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Abstract Approved: Rodney J. Sobieski

Pathogenic bacteria Salmonella, Shigella and Campylobacter were screened for in Topeka and Emporia Zoo animal populations. At the Emporia Zoological Park specimens were collected in three samplings from February to September, 1991, whereas sampling at the Topeka Zoological Park was conducted on four dates from May to August, 1991. A total of 170 fecal samples was collected from several species including birds, reptiles, primates and felines. In both zoos all accessible animals were included in an initial screening. Any animals shedding bacterial pathogens were also sampled in the further screenings, in addition to animals in close proximity to these, as well as animals on the same basic diet regimen as positive carriers. This method of sampling was used to determine if bacterial pathogens were still present, and if these pathogens had spread to nearby animals. Plus, if other bacterial pathogens were introduced during the study, they could also be identified in this way. The only bacterial pathogens isolated were serotypes of the genus Salmonella. This bacterium is typically associated with

foodborne illness and has been implicated in severe gastroenteritis and typhoid fever. However, all Salmonella isolates in this study were non-typhoidal. Results from this study may suggest that transmission has occurred, but increased sampling and further analysis of other variables such as diet will be needed to confirm transmission.

# Isolation and Identification of Bacterial Pathogens from Zoo Animal Populations

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# INTRODUCTION

*Salmonella*, *Shigella*, and *Campylobacter* are just a few of many bacterial pathogens that can affect and reside in animal populations. *Salmonella* and *Campylobacter* can be part of the normal flora in animals, but are often pathogenic in humans (Manual of Clin. Micro., 1985). Bacteria in the genus *Shigella* are unique because they are somewhat host specific and have been isolated from only human and primate sources (Manual of Clin. Micro., 1985).

The primary goal of this investigation was to determine if these pathogens were prevalent in Emporia and Topeka Zoo animal populations. This was accomplished through the use of differential and selective media, biochemicals, and serotyping. A secondary goal of this study was, after isolation of bacterial pathogens in these zoo animal populations, to see if the same pathogens were still being shed (i.e., carrier) and if other pathogens had been introduced. Additional samplings were conducted to determine if there was any indication of transmission to nearby animals.

The information from this study may be useful to zoo personnel in planning animals' diets and monitoring potential disease situations. Also this may be used in considering the health threat to animals transported between zoos.

## A. SALMONELLA

All species of the genus Salmonella are pathogenic (Joklik et al., 1988). Members of this genus are gram negative, flagellated nonsporulating bacilli which are facultative anaerobes with simple nutritional requirements (Difco Manual, 1984; Joklik et al., 1988). Organisms of this genus are invasive and lead to septicemia, gastroenteritis, and typhoid fever (Joklik et al., 1988). In this study, emphasis was on non-typhoidal Salmonella since humans are the only known reservoir of typhoidal Salmonella (Evans and Brachman, 1991). Non-typhoidal salmonellosis is a disease of clinical and public health importance. It has been estimated that over 2-4 million cases of salmonellosis occur in the United States each year, though only a small fraction are reported (Evans and Brachman, 1991).

The taxonomy of Salmonella is complicated by the development of several different nomenclatures over the past three decades (Joklik et al., 1988). Genetic studies performed on this genus have revealed that all Salmonella and organisms in the genus Arizona belong to the same species in a phylogenetic and evolutionary sense. Any differences in antigenic types, biochemical reactions, and host or geographic distributions are due to divergence within a single species, Salmonella enterica. Within this species there are now five subgroups based on DNA

hybridization studies (Joklik et al., 1988). These are referred to as Salmonella subgroup 1 with subspecies designation enterica; Salmonella subgroup 2 with subspecies designation salamae; Salmonella subgroups 3a and 3b with subspecies designations arizonae and diarizonae, respectively; Salmonella subgroup 4 with subspecies designation houtenae; and Salmonella subgroup 5 with subspecies designation bongori (Steele, 1980; Difco Manual, 1984; Gyles and Thoen, 1986; Joklik et al., 1988). The Centers for Disease Control (CDC) and other clinical laboratories report organisms as serotypes such as Salmonella, serotype typhimurium rather than using Salmonella enterica, subspecies enterica, serotype Typhimurium (Joklik et al., 1988).

Salmonellae are a group of hardy and resourceful bacteria that are resistant to drying and freezing and may survive for long periods in nutrient poor soil (Evans and Brachman, 1991). It has been determined that Salmonella can survive for over 200 days in contaminated soil, ten months in dust, five months in rodent feces, and more than four years in dried whole egg (Evans and Brachman, 1991). These bacteria multiply in food at temperatures ranging from 7 to 46°C and can survive at pH as low as 4.5 (Leminor, 1984; Timoney et al., 1988).

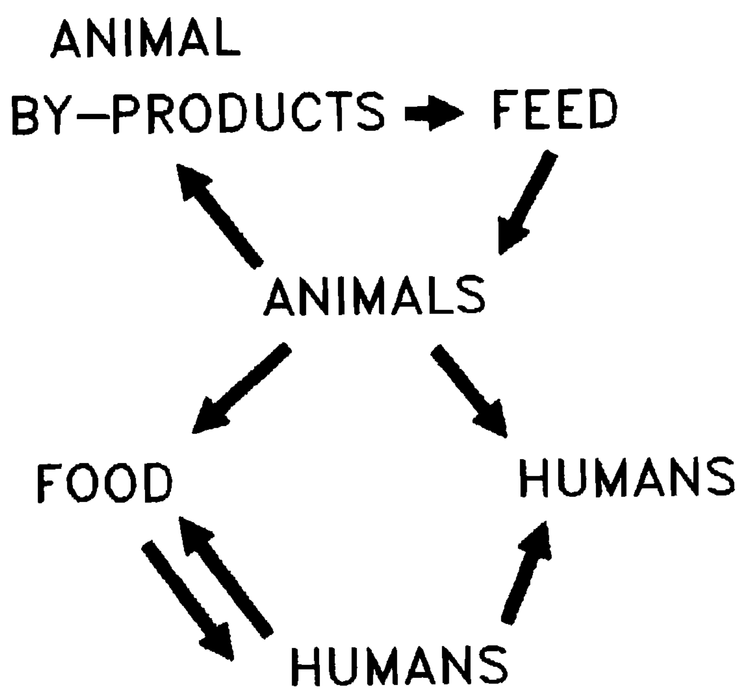
Salmonellae are pathogens in many lower animals, but some serotypes have been found to have a narrow range of

hosts. For example, the primary reservoir of Salmonella serotype pullorum, S. serotype gallinarum, and S. serotype heidlberg is chickens, the reservoir for S. serotype cholerae-suis is pigs, and S. serotype java, S.serotype urbana, and S. serotype litchfield are predominantly turtle associated (Evans and Brachman, 1991). However, all serotypes should be considered potentially pathogenic for humans and other animals. In addition to the narrow range of hosts they invade, many serotypes have very distinct patterns of virulence, antibiotic resistance, and geographic distribution which makes the epidemiology of this bacterium fascinating as well as complex (Evans and Brachman, 1991).

