

## **Investigation of the Outbreak of Fowl Cholera in the Coorong Region**

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## **Abstract**

**Objective** *Pasteurella multocida* is the aetiological agent of Fowl Cholera which affects a wide variety of domestic and wildlife avian species worldwide. This paper aims to document the Fowl Cholera outbreak which was first detected among waterfowl in the Coorong RAMSAR wetland, South Australia in January 2024.

**Design/Procedure** Post-mortem examination of collected carcasses was undertaken for gross pathology, bacterial culture, and histopathology. Frozen liver samples were sent to the Veterinary Pathology unit at the University of Melbourne for isolation and sequence typing of *P. multocida* to compare with other Australian isolates. Stakeholders relevant to the outbreak, including from management agencies, the local community, and First Nations, were interviewed to gain their perspectives on the outbreak.

**Results** The outbreak occurred from January 9<sup>th</sup>, 2024, to approximately March 24<sup>th</sup>, 2024; carcasses were mainly observed at the Pelican Point, North Lagoon, Coorong. The number of deaths was estimated between 500 to 1200 birds. The Coorong *P. multocida* isolates and previously sequenced avian isolates across Australia belonged to sequence type (ST) 20.

**Conclusions** The outbreak emphasised the need to implement preventative measures and raise public awareness to help manage outbreaks that may occur in the future. Genetic similarities of *P. multocida* across Australian states suggests the possible transmission between wildlife and free-range poultry farms, with the direction and mechanism of transmission not fully understood. Further study on potential risk factors specific to the nature of wetland and the importance of biosecurity is essential to maintain this valuable ecosystem in the future.

**Keywords** *Pasteurella multocida*; Coorong; RAMSAR wetland; Fowl Cholera; Avian Cholera

**Abbreviations** AI, Avian Influenza; APMV-1, Avian Paramyxovirus Type One; DEW, Department of Environment and Water; gDNA, genomic DNA; km, kilometre; LPS, Lipopolysaccharide; MALDI-TOF, Matrix Assisted Laser Desorption Ionization – Time of Flight; MVS, Melbourne Veterinary School; NPWS, National Parks and Wildlife Service; PCR, Polymerase Chain Reaction; PFGE, Pulse Field Gel Electrophoresis; PIRSA, Department of Primary Industries and Regions, South Australia; ST, sequence type

## Introduction

In 1881, Louis Pasteur first described *Pasteurella multocida* as the causative agent of Fowl Cholera (sometimes referred to as Avian Cholera) (Pasteur, 1880; Pasteur, 1881). The vaccine developed by Pasteur at the time for this disease was a pioneering milestone in the field of immunology (Berche, 2012). *P. multocida* has been classified into five capsular serogroups (A, B, D, E, F) and 16 somatic serotypes (1-16), with serogroup A mostly isolated from acute Fowl Cholera cases (Harper et al., 2006). Bacteria enter the host system via the respiratory tract and cause a rapid and fatal septicaemia (Allen et al., 2024). Acute pasteurellosis may result in bird deaths within six to 12 hours of exposure (Friend et al., 1999). Chronic suppurative necrosis may also be observed at necropsy, however, clinical signs in live birds are short-lived, non-specific to *P. multocida* and thus not clinically relevant (Niemi, 2021). Fowl Cholera may also mimic other causes of sudden death in poultry such as Botulism, Avian Paramyxovirus Type One (APMV-1, also known as Newcastle Disease) and Highly Pathogenic Avian Influenza (Aiello et al., 2016).

Fowl Cholera is a significant infectious disease having a worldwide distribution (Blanchong et al., 2006). A recent French study conducted on seabirds at Amsterdam Island in the Indian Ocean over three decades reported its detrimental effects on (i) reducing total population; and (ii) reducing reproductive success rates (Jaeger et al., 2018). Furthermore, a Fowl Cholera outbreak in wild waterfowl was first reported in China in the Ordos Wetland, Inner Mongolia in 2007 - with 3,300 deaths of 11 wild waterfowl species documented over an 11-day surveillance period (Wang et al., 2009). Most strikingly, however, this disease has had severe impact especially on wild waterfowl in North America, with frequent mass mortality events (Botzler, 1991). For example, over 30,000 deaths of primarily diving ducks were reported to be caused by *P. multocida* in an outbreak which

took place in the largest estuary in the United States of America, the Chesapeake Bay, in 1978 (Montgomery et al., 1979). Despite these documented events, there are limited records of the physical characteristics and clinical signs prior to death, and the epidemiology including causal factors leading to these epizootics is not well understood.

Mortality associated with Fowl Cholera also imposes a huge impact on the poultry industry, especially in broiler breeders due to the reduction of eggs hatched and an overall decrease in fertility (Huberman & Terzolo, 2016). Choudhury et al. (1985) and Morris & Fletcher (1988) estimated the mortality rate to range between 25-35% with an income loss of US\$0.015/kg meat. In early stages of the disease outbreak, mortality rates can range from 5% to 20%, with potential risks of further increasing as the disease progresses (Pilatti et al., 2016).

Isolates of *P. multocida* are found in a wide range of avian species, including chickens, turkeys and waterfowl (Botzler, 1991), while the virulence of the organism is strain-dependent (Petersen et al., 2001). The results of a challenge experiment in which turkeys and chickens were intratracheally infected with isolates from a waterfowl cholera outbreak theoretically supports the idea that *P. multocida* can be cross-infected among different avian species and wild birds can serve as a carrier and reservoir of Fowl Cholera for domestic birds (Petersen et al., 2001).

The epidemiological approach of strain typing the bacterium responsible for outbreaks facilitates research into disease transmission in wildlife, since the site of exposure is always distant from the location where birds are found dead due to seasonal migration patterns (Friend et al., 1999). Whole genome sequencing has been used widely in recent times to explore the strain of *P. multocida* causing an outbreak as well as determining overall virulence by screening for known virulence-associated genes (Peng et al., 2021).

