SALMONELLA TYPHIMURIUM IN THE ZOO SETTING: CONSIDERATIONS OF DISEASE RISK IN CAPTIVE BREEDING FOR CONSERVATION PROGRAMS

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There are many examples of captive breeding in zoo settings to aid the conservation of species which are threatened or endangered. Captive breeding of many species for release comes with a plethora of positive and negative side effects including improved adaptation to a changed local environment, expansion of genetic variation and increase in population or maladaptation to an unchanged environment, introduction of disease, poor predator recognition and poor foraging ability.

Zoos South Australia (ZoosSA) has been involved with a rescue effort in collaboration with South Australia's Department of Environment and Heritage (DEH) to maintain a back up population of Malleefowl (*Leipoa ocellata*) from the Ferris MacDonald Conservation Park which borders Monarto Zoo. Within this park is a unique and isolated group of Malleefowl which, from active mound monitoring, may number only 2-3 breeding pairs. Because this park is so isolated, with extreme fire risk throughout the summer period, efforts are being made to ensure these unique genetics are maintained . Keepers from ZoosSA, alongside rangers from DEH, have been monitoring mounds and collecting eggs for incubation to create a back up population, with the aim of establishing a free ranging population at Monarto Zoo. However, bringing these individuals into captivity may potentially expose them to bacterial, fungal and viral agents commonly seen in zoos. *Salmonella* is one such pathogen; this paper will present two cases which illustrate this risk.

***Salmonella* species are well recognized pathogens in people, livestock, wild mammals, birds and reptiles, and even insects. They are common pathogens in zoological parks where many species are kept in close approximation. Salmonellosis is the most widespread zoonosis in the world[1]. They are straight, small gram negative rods 0.7-1.5 x 2.0-5 µm. Most strains are motile but some non-motile forms do occur and all grow on common media. Propagation can occur outside the host if the correct ambient temperature and nutrients are available[1].**

Most vertebrates are able to be infected with *Salmonella* spp. Host susceptibility and development of a carrier state varies widely among species. Free ranging birds can be subclinical carriers and serve as reservoirs for an aviary[3]. However, birds probably play a minor role in the epidemiology of *Salmonella* infection in mammals and the probability of humans or domestic animals contracting *Salmonella* from wild animals is low[1]. Despite this, they are able to act as point sources of infection to other avian species. In captive situations, a high standard of husbandry and hygiene, with attention to rodent control and fecal contamination, should minimize the risk of disease[4]. However, salmonellae are ubiquitous and *S.* Typhimurium is one of the most widespread bacterial pathogens

in the world[1]. Thus, cases of salmonella are still relatively common in zoological parks where large aggregations of multiple species exist.

***Salmonella* Typhimurium is the most common psittacine and free living avian isolate[4]. Examples in the literature of death and disease from *S* typhimurium abound in both psittacine and non-psittacine birds[2,5,6,7]. Transmission is via ingestion of contaminated food or water or by direct contact with aerosolized faecal or feather dust, where it can survive for extended periods. Faeces of chronically infected carrier birds are one of the most common sources of infection. Food stuffs handled improperly are also common sources. *Salmonella* Typhimurium can penetrate the eggs of chickens to the chorioallantoic membranes and yolk in 10 minutes. Moisture enhances penetration but is not essential[4].**

The disease may be peracute, acute, to chronic or subclinical, depending on the condition of the host and a number of other factors. Clinical signs include depression, lethargy, weight loss, anorexia, diarrhea, lameness, abscess formation and poor hatching or fledging mortality and sudden death[4]. Classical lesions include hepatomegaly, splenomegaly, pneumonia, catarrhal to haemorrhagic enteritis and in some cases, meningitis and osteoarthritis. Granulomatous osteomyelitis has also been described. Diagnosis relies on culture of affected organ systems or faeces and treatment is prolonged (3-8 weeks) often without eliminating the organism from the host[4].