Infection by salmonellae can occur through a variety of routes, including foodborne, animal to animal contact, and waterborne (Fig. 1). It is believed that 90% of Salmonella infections that occur are foodborne (Evans and Brachman, 1991). There are usually two errors that promote a foodborne outbreak: one that permits the contamination to occur, and another that permits sufficient bacterial growth to reach an infectious level (Brown, 1982; Evans and Brachman, 1991).

The incubation period after ingestion of Salmonella ranges from 8-48 h (Difco Manual, 1984). Salmonella serotypes express different levels of pathogenicity depending on the host they invade (Evans and Brachman,

Figure 1. Cycles of infection for salmonellosis


ANIMAL
BY-PRODUCTS
FEED
ANIMALS
FOOD
HUMANS
HUMANS

1991). The clinical expression of infection is directly affected by the serotype and strain of bacteria, the health of the gut flora in the host, and the dose, vehicle, and route of transmission.

After ingestion, bacteria travel through the gastric acid barrier and multiply in the small intestine. Salmonellae penetrate the intestinal mucosa, are ingested by macrophages and may multiply in the mesenteric lymphoid tissue (Gyles and Thoen, 1986; Evans and Brachman, 1991).

Since Salmonella can inhabit the intestinal tract, excretion of feces may result in contamination of water, food, and the environment (Wray and Sojka, 1977: Leminor, 1984). Fertilizers and feeds containing animal products are sometimes a source of infection for animals (Gyles and Thoen, 1986). Fish meal, bone meal, and meat meal have all been shown to be frequently contaminated with Salmonella. Contaminated milk and milk products are other sources of this bacteria, particularly for calves (Gyles and Thoen, 1986).

Although Salmonella may survive for several years in the environment, it is the carrier state that provides the major source of infection for animals and humans (Gyles and Thoen, 1986; Joklik et al., 1988). Carrier animals can shed large numbers of Salmonella up to $10^5$/g of feces (Evans and Brachman, 1991). Asymptomatic carriers develop as a result of the interaction of several factors including

the serogroup of Salmonella, age of the animal, and number of bacteria ingested. Young animals often shed Salmonella only during convalescence whereas adults are more likely to become chronic shedders. A dose of Salmonella insufficient to cause disease may also result in the carrier state (Gyles and Thoen, 1986; Joklik et al., 1988; Timoney et al., 1988).

## B. SHIGELLA

Members of the genus Shigella are gram negative, nonmotile, noncapsulated, lactose-negative facultative anaerobes that form circular transparent colonies when grown under aerobic conditions on ordinary nutrient media (Difco Manual, 1984; Manual of Clin. Micro., 1985). These bacteria, which are closely related to Eschericia coli, are members of the family Enterobacteriaceae and tribe Eschericeae (Evans and Brachman, 1991).

All members of this genus are able to cause a disease syndrome called bacillary dysentariae or shigellosis. This is a syndrome that is manifested by the passage of small volume, bloody mucoid stools, associated with abdominal cramps, and tenesmus (Evans and Brachman, 1991). One of the most important properties of this bacterium is the ability to penetrate mammalian epithelial cells (Joklik et al., 1988). With this invasion the organism is able to secure a site for multiplication. Shigellae are

sequestered away from the host's antibacterial factors including antibody, complement, and phagocytic cells. Disease may be initiated by a dose as low as 200 bacterial cells (Gyles and Thoen, 1986).

The genus Shigella is divided into four serogroups, each consisting of a species which contains distinctive type antigens with considerable variation. Serogroup A, Shigella dysentariae, contains ten serotypes; serogroup B, S. flexneri, contains 6 serotypes; serogroup C, S. boydii, contains 15 serotypes; and serogroup D, S. sonnei, contains 1 serotype (Difco Manual, 1984).

Shigellae are basically host adapted and as a result the only natural hosts of Shigella are humans and non-human primates. Fecal contaminated fingers, food, and flies serve to spread the bacilli through a population. A common means of transmission is improperly disposed of Shigella-contaminated feces. In areas of substandard sewage treatment, flies can pick up the pathogens and transmit them to food which is then ingested by susceptible hosts (Gyles and Thoen, 1986; Joklik et al., 1988; Evans and Brachman, 1991).

## C. CAMPYLOBACTER

Members of the genus Campylobacter are slender, gram negative, spiral or curved rods. These organisms are microaerophilic and require a low oxygen tension and

increased $CO_2$ level for growth (Manual of Clin. Micro., 1985; Ross, 1986). The major pathogen, Campylobacter jejuni, does not ferment or oxidize carbohydrates, rather energy is obtained from the metabolism of amino acids (Banwart, 1989). Colony color ranges from brown to tan to gray and this bacterium may show swarming patterns similar to those of Pseudomonas or Proteus on Campy Blood Agar (Difco Manual, 1984). This bacterium can cause diarrhea and systemic illness in humans, and a number of diseases in wild and domestic animals (Evans and Brachman, 1991). Due to the similarity in morphology to the vibrios, these organisms were originally classified as Vibrio fetus in 1909 (Joklik et al., 1988). The genus name Campylobacter (Greek for "curved rod") was later proposed as a new name because these organisms differ biochemically from true members of the genus Vibrio (Evans and Brachman, 1991).