A recent Australian study documented the presence of a unique *P. multocida* multilocus sequence type (ST) 20 in both local poultry farms and wild waterfowl between 2009 and 2022 (Allen et al., 2024). According to the PubMLST database, no ST20 isolates have been reported outside Australia (Smith et al., 2021). Pulse Field Gel Electrophoresis (PFGE) profile analysis was performed on isolates, and the dendrograms generated from it showed that isolates from free-range farms and from wildbirds fell into the same cluster, exhibiting more than 85% similarity in band profiles, except for one outlying free-range isolate (Allen et al., 2024). The two wildbird (waterfowl) isolates were obtained from a Chestnut Teal (*Anas castanea*) reported dead in March 2013 in a wetland at Werribee, Victoria, Australia and a Black Swan (*Cygnus atratus*) reported dead in May 2013 at Lake Tooliorook, Victoria, Australia, approximately 120 kilometres (km) West of Werribee Wetland. *P. multocida* was isolated from the livers of both wild birds. These isolates were approximately 98% similar and showed >95% similarity within the same cluster, with three strains originating from two free-range chicken farms in Queensland and Victoria. Repetitive sequenced based polymerase chain reaction assays also supported the findings from PFGE analyses. Whole genome sequencing by Illumina platform was also conducted to analyze the two Victorian wild bird isolates and the five strains from the Victoria and Queensland free-range farms and they all belonged to ST20 (RIRDC scheme). These contributed to a total of 25 ST20 isolates stored at the Melbourne Veterinary School (MVS) Clinical Microbiology Diagnostic Laboratory, Werribee. To investigate the phylogenetic structure of the ST20 isolates, the 25 isolates were then compared to 280 *P. multocida* genomes from 23 countries from the GenBank and 94 Queensland strains of unassembled Illumina sequences by construction of a Maximum Likelihood phylogenetic tree with core single nucleotide polymorphism aligned. Both results showed ST20

isolates formed a single phylogenetic clade, possessing either Lipopolysaccharide (LPS) type one or type three loci. These results illustrate the uniqueness of the genetic similarities of *P. multocida* in free-range farms and wild birds, as well as their marked differences from other avian isolates, not restricted to chickens, from different farming systems. Molecular diagnostic techniques were shown to be valuable tools to aid in understanding transmission patterns and to contribute to epidemiological investigations. Diversity of the public *P. multocida* genome library and knowledge of the prevalence of *P. multocida* in Australia were expanded (Allen et al., 2024).

The outbreak of Fowl Cholera documented in the present study occurred from January to March 2024 in the Coorong region, South Australia, where mortality of waterfowl was reported. The Coorong, and Lakes Alexandrina & Albert Wetlands were recognised as a Ramsar Wetland of International Importance in 1985. This area contains 23 unique wetland types – ranging from freshwater to saline, dense vegetation to open water, and temporary to permanently inundated – supporting a large variety of waterbird species (Prowse, 2020; Prowse et al., 2022). In 2012, within the 307 bird species recorded in this area, 119 were wetland bird species (O'Connor et al., 2012; Information Sheet on Ramsar Wetlands (RIS), 2013).

Here we report the sequence typing of the *P. multocida* isolated from the Fowl Cholera outbreak in wild waterfowl in the Coorong and their relationship to previously obtained ST20 isolates. We also aim to canvas the perspectives and understand the viewpoints of different stakeholders responding to the outbreak. They include the management agencies, the local residents and the First Nation community. We also discuss the potential risk factors that may contribute to the

outbreak. In addition, we identify current and future possible management actions to address outbreaks to facilitate disease preparedness for future occurrences.

## **Materials and Methods**

### **A. Veterinary diagnostic investigation**

A total of four frozen and eight freshly dead Grey Teals (*Anas gracilis*) were collected by a member of the public and sent to Gribbles Veterinary Pathology Laboratory at The Department of Primary Industries and Regions (PIRSA), Glenside, South Australia by a Department for Environment and Water (DEW) National Parks and Wildlife Service (NPWS) Ranger. The following diagnostic investigations were undertaken.

#### 1. Testing for exotic disease by Polymerase Chain Reaction (PCR)

Prior to post-mortem, choanal and cloacal swabs were collected from the eight freshly dead ducks and subjected to PCR to rule out two notifiable exotic diseases – Avian Influenza (AI) and APMV-1.

#### 2. Gross pathology

Post-mortem examinations were performed on six freshly dead ducks. Fresh tissue samples collected included brain, proventriculus, ventriculus, gut, kidney and liver, which were either stored at -20°C or submitted for microbial culture. Samples including brain, trachea, heart, lung, liver, spleen, gut, and kidney, were preserved in formalin for histopathological sectioning. Gross lesions were described and compared to assess agreement with the typical pathology associated with Fowl Cholera.

#### 3. Microbiology

Samples were obtained from faeces, kidney and liver. All samples were grown on chocolate agar, Columbia Nalidixic Acid agar, MacConkey Agar in both 5% CO<sub>2</sub> and

37°C aerobic incubators. The plates were read at 24 hours and then at 48 hours. *P. multocida* does not grow on MacConkey agar but grows well on chocolate agar to show circular colonies. Organisms were identified using the Matrix Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) mass spectrometry at Australian Clinical Laboratories, Adelaide Airport. Mixed-growth culture was also followed up by induction of a purity plate to obtain single colonies.

#### 4. Histopathology

Tissues preserved in formalin from gross pathology were dehydrated and embedded in paraffin. Paraffin-embedded tissues were sectioned at 4–6 micron and stained with routine haematoxylin and eosin (H&E) for histopathological studies (Tripathi, Srivastava & Kumar, 2009).

