Avian botulism mass mortality from Sambhar Salt Lake, Rajasthan, India

The first report of mass mortality in migratory birds was received by the Rajasthan Forest Department on 10 November 2019. A joint team of forest, revenue and animal husbandry officials visited the Sambhar lake area the same day, and samples were collected to test for bird flu. It was decided on the spot that carcasses would be collected and buried in deep pits with limestone due to the fear of disease spread. Due to the threat of bird flu, the local staff was advised to adopt appropriate prophylactic measures such as the use of masks and gloves during their field visits and while handling the carcasses. Preliminary findings gave a negative result for bird flu. Thereafter, water samples and biological samples were sent to multiple Government laboratories for further analysis.

The Sambhar Salt Lake (26°52′–27°02′N, 74°54′–75°14′E) is situated in the Jaipur, Ajmer, and Nagaur districts of Rajasthan, India, spread over 230 km^2 area (Figure 1). It is India's largest inland saline wetland and has been recognized as a wetland of international importance and was designated as a Ramsar Site in 1990. The Lake is fed by four seasonal rivers, namely Mendha, Rupangarh, Kharian and Khandel, forming a catchment area of 5700 km^2. The lake has an asymmetrical drainage pattern; the northern and eastern portions are poorly drained, whereas the western and southern parts serve as a confluence of streams. The Lake is an extensive saline wetland, with water depth fluctuating from 60 cm (24 in) during the dry season to about 3 m (10 ft) at the end of the monsoon season.

The Lake is a revenue area, part of which has been leased out for salt-making, accounting for 9% of the total salt production in India. The salt production is mostly managed by a Government-owned company and the State Government of Rajasthan.

The Sambhar Salt Lake is rich in biodiversity; being an inland salt lake, its unique ecosystem supports a highly specialized group of organisms. Both the lesser flamingo and greater flamingo are the flagship species of this Lake. It is probably the most important site for flamingos in the Indian subcontinent, outside the Rann of Kutch[1]. This Lake is also an important bird area and reportedly hosts a large number of migratory birds every winter. The migratory birds that arrive at Sambhar either use the Lake as a stopover site en route to their wintering grounding areas in peninsular India or beyond, or spend the entire winter months there.

A total of 83 species of waterfowl have been recorded in Sambhar[1]. Some commonly reported waterfowl species are greater flamingo, lesser flamingo, northern shoveler, common coot, northern pintail, gadwall, mallard, garganey, common teal, pied avocet, Kentish plover, and ruff.

The entire rescue operation was aimed to remove the source of infection, viz. dead birds from the site to avoid feeding healthy birds on carcasses, and to prevent ingestion of preformed botulinum toxin. As the disposal of dead birds had to be carried out quickly, the species-wise record of birds that had died could not be maintained. However, all birds were waders and dabbling duck groups. Northern shoveler, Kentish plover, black-winged stilt and common coot were the most affected species.

Daily collection of dead birds was done and a total of 22,983 dead birds were collected in the Jaipur and Nagaur districts of Sambhar Salt Lake (Figure 2).

The combing operation was carried out using drones and inflatable boats. About 1293 birds were rescued from the Sambhar Salt Lake (Figure 3). The rescued birds were treated on-site and after stabilization they were shifted to the rescue centre. After recovery, the ringing of the birds was done and they were released into the wild. A total of 515 rescued birds were released in the wild after treatment.

It was noticed that the affected birds were of waders and dabbling duck groups, which are carnivorous and omnivorous respectively. Flamingos and other local birds were not affected. Also, in the freshwater lake Ratan Talab near the affected site, no bird mortality was reported. The dead birds belonged predominantly to the three orders mentioned below.

(a) Order Charadriiformes.

(i) Waders: 16 species, viz. curlew sandpiper, marsh sandpiper, common sandpiper, little-ringed plover, ruff, black-winged stilt, Kentish plover, red-wattled lapwing, white-tailed lapwing, little stint, Temminck's stint, pied avocet, common redshank, common greenshank, dunlin and Eurasian curlew. Most of them feed by probing into the mud or by picking items off the surface.