Campylobacter grows best at body temperatures of warm-blooded hosts. Common hosts of Campylobacter are dogs, swine, cattle, and other mammals (Prescott et al., 1982). The most common type found in cattle is C. venerealis. The natural habitat of this bacterium is in the glans penis and prepuce of sexually mature bulls and the genital tract (vagina, cervix, uterus, oviducts) of cows (Prescott, 1982; Evans and Brachman, 1991). This organism is responsible for causing abortion and sterility in cattle. C. jejuni is common in the intestine of many vertebrates (pigs, calves,

sheep, poultry, dogs, cats, horses, rodents) and also in human populations living in close contact with animals and their wastes (Prescott, 1982; Difco Manual, 1984; Joklik et al., 1988). Wild birds, particularly waterfowl, are also common carriers of this infection (Prescott, 1982).

C. jejuni can cause acute gastroenteritis, systemic infections, and abortion in animals (Prescott, 1982). Viability of organisms under environmental conditions is temperature-dependent (Blaser et al., 1980). C. jejuni will survive for weeks in water, feces, urine, and milk when kept at 4°C; however, at 25°C, viability persists for only a few days or less (Blaser et al., 1980). Campylobacter, like Salmonella, is sensitive to low pH and will not survive longer than five minutes at pH less than 2.3. At neutral or alkaline pH, especially in bile, organisms may multiply and survive up to three months at 37°C (Blaser et al., 1980).

Modes of transmission of Campylobacter appear to be similar to that for other known enteric pathogens. Increasing numbers of human outbreaks are being reported in which contaminated water or food, especially raw meat, have been implicated as the vehicle for transmission (Evans and Brachman, 1991). With Campylobacter excretion among domestic animals so common, it is not surprising that meat products are frequently contaminated with C. jejuni (Brown, 1982). Because the animal species with which humans come

into contact most frequently have been shown to excrete *C. jejuni* in their feces, the potential for human infection is great (Evans and Brachman, 1991).

# MATERIALS AND METHODS

## A. Collection of Specimens

Cary Blair media was used for the transport of fresh fecal specimens from the zoos to the laboratory. Cary Blair is a medium that allows for transport of organisms with little or no reduction in viability of bacterial cells within 24 h of collection (Difco Manual, 1984). Sampling of fecal specimens was carried out with the aid of zoo personnel. Samples were collected using sterile swabs and applicator sticks. Initially, screw capped test tubes (120mm x 14mm) were used, but these proved to be inadequate due to the small width. Therefore, larger screw capped vials (66mm x 30mm) were substituted to simplify collection. In the smaller tubes, 6ml of media was used and in the larger vials 10ml of Cary Blair was used.

## B. Primary Screening Media

All vials were mixed using a vortex to provide even distribution of the specimen in the vial. Plated media used in the initial screening process included Hektoen Enteric Agar, MacConkey Agar, Campy Blood Agar, selenite broth, and XLD (Xylose, Lysine, Dextrose) Agar. These media were used because of their selective and differential properties. All plated media received 1-2 drops of Cary Blair stool mixture with a pipette, and selenite enrichment

broths received one ml.

Hektoen Enteric Agar was used for the isolation and differentiation of *Salmonella* and *Shigella* from other enteric pathogens. MacConkey Agar detects gram negative lactose-fermenting bacilli. Both MacConkey and Hektoen Enteric Agar were incubated for 24 h at 37°C. Campy Blood Agar is a nutritionally rich medium used in the detection of *Campylobacter* species. In this medium the antibiotics, vancomycin, polymixin B, trimethoprin, cephalothin, and amphotericin B inhibit normal enteric flora while allowing for the selection of *C. jejuni*. Following inoculation plates were incubated at 42°C for 24-48h in an atmosphere containing 5% $O_2$, 10% $CO_2$, and 85% $N_2$ for development of mature colonies (Difco Manual, 1984; Joklik et al., 1988). Selenite broth was used in the detection of *Salmonella*. This broth enhances the growth of *Salmonella*, while reducing the growth of normal enteric flora. Following 24 h incubation at 37°C in selenite, XLD plates were inoculated by pipette with 1-2 drops of selenite broth and incubated for 24h at 37°C. XLD agar is a differential plating media designed to detect the presence of *Salmonella* and *Shigella* species from stool specimens. Plates with colonies typical of *Salmonella*, *Shigella*, or *Campylobacter* were kept for additional testing and further analysis.

## C. Secondary Screening

Following initial plating, at least two colonies appearing to be a *Salmonella* or *Shigella* were picked and transferred to Triple Sugar Iron Agar (TSI) and Lysine Iron Agar (LIA). TSI is used for the identification of gram negative enteric bacilli based on fermentation of dextrose, sucrose, and lactose, and for the production of hydrogen sulfide. LIA is a differential medium for the identification of *Salmonella* species based on the decarboxylation of lysine and the production of hydrogen sulfide. After inoculation, TSI and LIA media were incubated 24 h at 37°C. Following incubation, colonies were observed and those which appeared to be *Salmonella* or *Shigella* were confirmed biochemically. Appropriate colonies from Campy Blood Agar plates (oxidase positive) were reserved for gram staining and further analysis. Tests for *Campylobacter*, which are oxidase positive with gram negative helical rods, include rapid hippurrate hydrolysis and growth in nalidixic acid. Oxidase reagent and Gram Stain reagents (crystal violet, Grams Iodine, 95% ethyl alcohol, and safranin) were prepared in the lab at Emporia State.

## D. Biochemical Confirmation

Any bacterium appearing to be a *Salmonella* or *Shigella* was inoculated into a battery of biochemicals for further

identification. A computer coding identification system (CCIS) was incorporated into this protocol and provided a quick means of identification.