### **B. Epidemiological data collection**

A database of cases was built following interviews with PIRSA, Rangers and locals that responded to the outbreak. Information obtained included:

1. Species involved
2. Number of reported deaths and confirmed cases of Fowl Cholera
3. Location of the carcasses reported
4. Start and end dates: First and last reported cases, with the consideration of the seven-day incubation period (Carpenter et al., 1996).

### **C. Interview**

Stakeholders that were involved in or had a role in the response to the outbreak were interviewed. The framework of questions focused primarily on the suggested transmission patterns and management strategies employed to address the outbreak as follows.

Question included, but were not limited to:

Describing the management plan in place:

1. Is the interviewee involved in or aware of any management plan put in place regarding the outbreak? (e.g., surveillance, carcass collection)
2. How would they evaluate the management plan in terms of effectiveness?
3. From their perspective, what is the ideal management strategy for the outbreak, including preventative and remedial measures?
4. What is the major risk factor that should be targeted for future outbreaks?

#### **D. Molecular Epidemiology**

Two frozen liver samples were sent to MVS Clinical Microbiology Laboratory and were stored at -80°C. Samples were grown and inoculated on 5% Sheep Blood Agar for identification of *P. multocida* based on morphology and biochemical tests. *P. multocida* is identified microscopically as a Gram-negative, rod-shaped bacteria in Gram staining and bipolar in Leishman's staining, and biochemically, is positive for oxidase and the indole test and negative for the urease test (Panna et al., 2015). Bacterial colonies were subcultured to confirm purity for the extraction of genomic DNA (gDNA) using the QIAamp DNA Mini Kit. gDNA was sequenced by Illumina HiSeq at the Peter Doherty Institute, University of Melbourne. Adaptor removal, trimming of sequences, and preparation of libraries were performed as described previously (Allen et al., 2024). In addition, the isolates were sequenced on a Nanopore flowcell (FLO-MIN106 R9.4.1) fitted on a MinION MK1b device at MVS Clinical Microbiology Laboratory. The resulting fragments were assembled using either short read only or long and short read hybrid assemblies with Unicycler v0.5.0 (Wick et al., 2017). Assembled sequences were then assessed for sequence typing in accordance with both the Multihost and the RIRDC schemes from the PubMLST database (Jolley et al., 2018).

## Results

### Overview

The Fowl Cholera outbreak was first reported on 9<sup>th</sup> January 2024 and mortalities reported up until the 24<sup>th</sup> March 2024. Approximately 1200 deaths were documented, predominantly among Grey Teals. Most of the carcasses were found near Pelican Point (-35.609, 139.043) extending to the Murray Mouth, affecting a four km wide area within the North Lagoon of Coorong as shown in Figure 1. Local B noticed the first batch of dead Grey Teals in Coorong and reported it to Local A residing in the region. Assistance from PIRSA and DEW were soon sought by Local A for further investigation.

### Mortality count

Reported deaths by Locals A and B, one DEW Staff and DEW NPWS Rangers A and B over time are shown in Table 1. A total of thirteen species reportedly involved in the outbreak were as follows:

- Blue-winged Teal (*Spatula discors*)
- Grey Teal (*Anas gracilis*)
- Chestnut Teal (*Anas castanea*)
- Hardhead (*Aythya australis*)
- Musk Duck (*Biziura lobata*)
- Black Duck (*Anas superciliosa*)
- Australian Shelduck (*Tardona tadornoides*)
- Black Faced Cormorant (*Phalacrocorax fuscescens*)
- Great Crested Grebe (*Podiceps cristatus*)
- Seagull (*Larinae spp.*)
- Black Swan (*Cygnus atratus*)
- Australian Pelican (*Pelecanus conspicillatus*)
- Magpie-lark (*Grallina cyanoleuca*)

Table 1. Death of birds reported by the Locals, Rangers and DEW Staff. Please note that some affected species were not recorded, and specific details were not provided. Reporting showed significant diminishing of bird deaths after March. On 19<sup>th</sup> January, 2024, Ranger B observed a large congregation of live Great Crested Grebes at the Tauwitchere Barrage. On 26<sup>th</sup> February 2024, Ranger B reported one cormorant displaying lethargy and lack of coordination and several unwell ducks. This was excluded on this table as the birds were still alive.

| Date         | Mortality report  | Location   | Observer               |
|--------------|---|--|------------------------|
| 9-10/1/2024  | 12 dead ducks: one Mountain Duck, two Chestnut Teals, nine Grey Teals   | Mark Point Boat Ramp/Rumbelows Hut, near Pelican Point                   | Local B                |
| 11-17/1/2024 | Approximately four to five freshly dead duck on most days   | Mark Point   | Local B                |
| 16/1/2024    | Four Frozen and eight Freshly dead ducks were collected by Local B and were submitted to PIRSA by NPWS Rangers  | Not described (Between Ewe Island and Mark Point)                        | Local B                |
| 17/1/2024    | Four deaths washed up by boat moorings, including one Chestnut Teal   | Mark Point   | Local B                |
| 18/1/2024    | 71 dead birds in various stages of decomposition (Species not specified); 2 dead birds on adjacent roadway  | SA Water Barrage gates at Pelican Point to Rumbelows Hut (around two km) | Ranger A               |
| 18-28/1/2024 | Six to eight dead ducks per day, including one Australian Shelduck  | Middle of Coorong  | Local B                |
| 19/1/2024    | 115 dead birds, predominantly Teal ducks  | Near Tauwitchere Barrage   | Ranger B and DEW Staff |
|              | Five dead birds (species not mentioned)   | One km in either direction from Mark Point boat ramp                     |                        |
| 25-31/1/2024 | Up to 20 dead birds per day. On 30/1/2024, two dead Swans and one dead seagull were found in the shoreline from Ti-trees to Pelican Point.            | Pelican Point  | Local B                |
| 2/2/2024     | Well over 100 dead birds including a wide diversity of waterfowl, mucus discharged from nasal passages was observed. Dead magpie larks also observed. | Pelican Point (Less than 1km of shoreline)                               | Local A                |
| 20/2/2024    | Six freshly dead birds (species not mentioned) on water's edge and five dead birds on the adjacent road   | Same as 18/1/2024  | Ranger A               |

