with salmonellosis might pose to horses, cattle, and humans. To this end, serotypes of the *Salmonella* isolated from horses presented to the Texas A&M University Veterinary Teaching Hospital were compared to those found in the Cattle Egrets. These isolates were obtained from horses in the 3 yr following the studies in the Cattle Egrets, but as salmonellosis had been found in all Cattle Egret colonies every year, it was felt that livestock exposure would be expected to occur on a yearly basis. Twelve of the 17 serotypes of *S. enterica* subsp. *enterica* isolated from Cattle Egrets were also found in horses that presented with or developed diarrhea at Texas A&M University Veterinary Teaching Hospital. Isolates of these serotypes made up 50% of the isolates from horses at the hospital. However, it was expected that if Cattle Egrets were the source of these infections, salmonellosis in horses would occur most commonly in July–October when juvenile Cattle Egrets would be feeding in the same fields as horses. Instead, peak salmonellosis in horses occurred in February, after Cattle Egrets have migrated south and are no longer present in Texas in significant numbers. A second peak occurred in horses in the 2 mo prior to the time that nestlings would leave the nest. Based on these observations, it seems more likely that Cattle Egrets and horses acquire *Salmonella* infections from the same sources or that some *Salmonella* infections in Cattle Egrets may come from fecal contamination of pastures from *Salmonella*-contaminated horse feces. The possibility that Cattle Egrets might infect horses at one time of the year and that clinical disease may not develop in the horse until triggered by other factors cannot be ruled out.

A similar pattern was seen when *Salmonella* serotypes isolated from Cattle Egrets were compared to *Salmonella* serotypes isolated from dairy and feedlot cattle from Texas and surrounding states. There was some overlap of serotypes, but the common serotype found in Egrets was not present in cattle, and the most common serotypes found in cattle did not occur in egrets, or did so rarely.

Although our data do not suggest an exchange of the 4,5,12:i-monophasic serotype between Egrets and cattle and horses in Texas, the 4,5,12:i-monophasic serotype is infrequently but consistently isolated from poultry, cattle, horses, and pigs in other regions of the USA (Centers for Disease Control, 2006). Thus Cattle Egrets should at least be considered to be a source of *Salmonella* infections when this serotype is isolated from livestock.

Salmonella enterica subsp. *enterica* serotype 4,5,12:i-monophasic is a common cause of food-borne illness in Spain and is believed to be associated with contaminated pork (Echeita et al., 1999; Astorga, et al., 2007). This serotype has also been found to be a cause of food-borne illnesses in New York, New York, USA, and in Luxembourg (Agasan et al., 2002; Anavisit et al., 2005; Mossong et al., 2007). The isolates in all of these outbreaks differed from the 4,5,12:i-monophasic isolates in this study as they all demonstrated resistance to multiple antibiotics. The determinate type of the New York isolates was not reported. The Spanish isolates were DT U302 (Astorga et al., 2007). The Luxembourg isolates were DT 193, giving them the same determinate type as the common *S. enterica* serotype 4,5,12:i-monophasic isolates from the Cattle Egrets (Mossong et al., 2007). Although the Cattle Egret 4,5,12:i-monophasic isolates are distinguishable by antibiotic resistance patterns or determinate type from those causing disease in people, the chick-infection trials suggest that they are potential zoonotic pathogens. The majority of Cattle Egret colonies in this study

were close to or in direct contact with human habitations. Public health authorities should consider that fecal matter and dead nestlings associated with these colonies have the potential to be heavily contaminated with *Salmonella* and should be considered a public health threat.

The ultimate source of the 4,5,12:i- monophasic isolates remains unknown. Food-borne outbreaks have been associated with pork, and this serotype has been isolated from pigs (Echeita et al., 1999; Astorga et al., 2007). Feral pigs are widespread in Texas and throughout much of the USA and thus may have the potential to be a source of this serotype. Given that these isolates show no antibiotic resistance, it is likely that they are from a feral animal source.

In conclusion, salmonellosis is expected to be present, to some degree, in nestling Cattle Egrets in all colonies in Central Texas. Under appropriate conditions, the prevalence of salmonellosis can exceed 50% and would appear to be a significant cause of morbidity and mortality in Cattle Egrets. The most common serotype found in the nestling Egrets (4,5,12:i-monophasic) is highly pathogenic for them and is potentially a public health threat. We did not find evidence that Cattle Egrets are an important source of salmonellosis for livestock, although they should be considered as a source if an outbreak of the 4,5,12:i-monophasic serotype were to occur. Whether salmonellosis will be found in Cattle Egret colonies in other geographic areas and the potential relationship of the 4,5,12:i-monophasic serotype to feral swine is unknown, but these questions merit additional research.

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