The CCIS system corresponds to the Roche Enterotube II. Roche Diagnostic Systems, Inc. developed this CCIS which employs the latest taxonomy to identify members of the family Enterobacteriaceae. In this method 15 biochemical tests are used in the confirmation of bacterial isolates. Included in these tests are: D-glucose, lysine decarboxylase, ornithine decarboxylase, hydrogen sulfide (determined from TSI and LIA), indole, adonitol, lactose, arabinose, sorbitol, dulcitol, phenylalanine, urea, Voges-Proskauer (VP), and citrate. For <u>Salmonella</u> serotype <u>arizona</u> confirmation malonate broth was also used. All reagents (Kovacs and VP reagents) were prepared in our lab. All biochemicals were prepared in test tubes which corresponded to the commercially prepared Enterotube II. An ID value was derived from a listing of the positive and negative reactions in the Enterotube II system. A five digit ID value is located in the Enterotube II biocodes section (Roche, 1988), and this number identifies the bacterium in question along with atypical tests. If at any time a particular bacterium could not be located in this system, the isolate was re-streaked on an appropriate screening plate for reisolation and the identification process was repeated. Also the Kansas Department of Health

and Environment policy and procedure manual for isolation of Salmonella, Shigella, and Campylobacter (1990) was referenced for other biochemical tests that would aid in confirmation.

### E. Salmonella Serotyping

Following positive biochemical identification of a member of the genus Salmonella, typing was needed to determine each serotype isolated. All Salmonella serotyping was performed at the KDHE lab with one isolate sent to CDC and the University of Missouri at Columbia for confirmation. Organisms were grown on tryptose agar slants (for preparation of the O antigens), and then grown in a flagellar broth of 50% Trypticase Soy Broth (TSB) and 50% Tryptose (TRY) for preparation of the H antigen. In the serological examination, the O antigens were identified first. To prepare O antigens, a tryptose slant was inoculated with the culture and grown 24 h at 37°C. The growth was then suspended in 1.0 ml of 95% ethyl alcohol. The preparation of the organism's O antigen was placed in a 60°C water bath for 60 minutes to inactivate the flagellar H antigen. Tubes were then centrifuged at 2580 rpm for 10 minutes, and the alcohol was decanted. Alcohol was drained by turning tubes upside down for 15-30 minutes at 37°C in an air incubator, then 0.5 ml of phenolized saline solution was added and the tubes were shaken vigorously to resuspend

the bacteria (Murlin, 1991).

The O antiserum was used for a slide agglutination test. One drop of alcohol treated O antigen was placed on a slide. A single drop of an equal amount of antiserum was added to the drop of O antigen, mixed with a toothpick, and gently tilted back and forth until granular agglutination occurred. Of all Salmonella cultures serotyped by the CDC, 98% belong to the first eight O groups Murlin, 1991). For this reason all cultures were tested for the first eight O groups. If no agglutination occurred in the first eight O groups, the O antigen was tested in eight additional O pools (polyvalent antiserum) which contained the remaining O antiserum (Murlin, 1991).

For final identification of Salmonella serotypes within a group, it was necessary to determine the H antigens and the phase of the organism. A flagellar broth (50% TSB and 50% TRY) was inoculated and grown for 24h at 37°C. The culture was inactivated by using an equal volume 0.6% formalin in saline. This provided the antigen used in the serotyping process. Antigen, 0.5 ml, was added to a Kahn type serological tube, and 0.5 ml of the appropriate serum dilution was added and incubated in a water bath at 50°C for 1 h (Difco Manual, 1984).

Certain H antigens occur in combination with O antigens, therefore initial determination of the O antigen leads to the appropriate H antisera to test first. There

are 59 H antisera, and in order to prevent cross reactions, H single factor antisera must be absorbed before use. An interesting characteristic about Salmonella is that they can either be monophasic or diphasic. Most Salmonella have a first and second phase. Sometimes both phases of a diphasic culture will be immediately apparent. However, often only one phase can be detected, especially among cultures recently isolated from singular colonies. When only one phase was detected, the culture was inoculated into a semisolid agar (Jordan's Semisolid) to which the antiserum of this phase had been added, this ties up the first phase exposing the second phase. The agar was melted, cooled and poured into a petri plate with 1-2 ml of sterile serum added. After mixing well, the agar was allowed to harden and was inoculated at one end of the plate. Following 24h incubation at 37°C, a sterile cotton swab was used to obtain inoculum from the opposite part of the petri plate from the original site of inoculation. The purpose of this is to enhance motility. Organisms which are most motile migrate farthest from the original site of inoculation. Inoculum was transferred to another flagellar broth where it was incubated 4-6 h at 37°C. The culture was inactivated by using an equal volume of 0.6% formalin in saline. Following inactivation, the second phase was determined in the same manner as the first phase (Murlin, 1991).

## F. SAMPLING

The first task was to collect fresh fecal samples from all accessible animals in both zoo populations to determine if bacterial pathogens were present. Following each sampling, specimens were taken to the lab within three hours after collection for analysis. On a few occasions zoo personnel at Emporia participated in collecting samples since the defecation times for some animals were early in the morning or late at night. Samples collected by zoo personnel were taken to the lab for analysis within 24h of collection. Animal species sampled in this study dates sampled, and number of samples collected/cage are presented in the Appendix.

Any animals from which bacterial pathogens had been isolated previously were sampled again. In this study 44 animal species were sampled from the Emporia Zoo and 32 animal species were sampled from the Topeka Zoo. Animals sampled included those in close proximity to a positive shedder as well as animals on the same basic diet regimen as a positive carrier. Some animals were sampled that had been negative in the initial screening process to determine if pathogens had been missed or introduced into the zoo populations after the beginning of the study. Of the animals sampled in the study, at no time was a report of ill health of any animals involved in the investigation.

## RESULTS

No *Campylobacter* or *Shigella* were isolated in this study. Thirty-two *Salmonella* serotypes were identified, 15 from the Emporia Zoo and 17 from the Topeka Zoo. Seven different serotypes were isolated during the three screenings at the Emporia Zoo (Tables 1,2, and 3). Of the 15 that were positive, eight were isolated from avian species, seven from reptiles, and none from mammals.

Five different *Salmonella* serotypes were isolated during the four screenings at the Topeka Zoo (Tables 4,5,6,and 7). One of those serotypes was from avian species, four were from reptiles and 12 were from mammals. The number of isolates noted represents the number of positive fecal samples collected per date.

Other types of bacteria commonly isolated from animals from both zoos included members in the genera *Proteus*, *Providencia*, *Morganella*, *Eschericia*, *Citrobacter*, and *Serratia*. In one of the samples taken from the African lions in the June, a hydrogen sulfide producing *Eschericia coli*, and a lactose negative *E. coli* were isolated which did not appear in other animal fecal samples.