|              |  |   |                        |
|--------------|--|---|------------------------|
| 26/2/2024    | 333 dead birds in varying stages of decomposition, 17 very freshly dead birds (estimated under two days old). An additional 13 dead ducks floating on the water or ashore. Most deaths were Teal ducks, but a higher proportion of Shelducks, especially in the fresh specimen, was noticed, accompanied by few Cormorants and Musk Ducks. | Same as 19/1/2024                                 | DEW Staff and Ranger B |
| 18/3/2024    | Lots of old carcasses (not counted), four carcasses looked less than one week old, zero fresh with eyes intact   | Pelican Point/<br>Tauwitchere site                | DEW Staff and Ranger B |
| 18-24/3/2024 | Number of freshly dead ducks rapidly declined  | Ewe Island to Mark Point (includes Pelican Point) | Local B                |



Figure 1. (a) shows a map of Australia; (b) shows a map of the Coorong RAMSAR site; (c) shows a segment of the North Lagoon, which includes the Pelican Point, the Tauwitche Barrage and the Ewe Island Barrage. As reported by Local A and B, the bird deaths were predominantly described at the closed area to line extending directly from Mark Point, and Mark Point to Long Point, respectively. Purple solid line on (c) indicated the main area Ranger A surveyed on 18th January 2024 and 20th February 2024. Green dashed lines on (c) indicated the main area the DEW Staff and Ranger B surveyed on 19th January 2024 and 26th February 2024. Blue circles on (c) indicated individual birds either washed ashore or floating, they were observed by the DEW Staff and Ranger B on a boat on the same day.



Figure 2. Deceased Australian Shelduck (*Tardona tadornoides*) on the left and Grey Teal (*Anas gracilis*) or female Chestnut Teal (*Anas castanea*) on the right were spotted on the shoreline from Pelican Point to the Rumbelows Hut. Photos were taken by Ranger A on 18<sup>th</sup> January 2024.



Figure 3. Deceased Black Swans (*Cygnus atratus*) on the left and Seagull (*Larinae spp.*) on the right were spotted on the shoreline from Pelican Point to the Rumbelows Hut. Photos were taken by Local A on 2<sup>nd</sup> February 2024.



Figure 4. Deceased Musk Duck (*Biziura lobata*) on the left and Black Faced Cormorant (*Phalacrocorax fuscescens*) on the right were spotted on the shoreline from Pelican Point to the Rumbelows Hut. Photos were taken by Local A on 2<sup>nd</sup> February 2024.

### Diagnostic findings

1. Testing for exotic disease by PCR

Type A influenza, specifically the H5 and H7 subtypes, are tested by PCR. The low pathogenic Avian influenza H7 subtype was detected in a small number of samples. This subtype is commonly present in wild bird populations and deemed to be a normal finding. No H5 (Highly pathogenic) was detected. PCR testing was also negative for APMV-1.

2. Faecal culture and culture on kidney and liver

Faecal culture results were positive for *Clostridium perfringens*, *Clostridium sordellii*, and *Fusobacterium varium*, which are common gastrointestinal inhabitants and considered normal findings. *Clostridium botulinum* was not reported in all samples.

Cultures from all six kidney and liver samples exhibited heavy growth of *P. multocida*.

While some samples also showed growth of *F. varium*, *Enterococcus faecalis*, and *C.*

*perfringens*, these organisms are typical gastrointestinal inhabitants and are regarded as common post-mortem invaders that colonise tissue after death due to the breakdown of intestinal tight junctions. Since *P. multocida* does not normally inhabit the gastrointestinal tract, its presence in the liver is considered indicative of septicaemia.

### 3. Gross pathology

All six freshly dead bird samples were in good condition with no physical lesions such as gun shots observed. No gross findings within the kidney, liver, spleen, heart, and brain were observed. A moderate amount of grey-green gritty ingesta were shown in the intestines of all birds. Only two birds had red intestinal intraluminal fluid. The lungs appeared diffusely pink, except for one bird, which exhibited hypostatic congestion on one side. Clear air sacs were exhibited. No significant findings indicative of acute cause of death could be ascertained from the gross post-mortem appearance.

### 4. Histopathology

Most birds exhibited moderate to marked autolysis of the intestines, which is considered a normal post-mortem finding. No significant abnormalities were observed in the lungs, kidneys, trachea, or heart, except for autolysis and mild lymphoplasmacytic and neutrophilic infiltration of the mucosa in the trachea of some birds. All six samples showed random multifocal necrotic lesions in the liver, accompanied by granulomatous or pyogranulomatous inflammation and fibrin accumulation. Fine, rod-shaped bacteria were also identified within the necrotic lesions. The koilin layer of the gizzard in five out of six birds exhibited multifocal degeneration, with infiltration of macrophages and multiple aggregations of rod-shaped bacteria. Multifocal lesions in the brain, along with colonization by rod-shaped bacteria, were observed in two samples. A trematode parasite

was found in the proventricular mucosa of one sample. Necrosis of the spleen, with colonization by rod-shaped bacteria, were also noted in several bird samples. These findings are suggestive of septicaemia being the likely cause of death with infiltration of rod-shaped bacteria potentially traveling via the portal vein from the ventriculus to the liver, and subsequently spreading to the spleen.