(ii) Gulls: Four species, viz. Pallas's gull, black-headed gull, brown-headed gull and steppe gull. The diet of gulls (Laridae) is highly varied and includes fish, small birds, rodents and a wide range of invertebrates (by active predation), as well as carrion garbage and vegetable material.

(b) Order Anseriformes.

Family Anatidae: Seven species, viz. northern shoveler, common teal, ruddy shelduck, garganey, mallard, gadwall and common pochard. They feed on aquatic

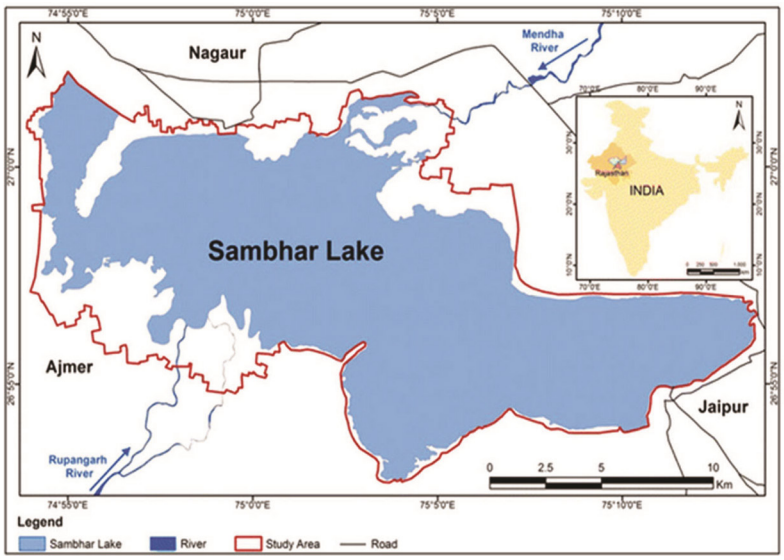

Figure 1. Location map of the Sambhar Salt Lake, Rajasthan, India[10].

invertebrates, plankton, algae, mollusk, aquatic insects, crustaceans and small fish.

(c) Order Gruiformes.

Family Rallidae: Two species, viz. common coot and common crane. The former is omnivorous, feeding primarily on the vegetal matter such as shoots, seeds of aquatic and terrestrial plants, algae and grasses. It also consumes animal food such as worms, leeches, molluscs, shrimp, insects and spiders. The latter is also omnivorous. It consumes plant matter such as roots, rhizomes, tubers, stems, leaves, fruits and seeds. The animal feed includes insects, especially dragonflies, snails, earthworms, crabs and spiders.

The rescued birds were reported to belong to 30 species (Table 1). However, the lesser flamingo was an exception, as only one bird of this species was rescued and it was injured. Hence birds affected by avian botulism were predominantly from 29 species belonging to three orders.

Table 2 provides a summary of the findings.

Mass mortality events (MMEs) happen regularly worldwide. Outbreaks of avian botulism type C have been reported in the United States, Italy, Poland and South Korea[2–5]. In India, such events are a rarity and have not been reported earlier. The cause of the recent deaths of 22,983 birds in Sambhar Salt Lake was found to be due to avian botulism caused by *Clostridium botulinum*.

Between 1978 and 2008, 13 avian botulism outbreaks were recorded in the wetland of Mancha Hu'meda in Central Spain, and around 20,000 individuals of more than 50 species from 18 families were found dead. The most frequently affected family was Anatidae. In general, wild birds most affected by botulism are water birds and shorebirds. In Mancha Hu'meda, 69.3% of the recorded deaths was from Anatidae and 12.6% shorebirds. Similar results were reported in South Korea by Woo *et al*.[6] in 2010. Mehdi *et al.*[2] reported a high percentage of Anseriformes (56) and a lower percentage of shorebirds (6.6) death in New York, USA[7].