Table 1. Salmonella Isolated From the Emporia Zoo on 6 February 1991.

| Bacterium | No. of Isolates | Reservoir |
|---|---|---|
| S. serotype anatum | 2 | Sarcorhamphus papa (King vulture) |
| | | Ara ararauna #1 (Blue gold macaw) |
| S. serotype panama | 2 | Lichanura trivrigata (Baby common boa) |
| | | Lichanura trivrigata (Adult common boa) |
| S. serotype arizona | 1 | Geochelone denticulata (Red-footed tortoise) |

Table 2. *Salmonella* Isolated From the Emporia Zoo on 3 May 1991.

| Bacterium | No. of Isolates | Reservoir |
|---|---|---|
| *S.* serotype *anatum* | 2 | *Ara ararauna* #1 (Blue gold macaw)<br>*Ara ararauna* #2 (Blue gold macaw*) |
| *S.* serotype *montevideo* | 1 | *Sarcorhamphus papa* (King vulture) |
| *S.* serotype *angola* | 1 | *Lichanura trivrigata* (Baby common boa) |
| *S.* serotype *arizona* | 1 | *Lichanura trivrigata* (Adult common boa) |

* from a different cage

Table 3. *Salmonella* isolated from the Emporia Zoo on 9 September 1991.

| Bacterium | No. of Isolates | Reservoir |
|---|---|---|
| *S.* serotype *montevideo* | 1 | *Sarcorhamphus papa* (King vulture) |
| | | *Ara chloroptera* (Green red macaw) |
| *S.* serotype *arizona* (group 3a) | 2 | *Lichanura trivrigata* (Adult common boa) |
| | | *Lichanura trivrigata* (Baby common boa) |
| *S.* serotype *menston* | 1 | *Cacatua galerita* (Cockatoo) |

Table 4. *Salmonella* Isolated From the Topeka Zoo on 30 May 1991.

| Bacterium | No. of Isolates | Reservoir |
|---|---|---|
| *S.* serotype *agona* | 1 | *Erethizon dorsatum* (Porcupine) |
| *Salmonella* serotype *tennessee* | 1 | *Vultur gryphus* (Andean condor) |
| *Salmonella* serotype *arizona* (group 3a) | 1 | *Gekko gecko* (Tokay gecko) |
| *Salmonella* serotype *uganda*[a] | 1 | *Panthera leo* (African lion) |

[a] KDHE typed as a *Salmonella sinstorph*

Table 5. *Salmonella* Isolated From the Topeka Zoo on 4 June 1991.

| Bacterium | No. of Isolates | Reservoir |
|---|---|---|
| *S.* serotype *eastbourne* | 2 | *Panthera leo* (African lion) |
| *S.* serotype *arizona* (group 3a) | 1 | *Gekko gecko* (Tokay gecko) |

Table 6. *Salmonella* Isolated From the Topeka Zoo on 2 July 1991.

| Bacterium | No. of Isolates | Reservoir |
|---|---|---|
| *S.* serotype *agona* | 4 | *Panthera leo* (African lion) |
| | 1 | *Mephitis mephitis* (Striped skunk[a]) |
| *S.* serotype *arizona* (group 3a) | 1 | *Gekko gecko* (Tokay gecko) |
| *S.* serotype *eastbourne* | 1 | *Felis lynx* (Lynx) |

[a] nonresident transient of the zoo

Table 7. *Salmonella* Isolated From the Topeka Zoo on 29 August 1991.

| Bacterium | No. of Isolates | Reservoir |
|---|---|---|
| *S.* serotype *agona* | 1 | *Panthera leo* (African lion) |
| *S.* serotype *eastbourne* | 1 | *Panthera tigris* (Siberian tiger) |
| *S.* serotype *arizona* (group 3a) | 1 | *Eunectes notaeus* (Yellow anaconda) |

## DISCUSSION

In my screening for Salmonella, Shigella, and Campylobacter in zoo animal populations, the only pathogens found were members of the genus Salmonella. At the Emporia Zoo eight Salmonella serotypes were isolated from avian species, seven from reptiles, and none from mammals. At the Topeka Zoo one Salmonella serotype was isolated from avian species, four reptiles and 12 from mammals.

In this study the only serotype isolated in each sampling was S. serotype arizona (group 3a). The fact that this Salmonella serotype was isolated is not surprising since many of the reptiles sampled in this study are known common carriers. Before restrictions were placed on the sale of turtles and other small reptiles, these animals were significant sources of infection in children (Joklik et al., 1990).

At the Topeka Zoo, Salmonella was isolated in all screenings. In some cages serotypes that were isolated varied considerably between samplings. This was especially true in the African lions containment area. In the first screening, S. serotype sinstorph was isolated and serotyped in the KDHE laboratory. Since this is a very rare isolate, it was sent to the CDC for further testing. Upon further analysis, the CDC indicated the actual serotype was S. serotype uganda. The reason given was that a cross reaction had occurred in the first phase involving KDHE

Salmonella antisera. A further confirmation was made by sending the isolate to the University of Missouri at Columbia. The isolate was also reported as S. serotype uganda. This serotype was never reisolated but is noteworthy because it is also a very rare isolate. Only 114 isolates of S. serotype uganda were reported from 1980-1990 (CDC Report, 1991). Other serotypes isolated from the African lions' cage included S. serotype eastbourne and S. serotype agona. Salmonella was also isolated in other containments but did not show the same variety of serotypes isolated. Most of the serotypes isolated in this study are similar to those results reported by the CDC from non-human sources (Table 8).