### **Molecular Findings**

Both Coorong isolates from the two liver samples belong to ST20 (RIRDC scheme) and ST159 (multi-host scheme), which are predictably capsular type A and LPS type one or three. These results contribute to a total of 27 ST20 isolates stored in MVS Clinical Microbiology Diagnostic Laboratory and marked the first ST20 isolation from wild birds in South Australia.

### **Viewpoints from stakeholders involved in the outbreak**

In total, 12 interviewees were consulted: Two locals (Locals A and B), a spokesperson from PIRSA, two DEW NPWS Rangers (Rangers A and B) and First Nations working On-Country Rangers (six members and one manager).

### **Response to the outbreak**

The spokesperson from PIRSA said that the resources allocated for wildlife for management agencies were very limited. Minimal legislation specified which agency should be responsible for wildlife. Notifiable diseases which present a significant risk to poultry industry, such as AI and APMV-1, was on PIRSA's priority whenever bird mortality is reported. The outbreak has not raised significant concerns in the commercial poultry industry, as its impact has been largely confined to wild waterfowl.

Locals A and B expressed jointly that the responses from management agencies on the outbreak were inadequate and ineffective. The strength and scope of the locals' work were limited due to the lack of funding. For example:

*“The government was very slow to respond to the outbreak.”*

*“PIRSA took too much of the responsibility.”*

*“It would be more effective to distribute funding resources among other stakeholders.”*

Roles of the Rangers were as mentioned in the “Results”.

The First Nations members were not involved in the response to this outbreak, but are actively working in the region, primarily between Parnka Point to Long Point, Southeast to the Pelican Point.

### **Management currently in place**

PIRSA encourages the public to report any spotting of bird carcasses via the emergency disease hotline or social media platforms. In addressing disease outbreaks, they have previously organised webinars and distributed factsheets to inform the industry and educate on biosecurity, while hunters were advised to report and keep away from dead bird carcasses in the recent outbreak.

Locals A and B raised biosecurity concerns as they reported evident predation on carcasses but minimal actions from the management agencies were addressed to this issue. Local A was worried about the Osprey nests and the Cape Barren Geese (feeding in the paddocks) near the location of outbreak, being infected through scavenging of carcasses. They agreed collecting carcasses could help reduce cross-contamination and possibly the extent of the outbreak. In addition, they have expressed their frustration towards the current disease reporting protocols, which discourages them to report unusual findings. For example:

*“There were too many hotlines.”*

*“South Australian Government said we did not report correctly.”*

### **Ecological Value of Coorong**

“... but it has a substantial impact on Coorong, an ecologically important region,” said by the spokesperson from PIRSA. Despite the fact that this outbreak was not described as significantly affecting the poultry industry, the impact on the Coorong was inevitably substantial.

Local A noted that the ecosystem of the Coorong has been severely impacted by drought events and the restructuring of water pipes from various regions in Australia to the Murray-Darling Basin. All waterfowl species have been affected, leading to a significant decline in their numbers over the years. The outbreak was particularly concerning for Local A, as mass mortality of birds due to disease has never been reported in Coorong before. Therefore, it is crucial to conduct an ecological assessment of the outbreak and the impact of *P. multocida* on wild waterfowl. Tracing the birds to observe potential transmission pathways will also be essential for developing an effective preventative plan.

The First Nations members expressed on how their ancestors treat Coorong as a hugely significant area. However, due to the loss of communication between generations, they were still not able to fully understand and work the same way their ancestors once did for the land.

### **Importance of communication between different parties**

The spokesperson from PIRSA mentioned the importance of establishing personal relationships among poultry and wildlife experts and veterinarians. This facilitates prompt reporting from the field and a timelier response from the management agencies.

Local A supported this idea and said, *“It would be helpful to include engagement with the local community, to help with disease surveillance.”* The local was discovering possibilities for offering

a local representative a role to connect and transfer information (i.e. death reports) from them to the management agencies. The representative will be more accessible for the local community.

The First Nations members mentioned about the importance of the strong connections they have between their crew, manager and one of the Rangers. This prompts the ability to respond quickly with the Rangers if events, such as a disease outbreak, occur. Within the First Nations community, information disseminates rapidly due to the well-maintained network of communication.

### **Potential risk factors contributing to the outbreak**

Most interviewees agreed that this outbreak could be multifactorial.

The spokesperson from PIRSA suggested that the density of the waterfowl population might be one of the risk factors, as highly infectious pathogens transferring among susceptible hosts can have severe consequences in areas with high bird density.

Local A mentioned all waterfowl hit peak number in January to February every year. Local A also mentioned that the impact on water quality can result in an outbreak. It was observed that the algal bloom caused by *Alexandrina minutum* and the incidence of fowl cholera outbreak may be related.

*“It was the same area as the cholera outbreak... I did not catch the red tides, but the environmental condition was favourable for the growth of them from our previous experience.”* Local A added

that the organic matter accumulated in the sediment and its anaerobic nature was due to years of low water flow in Coorong. The flooding in 2022 altered the stratification of the water column and released nutrients from the sediment. This favoured the growth and reproduction of *A. minutum*.

Toxins produced by the algae and reduction of food availability due to oxygen depletion in water can predispose waterfowl to disease and mortality. Regarding the causative relationship between agricultural contaminants and algal bloom, Local A commented that the chance was very minimal according to the water quality assessments. Another risk factor that raised Local A’s concern was

the exposure of contaminated sediments due to low tide and less freshwater input during the outbreak. Potential contaminants include persistent *P. multocida* and toxins released by *Clostridium botulinum* in the sediment.

Local B said, “*It is a compounding issue, but there may be a connection to the introduced algal bloom, which is very toxic.*” This was based on the observations of algal blooms in Lake Alexandrina, linked to high flow conditions during 2022-2024.