In January 2009, a 4 year old Mandarin Duck (*Aix galericulata)* presented with a closed mid-shaft fracture to the right tibiotarsus at Adelaide Zoo. The bird was in good body condition and was anaesthetised for the fracture to be radiographed, reduced and splinted. Radiographs revealed a multifocal granulomatous osteomyelitis. The fracture in the right leg was a pathological fracture from one such lesion. The bird also had a very unstable anaesthetic and was euthenased on radiographic findings. Subsequent post mortem findings revealed numerous round defined granulomatous lesions in long bones (metaphyses and diaphyses) including tibia, femur, radius, scapula, metacarpal, as well as encapsulated soft tissue granulomas in liver, kidney, lung and spleen. Histology revealed circumscribed nodules in long bones composed of centrally necrotic areas with laminar deposits of degenerating inflammatory cells surrounded by layers of multinucleated giant cells, histiocytes and heterophils. Large collections of rod-shaped bacteria were present throughout the necrotic debris. Similar lesions were embedded in the liver, kidney, and spleen, with no apparent pathology in tissue parenchyma. Differential diagnoses included mycobacteriosis, *Nocardia, Actinomyces*, mycotic granulomas, coligranulomas (*E. coli*) and botryomycosis *(Staphylococcus).* No acid fast bacteria were seen with ZN staining. Bacterial culture yielded a pure growth of *S.* Typhimurium.

Subsequently, all Mandarin ducks from the area were caught and radiographed to screen for granulomas. All radiographs were normal. Samples were not collected for culture and sensitivity testing.

Six months later one of three Malleefowl chicks hatched from eggs collected from Ferris MacDonald Conservation Park was found dead at 6 weeks old. The two other chicks in the same enclosure were unaffected. Gross post mortem revealed marked thick white-yellow fibrinous exudates coating air sacs over the heart, lungs, and liver. Large coalescing yellow-green exudative lesions in lung parenchyma were also evident. Differential diagnoses included Gram-negative septicaemia, *Chlamydophila* and *Erysipelothrix*. *Erysipelothrix rhusiopathiae* has caused mortality in captive Malleefowl populations in other institutions[8] and Erysipelas had been diagnosed in a black cockatoo at Adelaide Zoo 2 months earlier. Histology revealed the pericardial sac was markedly thickened, with fibrinoid material and mixed inflammatory cells present that were infiltrating the adjacent myocardial tissue. There were large collections of small bacterial rods present. There was oedema and large numbers of heterophils and plasma cells infiltrating the connective tissue and filling the airspaces in the lungs as well as large numbers of small bacterial rods present. Thickening, oedema and bacterial rods were also present in the airsacs, liver, spleen and digestive tract. The Malleefowl chick was diagnosed with fibrinopurulent air sacculitis, pneumonia, pericarditis, myocarditis and splenic hyperplasia. These were indicative of a severe systemic bacterial infection, Culture confirmed *S. typhimurium*.

***Salmonella* Typhimurium has historically been cultured from Red- Collared Lorikeets (*Trichoglossus haematodus rubritorquis*) that died acutely in an aviary bordering the quarantined Malleefowl aviary and the Mandarin Duck exhibit.**

The epidemiology of these cases of salmonellosis is not clear. Transmission from the parents in the mound is possible in the case of the Malleefowl, although this is unlikely due to the dry environment and the relatively acute nature of the lesions. To our knowledge, free-ranging Malleefowl have not been surveyed for *Salmonella*. Spread between enclosures via fomites, wind, and pest species is possible given the close proximity of the enclosures in question. Free ranging birds within the zoo could shed *Salmonella* in faeces, contaminating the various aviaries. Equally, *S.* Typhimurium is commonly isolated from humans, and a human source of infection could be considered.

Cage mates of the Malleefowl and Mandarin Duck did not show any evidence of *Salmonella* infection (although bacterial culture was not performed). It is possible that these individuals were exposed to a higher infective dose, and/or were stressed or otherwise immunocompromised relative to their cohorts.

These are relatively isolated cases, but they highlight the risk of disease transmission to highly endangered animals being reared in a captive setting. Zoos are frequently involved with such captive breeding efforts due to husbandry expertise and veterinary care. However, the close proximity to many other species, and the disease risk this presents, needs to be recognised. Maintaining high standards of husbandry, hygiene and quarantine are vital to the success of captive breeding programs.

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