It may be important for zoo managers to consider potential avenues for transmission of bacterial pathogens. In the initial sampling of the animals at the Emporia Zoo, a king vulture and a blue-gold macaw were positive carriers of S. serotype anatum (Table 1). In the secondary screening, the same blue-gold macaw and a different blue-gold macaw (different cage) were positive for carrying S. serotype anatum (Table 2). The king vulture which shed S. serotype anatum in the first screening, shed S. serotype montevideo in the secondary screen. In the final sampling at the Emporia Zoo (Table 3), S. serotype menston was isolated from a cockatoo. S. serotype montevideo was re-isolated from a king vulture from the same cage, and was

Table 8. Ten Most Frequently Reported Serotypes of *Salmonella* from Non-human Sources[a]

| Serotype | | Number | Percent |
|---|---|---|---|
| *S.* serotype *typhimurium*[b] | | 1,956 | 26.4 |
| *S.* serotype *cholera-suis* | | 563 | 7.6 |
| *S.* serotype *heidlberg* | | 432 | 5.8 |
| *S.* serotype *agona* | | 259 | 3.5 |
| *S.* serotype *montevideo* | | 258 | 3.5 |
| *S.* serotype *anatum* | | 244 | 3.3 |
| *S.* serotype *saint-paul* | | 220 | 3.0 |
| *S.* serotype *enteritidis* | | 197 | 2.7 |
| *S.* serotype *sandiego* | | 186 | 2.5 |
| *S.* serotype *newport* | | 165 | 2.2 |
| | Total | 4,480 | 60.5 |

[a] Data reported to CDC and U.S. Dept. of Agriculture (Evans and Brachman, 1991)

[b] *S. typhimurium* includes var. *copenhagan*

isolated for the first time from a green-red winged macaw.

In all samplings at the Emporia and Topeka Zoos, isolation of Salmonella serotypes was successful, but this does not definitively show that transmission occurred. However, serotypes isolated at the Emporia Zoo, were from animals in the same building that were in close proximity of each other. So, distance between cages may be one factor that may help in explaining how serotypes isolated from one animal in the initial screening were isolated from a different animal just a few feet away in a second or third screening. In some health facilities (e.g., Kansas Department of Health and Environment) it is standard practice to collect and analyze three negative fecal samples before an individual is diagnosed as not having that pathogen in question. In my study it would have been extremely easy for an animal to be a carrier and been reported as negative since only one sample would not account for an animal in the carrier state they may intermittently shed a pathogen (Evans and Brachman, 1990). If only one or two samples are collected from an animal, there is the potential that pathogens may be missed.

Diet is a possible mode of transmission for the dissemination of bacterial pathogens throughout zoo animal populations. Since some of those animals which tested positive eat the same basic diet, one variable which may explain the emergence of different serotypes is the

foodstuff which each animal consumes. Salmonellae probably reach the zoos by commercial feeding products (a frozen meat product, referred to as zoo food, and a dry product referred to as dry food) which are utilized as the major source of animal feed (Richter and Al-Sheddy, 1990). Other items such as fruits and vegetables are also used in feeding and may have some role in bacterial infection (Manual of Clin. Micro., 1985). Whereas raw meat for human consumption is from animals that have been inspected by government agencies and must meet certain criteria before slaughter (Silliker et al., 1980; Brown, 1982), meat used in zoo food is not inspected by any agency and is obtained from aging and dead cows and horses (Richter and Al-Sheddy, 1990).

Richter and Al-Sheedy (1990) addressed questions about microbiological quality and the safety of zoo food, and discussed the overall quality of zoo food, the presence or absence of bacterial pathogens, and proper defrosting times. Data obtained by these reasearchers indicated some zoo meat contains pathogenic organisms (Salmonella spp.) which can threaten the health of zoo animals. After defrosting zoo food at different temperatures, these researchers found 10°C for 24h was an optimal temperature and time to defrost frozen zoo meat without affecting the quality or safety of the product. When meat was defrosted at 37°C, the result was an increase in bacterial

populations producing a Standard Plate Count (SPC) > $10^8$/g, which is considered to be the level of putrification and slime production (Ayers, 1955; Richter and Al-Sheddy, 1990). Defrosting meat at 25°C permitted the microbial population to increase, but to lower levels than when thawed at 37°C. When meat was defrosted at 10°C, there was little change in the level of organisms, which was expected since only psychrophilic organisms can grow rapidly at this temperature (Richter and Al-Sheddy, 1990). Dry zoo food was also analyzed, and exhibited no potential health risk from microbiological pathogens and is regarded as a safe foodsource (Richter and Al-Sheddy, 1990).

Salmonellae may be incorporated into commercial feed products at the time of processing. Animal remains are rendered in feedstuff, which allows the salmonellae to survive and be consumed by other animals. Salmonellae can reach food from animal feces at the time of slaughter, from human feces or from water polluted by human or animal sewage. It is possible several species of *Salmonella* may be incorporated into one packaged meat product. If several animals are used in processing, all products can be ground up at the same time, thus incorporating many different serotypes of a pathogen (i.e., *Salmonella*) in meat products for animal consumption (Brown, 1982). Determining if more than one serotype is present in a fecal specimen could be accomplished by picking more typical

*Salmonella* colonies from plated media with the assumption that each colony could potentially be a different serotype. This method might indicate whether it is possible for an animal to shed more than one *Salmonella* serotype at a time.

One pathogen that is commonly found in meat products is *Campylobacter*. In my study *Campylobacter* was not isolated from any animal, which is similar to the findings of Richter and Al-Sheddy (1990). If *Campylobacter* is not present in the diet the risk of an animal shedding this bacterium in feces may be greatly reduced.

*Salmonella* may have broad epidemiologic consequences in animal populations. Zoo animal populations may be affected by resistant bacteria, incoming animals, and cleaning practices. Resistant bacteria may present a major challenge to zoo personnel in treating sick animals. For some time the incorporation of antibiotics and chemotherapeutics in feed has been beneficial to animal producers for growth promotion. However, the use of these therapeutic agents such as the penicillins, tetracyclines, and sulphamides in veterinary practices and in animal husbandry for fattening purposes and for prophalaxis where disease risks are high have resulted in the appearance of strains of bacteria that are resistant to a wide range of these agents (Brown, 1982). Antimicrobial resistance in *Salmonella* may result from resistance genes (R-factors) that may be transfer via plasmids or other transposable

genetic elements (Evans and Brachman, 1991).

Movement of animals from one zoo population to another has been identified as a means of dissemination of disease (Evans and Brachman, 1991). The common means by which infection is reduced is through quarantine, where animals are completely isolated from other animals as well as from the public. Usually during this time, animals are screened for intestinal parasites, however it is not common practice to screen for bacterial pathogens. This may present a problem with respect to subsequent transmission. This, however, may not be a major problem for a zoo that does not often trade animals or sell them for exhibit.