Ranger A mentioned the unique variability of salinity in different regions of Coorong may contain the outbreak to a certain location. The algal bloom occurred within Lake Alexandrina containing fresh water while the area of the outbreak and other parts of Coorong have brackish or hypersaline water.

Ranger B expressed his uncertainty towards the potential risk factors but observed the highest density of birds he had ever seen during his survey on 19<sup>th</sup> January 2024. The tide was reported to be very low on the day, encouraging lots of birds to feed on the mudflats. Regarding the same algal bloom reported by Local B, he mentioned that Lake Alexandrina is only five minutes flight from the area of outbreak. Both locations are within close proximity, yet the death of birds was only concentrated around Pelican Point; this made the Ranger assume a minimal connection between the algal bloom and the outbreak.

The First Nations members witnessed the low tide in January. They have mentioned the birds showing abnormal behaviours, such as carrying out out-of-season activities and congregating abnormally. However, the mechanisms of how the abnormal behaviours may contribute to the outbreak and when they saw them were not mentioned. They also were unsure if the emus, which were always present in the region, were involved in the outbreak. They also noticed the increased adoption of free-range farm system in several layer farms in Coorong.

## **Miscellaneous points**

The Spokesperson from PIRSA noted the high rain event on 8<sup>th</sup> January 2024 (one day prior to the first bird death report) with 33 millimetres recorded for Victor Harbor (approximately 40 km from the location of outbreak).

Local A did not see any deaths of the Pelicans in their breeding islands located within the South Lagoon, only in North Lagoon. Deaths of migratory waterfowl were not observed. Local A proposed that restoration of the Coorong ecosystem could alleviate stress predisposing to disease. Local A advocated the following: (i) Artificially add a bioavailable form of calcium carbonate (i.e. burnt lime) into the lagoons of the Coorong to neutralise the acid sulphate persisting in the sediment, (ii) Increase water flow down the Coorong by increasing the proportion of fresh water passing through the Tauwitchere Barrage from the Lake Alexandrina in the correct timing.

Ranger A expressed his concerns on the accuracy of the dead bird count. The count was likely reduced by the predation on freshly dead birds by foxes, cats, and birds. It could also be affected by the south-westerly to westerly wind, pushing deceased birds to the mainland shore (Pelican Point) from the large sand bar (opposite Pelican Point) densely populated by birds. As conditions change (change of wind directions), the severity of bird deaths could be masked. The impact of the wind was also noted by Ranger B on his survey on 19<sup>th</sup> January 2024. Carcasses could also be washed up on the opposite side of the Coorong by wind.

Ranger B reported one cormorant displaying lethargy and lack of coordination and several unwell ducks during his survey on 26<sup>th</sup> February 2024.

A First Nations member reported seeing odd congregation of Black Swans of approximately 100-200 individuals from Salt Creek to Mark Point in June 2024.

## **Discussion**

Below we discuss potential risk factors with respect to viewpoints from interviewees and approaches to manage future Fowl Cholera outbreaks.

### **Epidemiological analysis**

Epidemiological analysis is discussed under the framework referenced from the Manual of Procedures for Wildlife Disease Risk Analysis (Jakob-Hoff et al., 2014), to describe possible pathways of disease transmission and causal factors for the emergence of Fowl Cholera in Coorong. The framework includes McNew's disease triangle (McNew, 1960).

### **Host factors**

**Bird density** The most recent condition monitoring of the region conducted in January-February 2021 documented 135,599 waterbirds (representing 52 species) using the Coorong, with 23,195 waterbirds using the Murray Estuary which included Pelican Point (where the Fowl Cholera outbreak was recorded) (Paton et al., 2021). Grey Teals and Australian Shelducks were reported to be prominent in the region, and they were predominantly affected in the outbreak (Paton et al., 2021). As wild waterfowl aggregate where habitat is suitable, threshold for an epizootic of *P. multocida* can be exceeded more easily (Thomas et al., 2008). Bird densities and flock sizes were proven to have positive relationships with rate of infection (Combs & Botzler, 1991; Wobeser, 1997), hence there is increased risk of epizootics (Van Es & Olney, 1940). Stress of reservoir animals, which could also be birds, can increase with bird densities, further increasing shedding of bacteria (Wobeser, 1997).

**Diet** All waterfowl species reported in this outbreak were not endangered or migratory (Belbin et al., 2021). Apart from Seagull (*Larinae* spp.), all involved waterfowl species do not tend to feed on other waterfowl. A 2022 study reported on the diets of Chestnut Teal and Grey Teal. They mainly consisted of submergent and amphibious plants (52%), green algae and samphire. Black

Swan consume aquatic macrophytes and algae (Marchant & Higgins, 1990; Smith, Vernes & Ford, 2012). The diet of Great Crested Grebe includes Flatworms, Crustaceans, Mollusks, Fishes, etc. (Storer, 2000). Waterfowl species were not recorded in the diet. Gulls are omnivores and Silver Gulls are primarily scavengers. They feed on a range of food which includes carcasses, eggs and chicks of their own and other species (Barker & Vestjens, 1989; Smith, 1992). Scavenging carcasses and cannibalism within the species predominantly affected by this outbreak were not likely to be a source of transmission of *P. multocida*.

### **Environmental factors**

**Salinity** In the Coorong, salinity increases from the Murray Mouth (approximately 20 psu, brackish water) to Parnka Point, at the end of the South Lagoon (approximately 90 psu, hypersalinity) (Reeves et al., 2015; Priestley et al., 2022; Mosley et al., 2023). Pelican Point (location of outbreak) is approximately 15 km from the Murray Mouth, and the closest saline monitoring station manned by the South Australian Government is located in Tauwitchere. The station measured a median salinity of approximately 20 psu between 1998 and 2021 (Mosley et al., 2023). Regarding the relationship between salinity and the growth of *P. multocida*, Bredy & Botzler (1989) found that the addition of 0.5% (5 psu) NaCl greatly enhanced the survival of *P. multocida* but it is not known what saline concentration *P. multocida* can tolerate given that the level of water salinity in the Coorong is considerably higher. As the death of birds was concentrated only near Pelican Point but not in the Southern end of the North Lagoon and the South Lagoon, there is a possibility that *P. multocida* may not be able to tolerate the more hypersaline conditions in these areas.