C. botulinum produces a neurotoxin (BoNT), which is a fatally toxic compound causing flaccid paralysis and death. Except in Antarctica, avian botulism has been reported globally and cited as the most important

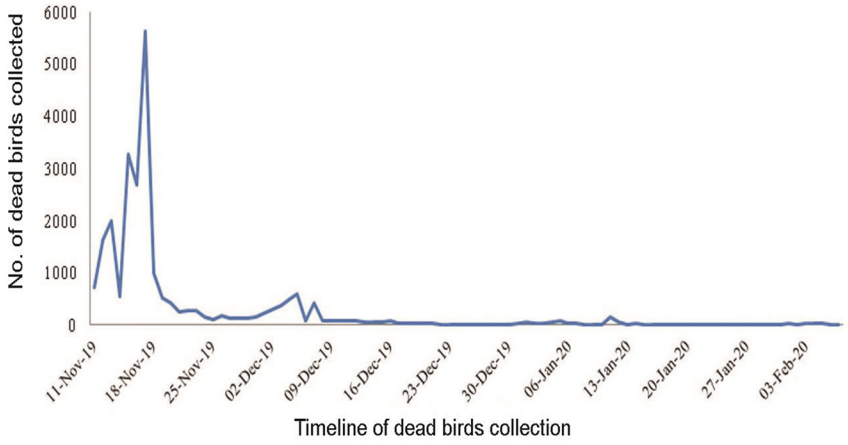

Figure 2. Mortality trend in migratory birds over a three-month period (November 2019 to February 2020) in Sambhar Lake, due to *Clostridium botulinum* toxicity. The highest number of death was reported in mid-November.

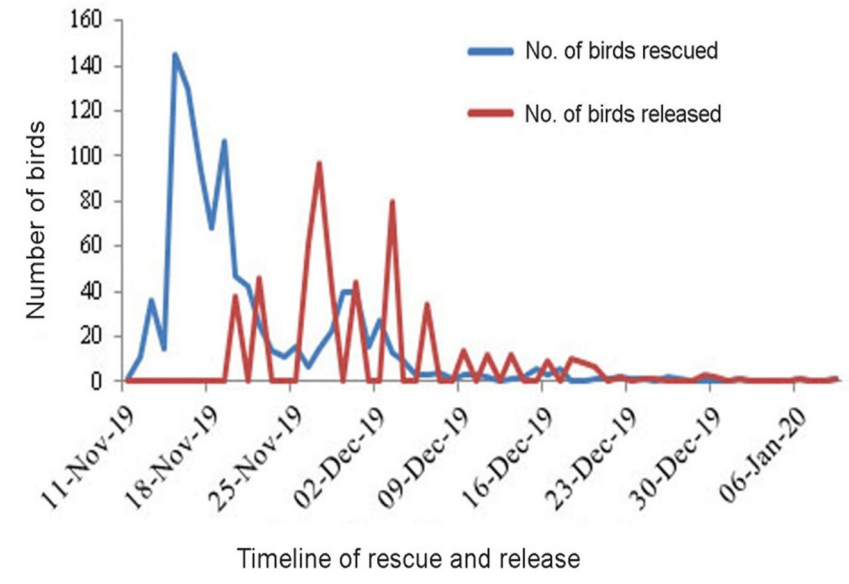

Figure 3. Rescue data of affected migratory birds over a two-month period (November 2019 to January 2020) in Sambhar Lake, due to *C. botulinum* toxicity. The spread of disease reportedly stopped in January 2020.