A potential problem that may exist for zoo populations may be non-resident transients. At the Topeka Zoo, a sample was collected from a non-resident transient skunk (Table 6) which was residing in the cranes' containment. S. serotype alamo was isolated from this skunk. This species was not found in any other animals at the zoo, but could be interpreted as a potential disease situation.

Other areas of epidemiologic concern are cleaning methods. Cleaning processes at both zoos involve rinsing floors of cages and pens with water. If during rinsing, feed trays and/or drinking trays become contaminated with fecal material, these trays may act as a source for transmission. The need to regularly rinse or disinfect

cages and trays may be necessary to minimize the risk of infection to other animals.

## CONCLUSIONS and RECOMMENDATIONS

It is apparent from this research that some zoo animals are infected with bacterial pathogens (*Salmonella* spp). This is not to say that the potential for other pathogens (i.e., *Shigella* and *Campylobacter*) is non-existent, but these were not isolated in this study.

With *Salmonella* being present in zoo animal populations, close monitoring of animals' health must be maintained. If a protocol similar to that used in this study were initiated by zoo personnel, it would provide the opportunity to monitor the bacterial flora in animal populations. With the high cost of some animal species it would appear that periodic sampling would be of value as a means of prevention and diagnosis.

If this study were repeated, more samples should be collected over a longer period of time. To get a better idea of whether transmission occurred, every animal should be sampled at least three times to verify that they were a carrier or not. Questions to be addressed in further studies include correlation of bacterial presence in both diet and feces to determine if diet is the major source of pathogens. With the potential for an animal to be infected with more than one *Salmonella* serotype, the need to pick more colonies is essential. A challenge in a study like this is fecal specimen identification. In a containment with more than one animal it is hard to collect a fecal

sample from each occupant at one sampling, and to know which animal produced the specimen.

Zoo practices that are recommended to maintain healthy animal populations include the following:

1. CLEANING: Proper sanitizing and disinfecting high risk areas (i.e., feedtrays, cages, and benches).

2. SCREENING: Utilizing similar techniques implemented in this study to screen animals transported between zoos as well as current animal residents.

3. TRANSIENTS: Minimizing non-resident transients that populate and cohabitate with zoo animal populations. Since animals carry disease, those which are not cared for by zoo personnel may act as a source of infection.

4. FOOD: Implementing thawing procedures addressed by Richter and Al-Sheddy (1990) for zoo food could decrease bacterial populations, therefore reducing the potential for dissemination. The use of sterile gloves and strict hand washing by zoo personnel could be beneficial to zoo animals as well as the workers. Care in food handling is another avenue that could be used to prevent and control the transmission of bacterial pathogens through zoo animal populations.

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# APPENDIX

Emporia Zoo Specimens Sampled

| Genus/species (Common Name) | Date Sampled | No. of fecal specimens/cage |
|---|---|---|
| *Lemur variegatus* | 6 February 1991 | 2 |
| (Black/white rough lemur) | 3 May 1991 | 1 |
| | 9 September 1991 | 1 |
| *Lemur catta* | 6 February 1991 | 2 |
| (Ringtail lemur) | 3 May 1991 | 1 |
| | 9 September 1991 | 1 |
| *Dacelo novaeguineae* | 6 February 1991 | 1 |
| (Giant kingfisher) | | |
| *Ara chloroptera* | 8 February 1991 | 1 |
| (Green-red winged macaw) | 3 May 1991 | 1 |
| *Ara ararauna* #1 | 6 February 1991 | 1 |
| (Blue-gold macaw) | 3 May 1991 | 1 |
| | 9 September 1991 | 1 |
| *Testudo hermanii* | 6 February 1991 | 1 |
| (Red-footed tortoise) | 3 May 1991 | 1 |
| *Ramphastos tucanus* | 6 February 1991 | 1 |
| (Red-billed toucan) | 3 May 1991 | 1 |
| *Erethizon dorsatum* | 8 February 1991 | 1 |
| (Porcupine) | | |
| *Branta canadensis* | 8 February 1991 | 3 |
| (Canada goose) | | |
| *Ara ararauna* #2 | 6 February 1991 | 1 |
| (Blue-gold macaw) | 3 May 1991 | 1 |
| | 9 September 1991 | 1 |
| *Ardeola ibis* | 8 February 1991 | 1 |
| (Cattle egret) | | |
| *Threskiornis aethiopicus* | 8 February 1991 | 1 |
| (Sacred ibis) | | |

| | | |
|---|---|---|
| Ara ararauna #3<br>(Blue-gold macaw) | 6 February 1991<br>3 May 1991<br>9 September 1991 | 1<br>1<br>1 |
| Ara ararauna #4<br>(Blue-gold macaw) | 6 February 1991<br>3 May 1991<br>9 September 1991 | 1<br>1<br>1 |
| Cynomys ludovicianus<br>(Black-tail prarie dog) | 3 May 1991 | 2 |
| Cereopis novahollandiae<br>(Ceropis goose) | 8 February 1991 | 1 |
| Cacatua galerita<br>(Sulpher-crested cockatoo) | 6 February 1991<br>3 May 1991<br>9 September 1991 | 1<br>1<br>1 |
| Speotyto cunicularia<br>(Burrowing owl) | 8 February 1991<br>3 May 1991 | 1<br>1 |
| Aix sponsa<br>(North American wood duck) | 6 February 1991<br>3 May 1991 | 2<br>1 |
| Lichanura trivrigata<br>(Common boa-adult female) | 6 February 1991<br>3 May 1991<br>9 September 1991 | 1<br>1<br>1 |
| Lichanura trivrigata<br>(Common boa-baby) | 6 February 1991<br>3 May 1991<br>9 September 1991 | 1<br>1<br>1 |
| Ammotragus lervia<br>(Aoudad) | 8 February 1991 | 3 |
| Tayassu tajacu<br>(Collard peccary) | 8 February 1991 | 1 |
| Lama glama<br>(Llama) | 8 February 1991 | 2 |
| Bovis taurus<br>(Longhorn) | 8 February 1991 | 1 |
| Bison bison<br>(Bison) | 8 February 1991 | 1 |
| Odocoileus hemionus<br>(Mule deer) | 8 February 1991 | 2 |