**Chemical toxins** Although the actual toxic tissue concentration of heavy metals in waterfowl is influenced by multiple factors, it is evident that certain heavy metals are associated with toxicities

in free-ranging waterfowl (Degernes, 2008). Environmental sources of heavy metals, particularly cyanide from industrial and mining sites, can bioaccumulate in aquatic ecosystems (Degernes, 2008). While acute effects from direct water ingestion are unlikely in the waterfowl species of interest in this study, bioaccumulation through the food web may lead to chronic toxicity, increasing their susceptibility to infection (Rogers & Wainwright). As a catchment of River Murray and River Darling, pesticides such as 1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane (DDT) could accumulate in the Coorong from chemical deposition from farms within the catchment area. Chronic cases of organochlorine toxicity are associated with symptoms such as weight loss, and the use of granular organophosphates has been linked to acute mortality in waterfowl (Degernes, 2008). Although lead shot has been banned in South Australia since 1992, lead deposition in wetlands remains a concern. Waterfowl can still access this lead, as evidenced by deaths of wild swans in 2000 due to lead poisoning even more than a decade after the ban on lead shot hunting (Degernes et al., 2006). The dredging project that resumed in November 2023 in the Murray Mouth aims to enhance connectivity between the Coorong and the Southern Ocean (DEW, 2024). While no direct ecological impacts have been observed in the Murray Mouth, the association remains undetermined. However, the removal of sediment from the bottom of lake bodies during dredging operations could be speculatively linked to the remediation of toxins, which may impact the health of waterfowl.

**Tide level** A low tide was reported in the region on 19 January 2024, by DEW Staff and Ranger B, which correlated with the low water levels and the higher accessibility of mudflats. Mudflats, defined as soft sediment areas with a water depth of up to 12 cm, provide a vulnerable habitat for foraging waterfowl (Bengera & Sharmab, 2013). A low tide allows waterfowl to access mudflats, which offer greater food sources, such as macroinvertebrates, thereby attracting waterfowl to stay

in the region (Rogers & Paton, 2009). This might contribute to the unusually large numbers of birds reported in the area, which, combined with the high population density, could impact the significant mortality rates. The density of birds accelerates disease transmission, leading to even more bird deaths, as previously discussed for host factors. While the low water levels and mudflats encourage foraging by waterfowl, the removal of sediment could enhance the remediation of heavy metals and other toxic substances, increasing their uptake by the birds.

### **Pathogen factors**

As this outbreak took place in Coorong, which is a wetland, *P. multocida* is likely to concentrate near the free water surface where bacteria can survive for several weeks (Potter & Baker, 1961). This layer of water is most severely disturbed by waterfowl when they carry out their daily activities such as bathing and hunting (Friend et al., 1999). This generates an aerosol form of *P. multocida*, which serves as a route of infection (Friend et al., 1999). Specifically for wetlands, ambient soil or water were proposed to be major reservoirs, where *P. multocida* can survive and produce recurring disease (Botzler, 1991).

*P. multocida* naturally occurs in all waterfowl species reported in the Coorong outbreak (Botzler, 1991). Waterfowl are the definitive hosts, after they die from pasteurellosis, as reported by the Rangers, carcasses can be washed up to land by wind. Scavengers, such as foxes and rodents, can pick up *P. multocida* by the ingestion of carcasses containing the bacteria on the shore. While scavengers do not necessarily show symptoms of the disease, they act as reservoir hosts for the bacteria. An interspecies chain for *P. multocida* transmission was reported in a wetland in California, where dead waterfowls were ingested by mice, and infected mice were ingested by owls and northern harriers, and ultimately ingested by living waterfowl again, creating a loop (Friend et al., 1999).

A non-linear relationship between concentration of *P. multocida* and dissolved protein in the wetland was reported by Blanchong et al. (2006). *P. multocida* concentration increased with increasing protein concentration until 12 µg/L, and relationship between the two variables was negative at higher protein concentrations. After infected waterfowl die from pasteurellosis, decaying carcasses can be a source of dissolved protein, which supports the growth of *P. multocida* and exacerbates the severity of the outbreak. However, further details regarding the interaction between dissolved protein and the bacterium were not elaborated.

### **Biosecurity measures**

The sequencing of the two isolates obtained from the Coorong outbreak as ST20, along with their clustering with 25 other avian isolates in Australia—including chickens, turkeys, and wild birds is a notable finding. This idea is supported by previous findings of ST20 in wild birds in Victoria in 2013 (Allen et al., 2024) and the increased prevalence of ST20 in free-range poultry farms in Queensland (Omaleki et al., 2022). The phenomenon of outbreaks simultaneously taking place in free-range farms and wetland was also supported by Combs & Botzler (1991). These indicated the possible interaction between *P. multocida* outbreak in various farm systems, particularly free-range farm, and the wild waterfowl in proximity. During our visit to a layer farm in South Australia, we observed several biosecurity issues persisting on the premises. It is evident that the prevalence of some pathogenic bacteria such as *P. multocida* is linked to the application of biosecurity measures (Aidaros, Hafez & El Bahgy, 2022). These issues may provide insight into how disease transmission may occur between poultry and wildlife.