Table 1. Number of *Clostridium botulinum*-affected migratory birds rescued species-wise from Sambhar Salt Lake, Rajasthan, India

Species	Number of birds rescued
Pied avocet	28
Common coot	60
Eurasian curlew	1
Ruddy shelduck	4
Dunlin	7
Gadwall	4
Black-headed gull	2
Brown-headed gull	1
Steppe gull	1
Pallas gull	2
Red wattled lapwing	14
Mallard	3
Kentish plover	351
Common pochard	5
Ruff	33
Common sandpiper	6
Marsh sandpiper	29
Wood sandpiper	4
Redshank	5
Northern shoveler	454
Black-winged stilt	119
Little stint	45
Common teal	68
Little-ringed plover	39
Lesser flamingo	1
Temminck's stint	1
Common crane	3
Garganey	1
White-tailed lapwing	1
Common greenshank	2
Total	1294

Table 2. Results of Sambhar Lake water and bird carcass biological sample analysis obtained from various Government institutions/organizations

Institution/Organization	Report
National Institute of High-Security Animal Disease, Bhopal	Negative report on avian influenza.
Wildlife Institute of India, Dehradun	(i) pH 6.2–9.2. (ii) TDS, specific conductance and salinity much above the permissible limit. (iii) Ions: Levels of sodium and fluoride are high, but not responsible for mortality. (iv) Heavy metals: All in tolerable limit, except aluminium and iron. (v) Pesticides Organophosphates and organochlorines: All within the permissible limit.
Rajasthan State Pollution Control Board	According to the Central Pollution Control Board water quality criteria, water is under 'D' class. Not suitable for freshwater fish.
Apex Centre for Animal Disease Investigation, Monitoring and Surveillance, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Science	Diagnosed as avian botulism.
Indian Veterinary Research Institute, Bareilly	Confirmed avian botulism.
Salim Ali Centre for Ornithology and Natural History, Coimbatore	No pesticide poisoning was found.
Bombay Natural History Society, Mumbai	Inconclusive
Defence Research and Development Establishment, Gwalior, Madhya Pradesh	(i) Soil and water samples were found negative for *Clostridium botulinum* toxin. (ii) Dead maggots, fresh maggots, dead birds, and intestine samples of birds were found positive for *C. botulinum* toxin.

avian disease causing bird mortality. During an outbreak, the mortality spreads exponentially through necrophagous flies. The maggots of necrophagous flies act as carriers of the toxin, which then spreads from the decomposing carcasses to other live birds[8]. However, an outbreak depends on many other environmental factors, which can help spread of *C. botulinum* spores. These environmental factors affect both the soil and the water properties, where the spores disperse. Sometimes carcasses of birds that have died due to other reasons can serve as a growth bed of *C. botulinum*, causing an outbreak among other healthy birds. Such growth of spores in the carcass can be augmented by either the temperature of the wetland or the decomposing organic materials, which provide a rich supply of nutrients. The susceptibility of some species or some individuals to BoNT may also be an important determinant.

Ailing birds showed symptoms like dullness, depression, anorexia, flaccid paralysis of legs and wings, gasping, soiling of the vent with severe watery, whitish to greenish diarrhoea, drooping of the neck, drooping of the third eyelid, no perching reflex, flight reflex and pupillary reflex.

Factors favouring the growth of *C. botulinum* were low salinity levels, temperature more than 25°C, pH between 7 and 9, dissolved oxygen level below 4, high chemical oxygen demand, presence of invertebrates[9], crustaceans and planktons, and the presence of maggot-infested carcasses in the lake area. Heavy rainfall during July and August 2019 also created a favourable environment for bacterial growth (Figure 4). Vidal *et al*.[7] have determined the effect of environmental factors on the spread of *C. botulinum* in the Mediterranean wetlands. The Indian tropical climate is similar to the Mediterranean region and can understandably create a favourable breeding ground for the spread of *C. botulinum* spores.

Rainfall in the year 2019 was the highest in the last decade. This might have led to the filling up of the Lake area, leading to the formation of new marshy areas and wetlands with a large congregation of migratory birds. Low salinity of water due to heavy rainfall created a conducive environment for the growth and proliferation of invertebrates, crustaceans and planktons[10], which harbour *C. botulinum* in their bodies. Receding water levels may have led to a mild increase in the salinity subtoxic levels, resulting in the death of crustaceans, invertebrates and planktons[11]. It has been reported that *C. botulinum* multiplies inside dead organisms, thereby accumulating toxins. During migration, the deaths of only carnivorous and omnivorous birds were reported and not herbivorous birds. After the death of these birds, the carcasses were infested with maggots known to bio-accumulate botulinum toxin. New birds that feed on maggots also succumbed to botulinum toxin, thus establishing the maggot – carcass cycle.