| | | |
|---|---|---|
| *Pavo crisatus*<br>(Peacock) | 8 February 1991 | 1 |
| *Cygnus cygnus*<br>(Whooper swan) | 6 February 1991 | 1 |
| *Anas rubripes*<br>(American black duck) | 8 February 1991 | 1 |
| *Cygnus olor*<br>(Mute swan) | 6 February 1991 | 1 |
| *Lophura nycthemera*<br>(Japanese pheasant) | 6 February 1991 | 1 |
| *Speotyto cunicularia*<br>(Great horned owl) | 6 February 1991 | 1 |
| *Sarcorhamphus papa*<br>(King vulture) | 6 February 1991<br>3 May 1991<br>9 September 1991 | 2<br>2<br>1 |
| *Nasua narica*<br>(Coati mundi-male/female) | 6 February 1991<br>3 May 1991 | 2<br>2 |
| *Cygnus atratus*<br>(Black swan) | 8 February 1991 | 1 |
| *Macropus rufogriseus*<br>(King island wallaby) | 8 February 1991 | 3 |
| *Amazona ochrocephala*<br>(Yellow headed amazon) | 6 February 1991 | 1 |
| *Haliaeetys leucocephalus*<br>(Bald eagle-male/female) | 8 February 1991 | 2 |
| *Otus asio*<br>(Screech owl) | 6 February 1991 | 1 |
| *Felis rufus*<br>(Bobcat) | 6 February 1991 | 1 |
| *Dromaius novaehollandiae*<br>(Emu) | 6 February 1991 | 1 |
| *Potos flavus*<br>(Kinkajou) | 6 February 1991<br>3 May 1991 | 1<br>1 |

| | | |
|---|---|---|
| *Taxidea taxus* (Badger) | 6 February 1991 | 1 |
| *Cygnus buccinator* (Trumpeter) | 8 February 1991 | 1 |
| *Anas cyanoptera* (Cinnamon teal) | 8 February 1991 | 1 |
| *Aix galericulata* (Mandarin) | 8 February 1991 | 1 |

Topeka Zoo Specimens Sampled

| Genus/species (Common Name) | Date Sampled | No. of fecal specimens/cage |
|---|---|---|
| *Panthera leo* (African lion) | 30 May 1991 | 2 |
| | 4 June 1991 | 4 |
| | 2 July 1991 | 4 |
| | 29 August 1991 | 5 |
| *Equus burchelli* (Grant's zebra) | 30 May 1991 | 2 |
| *Equus przewalskii* (Asian wild horse) | 30 May 1991 | 1 |
| *Panthera tigris* (Siberian tiger) | 30 May 1991 | 1 |
| | 4 June 1991 | 1 |
| | 29 August 1991 | 1 |
| *Articus binturong* (Binturong) | 30 May 1991 | 1 |
| | 2 July 1991 | 1 |
| *Felis lynx* (Lynx) | 30 May 1991 | 1 |
| | 2 July 1991 | 2 |
| *Elephas maximus* (Asian elephant) | 30 May 1991 | 1 |
| | 2 July 1991 | 1 |
| *Loxodonta africana* (African elephant) | 30 May 1991 | 1 |
| | 2 July 1991 | 1 |
| *Giraffa camelopardalis* (Reticulated giraffe) | 30 May 1991 | 1 |
| | 2 July 1991 | 1 |
| *Colobus quereza* (Black/white colobus) | 30 May 1991 | 1 |
| | 2 July 1991 | 3 |
| *Procavia capensis* (Rock hyrac) | 30 May 1991 | 1 |
| | 2 July 1991 | 1 |
| *Muntiacus reevesi* (Reeves muntjac) | 30 May 1991 | 1 |
| | 4 June 1991 | 1 |
| *Anthropoides virgo* (Demoiselle crane) | 30 May 1991 | 1 |

| | | |
|---|---|---|
| *Erethizon dorsatum* | 30 May 1991 | 1 |
| (Porcupine) | 4 June 1991 | 1 |
| *Felis concolor* | 30 May 1991 | 1 |
| (Puma) | 4 June 1991 | 1 |
| | 2 July 1991 | 1 |
| | 29 August 1991 | 1 |
| *Alopex lagopus* | 30 May 1991 | 1 |
| (Artic fox) | 2 July 1991 | 1 |
| *Macaca fuscata* | 30 May 1991 | 1 |
| (Japanese macaque) | 2 July 1991 | 1 |
| *Pongo pygmaeus* | 30 May 1991 | 2 |
| (Orangutan) | 4 June 1991 | 2 |
| | 29 August 1991 | 2 |
| *Gorilla gorilla* | 30 May 1991 | 2 |
| (Gorilla male/female) | 4 June 1991 | 2 |
| | 29 August 1991 | 2 |
| *Tragecephalus spekei* | 30 May 1991 | 1 |
| (Sitatunga) | 4 June 1991 | 1 |
| *Aonyx cinerea* | 30 May 1991 | 1 |
| (Otter) | | |
| *Taurotragus oryx* | 30 May 1991 | 1 |
| (Common eland) | | |
| *Echinops telfairi* | 30 May 1991 | 1 |
| (Hedgehog tenrec) | | |
| *Vultur gryphus* | 4 June 1991 | 1 |
| (Andean condor) | | |
| *Grus antigone* | 2 July 1991 | 1 |
| (Sarus crane) | | |
| *Eunectes notaeus* | 2 July 1991 | 1 |
| (Yellow anaconda) | | |
| *Ara ararauna* | 4 June 1991 | 1 |
| (Blue-gold macaw) | | |
| *Procyon lotor* | 30 May 1991 | 1 |
| (Raccoon) | | |

| | | |
|---|---|---|
| Mephitis mephitis (Skunk-nonresident) | 29 August 1991 | 1 |
| Nasua narica (Coati mundi) | 30 May 1991 | 1 |
| Boa constrictor (Common boa) | 30 May 1991 | 1 |
| Eptesicus fuscus (Fruit bat) | 30 May 1991 | 1 |
| Gekko gecko (Tokay gecko) | 30 May 1991 | 2 |
| | 4 June 1991 | 1 |

Signature of Graduate Student

Signature of Major Advisor

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Signature of Graduate Office Staff Member

May 18, 1992
Date Received