**Personnel and vehicle disinfection** Personnel, including staff, health professionals and visitors, can act as mechanical vectors through their footwear, clothing, and vehicles. During our visit, we noticed several biosecurity shortcomings on the farm, such as the lack of foot baths and logbooks

to track traffic and personnel movement in and out of the premises. The fact that we were allowed to drive our own vehicles with no restrictions at all near the shed indicated no designated farm vehicles were present. Parking lots for vehicles should be designed to be sufficiently distanced from production areas (Zhouet al., 2020). External personnel visiting the farm must adhere to strict regulations regulated and recorded in a proper sign-in/sign-out visitor log, while visits should only occur upon necessity and permission. Visitors should be equipped with protective clothing and footwear, and hands must be sanitized before entering (Zhouet al., 2020).

**Wild animals** Four flying finches were spotted in one of the chicken sheds. Studies investigated on turkeys suggested that free-flying birds could be a source of *P. multocida* for turkeys (Rhoades & Rimler, 1984; Snipes et al., 1988). Although reports on the presence of wild animals as reservoir hosts on farm focused on turkeys, chickens are also definitive hosts which can be infected. Simultaneously, the study demonstrated that wild birds using and grazing on farmland and water surfaces were more susceptible to Fowl Cholera (Combs & Botzler, 1991), resulting in bidirectional disease transmission. One dead mouse was spotted next to the entrance of the chicken shed. This indicated the presence of rodents on free-range farms and indicated the possibility of the bird-rodent-bird transmission pathway, as mentioned in the “Pathogen” section. A study reported that small mammals, such as mice, were present in all 64 chicken farms with different farm types which includes free-range broilers and layers in Sydney and Queensland (Scott et al., 2018). Rodents can scavenge dead waterfowl and transmit *P. multocida* by non-fatal bites to free-range poultry (Friend et al., 1999). Feral cats and foxes are frequently spotted in the Coorong coastal region. While feral cats were evident to be capable of harbouring the same strain of *P. multocida* (ST20) as infected broiler s(Singh et al., 2014), they can serve as a reservoir and carrier of pathogen between farm system and wild birds.

**Vaccination** Due to multiple serogroups and serotypes of *P. multocida*, some farms opt for autogenous vaccines to specifically act on the genotype of *P. multocida* present on the farm. However, field evidence is present to suggest although autogenous vaccines have higher efficacy than commercial vaccines, presence of stress among the flock lead a wide range of consequences including vaccine failures (Alberts & Graham, 1948; Singh et al., 2014).

### **Management strategies**

**Poultry wastewater treatment** Wastewater from poultry farms contains high levels of fat, protein, macronutrients, etc., which may result in eutrophication if not properly disposed. Vaishnav et al. (2023) compared biological, physico-chemical and membrane-based processes to treat poultry wastewater; and treating by biological methods are the most cost-effective. For example, farms can introduce genetically modified microbes such as modified *Deinococcus radiodurans* to remove mercury and the combination of *Mesorhizobium huakuii* and *Arabidopsis thaliana* to remove cadmium from wastewater (Meruvu, 2021; Luo et al., 2020; Vaishnav et al., 2023). However, apart from removing harmful components from the wastewater, if suitable treatments are followed by land disposal, wastewater with high nutrient values can be utilised to enhance soil fertility and productivity (Artukmetov et al., 2021).

**Carcass disposal** Although the Carcass Management Guidelines published by the Food and Agriculture Organisation of the United Nations is not legal binding, farmers should respect and abide by the guidelines regarding the methods of proper carcass disposal. Carcass management options include (i) Burning, (ii) Deep burial, (iii) Above-ground burial, (iv) Composting and (iv) Ancillary activities (Miller et al., 2020). Details are included in Appendix A to D. Guidelines for standard transport vehicles for carcasses are also included, vehicles (trucks, containers or dumpsters) should be closed and leak resistant (Miller et al., 2020). Dropping carcasses from

vehicles on roadways build a potential transmission pathway and a platform for reservoir hosts to be infected and carry pathogens to new hosts. We suggest the government provide printed copies of the guidelines to the veterinarians for them to distribute to farms they are visiting. This can raise the farmers' awareness of biosecurity with guidelines to follow.

**Wild animals on farm** During our farm visit, we saw broken sections of walls which were inadequately maintained. This possibly led to the presence of flying finches. Sheds housing poultry animals should be wild bird proof. In addition, trees and shrubs around the sheds should be non-flowering to reduce attractiveness to wild birds. Rodents such as rats can dig holes to enter the shed therefore it is harder to have sheds rodent-proof. Drainage is also crucial in preventing the attraction of waterfowl and minimizing the introduction of microorganisms associated with stagnant water (Zhou et al., 2020).

**Communication platform** As mentioned by the PIRSA spokesperson and a local, communication between different parties is essential in managing disease outbreaks. Apart from PIRSA's emergency disease hotline, we believe groups on social media platforms such as WhatsApp or Facebook Messenger can be set up for different areas of ecological importance in South Australia, not restricted to Coorong. Each group should include at least one representative from each management agency, locals including the First Nations Community and people of expertise to the region, such as ecologists and fisher people. Text messages or phone calls for reporting different sightings (i.e. bird deaths) can then be attended to by the personnel responsible timelier and ultimately respond to the situation efficiently and effectively.

**Local response team** Locals have expressed concerns regarding the response from the government for the outbreak. We suggest the government or management agencies to start a training programme for veterinarians and personnel of expertise and form a local emergency

disease response team. They will be allocated into teams based on their locations. The content of the programme can include (i) criteria for selection of carcasses for diagnostics, (ii) detailed steps for basic on-site specimen collection, for example, nasal swabs, (iii) how to educate locals if they witness animal carcasses (i.e. report and not to contact carcasses). Upon completion, eligible participants will be certified and granted the permission by the government to service in their local response teams. This can reduce the time it takes for the carcasses to be sampled hence achieve better quality diagnostics results. In addition, the team can work together with management agencies to provide timelier response to an outbreak.

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