The mortality has also displayed a lack of ground coordination of various Government line departments towards the safe keeping of the Lake habitat as a prime conservation site. The wetland health status of Sambhar as a Ramsar site has been poor for several years. As this Lake is not under the direct administrative control of the Forest or Animal Husbandry Department, conservation-oriented management is lacking in the area. The State Government has established a Standing Committee for the Management of Sambhar Lake for effective monitoring. Also, a Sambhar Lake Management Agency was set up in 2021 under the State Forest Ministry for the protection, conservation and all-around development of Sambhar Lake.

Each water bird species exhibits varying vulnerability towards botulism toxicity, which also depends on its arrival to the wetland, climatic/edaphic factors and feeding habits. Thereby, the possibility of a disease outbreak depends on the presence of vulnerable species within the small time frame of spore production and spread in the wetland environment. In the present case, according to data on the rescued birds, highly vulnerable species belonged to three orders, i.e. Charadriiformes (53.51%), Anseriformes (41.60%) and Gruiformes (4.87%). The most affected species were northern

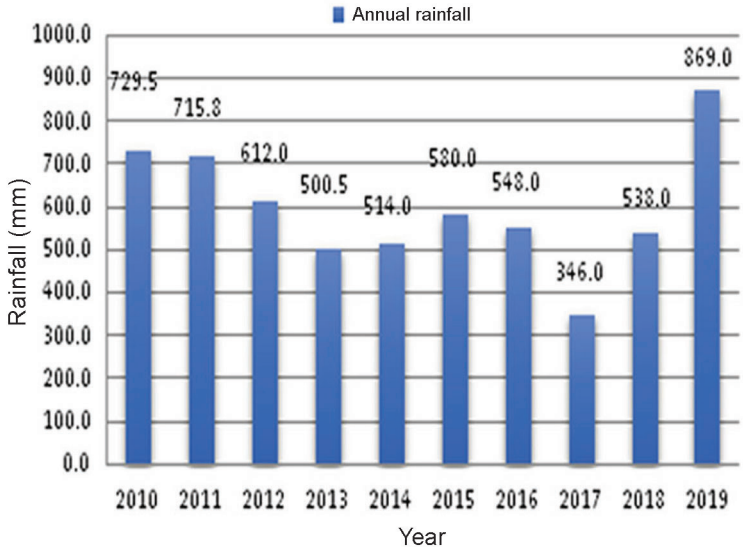

Figure 4. Yearwise annual rainfall data of Sambhar Lake during 2010–19. Maximum rainfall was received in 2019 (ref. 12).

shoveler (35.11%) and Kentish plover (27.15%). Thus feeding habits of three orders, i.e. carnivorous and omnivorous were the most affected. It can thereby be inferred that, botulism bacteria (*C. botulinum*) were already present in the Sambhar salt lake area, however, there were not much proliferation and spread of spores previously. In 2019, the high rainfall raised the water level of the lake area by filling previous comparatively drier areas. The bacterial spores already present in those newly waterlogged areas proliferated massively, causing the high rate of mortality. Additionally, there has been a cycle of increase and decrease in water level due to three spells of rainfall, leading to increased anaerobic conditions which promote these bacteria. Lastly, changing levels of salinity might have promoted bacterial growth. This case is an eye-opener to ensure future monitoring and conservation of the Sambhar Lake Ramsar site.

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KAVITA SINGH[1]
MONALI SEN[2,*]

[1]*Rajasthan Forest Department, Raj Bhawan, Civil Lines, Jaipur 302 007, India*
[2]*Rajasthan Forest Department, Wildlife Institute of India, Chandrabani, Dehradun 248 001, India*
**For correspondence.*
e-mail: monal7th@gmail.com