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Cyanobacterial toxins and bacterial infections are the possible causes of mass mortality of lesser flamingos in Soda lakes in northern Tanzania

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Abstract

During the mass die-off of lesser flamingos in Soda lakes of Tanzania in 2000, 2002 and 2004, clinicopathological and toxicological investigations were made in order to elucidate the likely cause of mortality. Water and tissue samples were collected from the lakes and from dead flamingos respectively. While water samples were analyzed for pesticide residues, tissues were analyzed for pesticide residues and cyanotoxins. The significant pathological lesions observed in fresh carcasses included oedema in lungs, enlarged liver, haemorrhages in liver with multiple necrotic foci, haemorrhages in kidneys and haemorrhages in intestines with erosion of mucosa. Analysis of cyanotoxins revealed presence of neurotoxin (anatoxin-a) and hepatotoxins (microcystins LR, RR). Concentrations of microcystins LR were significantly higher ($P = 0.0003$) in liver than in other tissues. Based on clinicopathological findings and concentrations of the detected cyanotoxins, it is suspected that cyanobacterial toxins concurrent with secondary bacterial infection were the likely cause of the observed mortalities in flamingos.

Keywords: Bacterial infection; cyanobacteria toxins; lesser flamingos; Soda lakes; Tanzania

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Introduction

Lesser flamingo (*Phoenicopterus minor*) is endemic in Africa south of Sahara and categorized on the IUCN Red list as ‘near threatened’ or ‘vulnerable’ across its entire range (McCulloch et al., 2003). Lack of enough breeding sites and the vulnerability of breeding to both natural and anthropogenic disturbance have a significant impact on population growth (Anderson, 2000; Simons, 2000; Araoz et al., 2010; Kotut et al., 2010). This species is a phytoplankton feeder that consumes mainly the Cyanobacterium (*Arthrospira* sp) that prevails in soda lakes (Metcalf et al. 2013).

As lesser flamingos can live for 30-50 years (http://www.thebigzoo.com), they are liable to suffer from the long-term accumulation of pollutants and toxins in their tissues. Furthermore, this species undertakes extensive movements between Soda lakes of East Africa, and thus are likely to bioconcentrate the particulate pollutants and toxins through filtering and feeding on cynobacteria over a large geographical area (Simons, 2000). Although several contributing factors are suspected, the understanding about the extent of the candidate toxicant list of relative toxicities to flamingos has been established (Araoz et al., 2010; Metcalf et al., 2013). The known toxin producing cyanobacteria include *Anabaena* sp., *Aphanizomenon* sp., *Nodularia* sp. which produce hepatotoxins (microcystins and nodularins) that cause liver damage, and *Arthrospira* sp. and *Oscillaria* sp. which produce neurotoxins (anatoxins-a, s) and result in nerve damage (Creekmore, 2001; Ballot et al. 2004; Metcalf et al. 2013). Bird

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species, which have been observed to die from the toxic cyanobacteria through contaminated water, include lesser flamingos, free-ranging ducks, geese, eared grebes, gulls and songbirds (Creekmore, 2001; Metcalf et al., 2008).

The concentration of *Arthrospira* sp. which is the predominant algae species in Soda lakes of Tanzania during algal bloom has been found to vary from over 700 thousand to 150 million filaments per litre in different Soda lakes and in different sites within the lake (Lugomela et al., 2006). The concentration of other cyanobacteria species range from 1000 to 3000 filaments per litre and that of diatoms range from 10 to 150 thousand filaments per litre (Lugomela et al., 2006). Cyanobacteria bloom occurs during low precipitation or drought and/or if the level of nitrogen and phosphorus in water increases as a result of anthropogenic activities (Kotut et al., 2010).

A mass die-off of lesser flamingos was observed for the first time in Tanzania at Empakaai Crater in August 2000, when more than 1,000 birds were estimated to have died. In 2002 high mortality of lesser flamingos was noted in Lake Natron which is the most important breeding site for flamingos in Eastern and Southern Africa (Simons, 2000; Tuite, 2000). Efforts were made to find the cause of the problem, thus water and tissue samples from the affected areas were collected and submitted for analysis of pesticide residues at the Government Chief Chemists' laboratory in Dar es Salaam, Tanzania. Since some of the flamingos were presenting central nervous signs and pathological features suggested bacterial infection, attempts were made to assess possible toxins in the Soda lakes that might have originated from cyanobacterial toxins and histopathological examination for bacterial involvement. The objective of this investigation was to find out the possible cause of the flamingo mortality for sustainable conservation of the threatened species.

Materials and Methods

Study locations

Empakaai Crater is an alkaline lake formed from the volcanic activity and is situated north of Lake Manyara but on the rim of the rift (Fig. 1). The caldera is about 6 km wide and nearly half of its floor is occupied by lake and the depth of the lake is about 85 m which is very unusual for a Soda lake. The steep walls of the caldera clothed in forest rise in some places to almost 300 m above the floor. The trees are a mixture of Nuxia, fig (*Ficus benjamina*) and crotons and one of the most remarkable trees that grow here is *Hagenia abyssinica* which is seldom found below 3000 m (Hanby and Bygott, 1999). The common birds in the lake are flamingos, Cape Wigeon and Black-winged Stilts. The Empakaai Crater shore is often used by Maasai pastoral community for cattle grazing during dry season.

Lake Manyara and Lake Natron are Soda lakes along the Great Rift Valley and situated about 120 km apart with the latter being in the northern part of the valley (Fig. 1). Lake Manyara is a part of the Lake Manyara National Park situated on the Great Rift Valley. The lake occupies about 230 km^2 out of the total park area of 330 km^2. The park contains a large variety of habitats including the rift wall, the ground water forest which is the water catchments for the lake, acacia woodland and open grassland. Other source of fresh water which drains into the lake is from Ngorongoro forest and crosses through a small irrigation scheme in the nearby Mto-wa-mbu village. The area is semi-arid with warm weather due to low altitude of less than 1200 m above sea level.

Lake Natron is within Lake Natron Game Controlled Area and extends to the border with Kenya. It is the most important breeding site for flamingos in Eastern and Southern Africa due to presence of a number of rocks within the lake inaccessible by terrestrial predators (Simons, 2000; Tuite, 2000). The weather is hot and is a semi-arid area with volcanic soils dominated by acacia wooded grassland. The alkaline and fresh water streams from the rift drain into the lake and the surrounding area is inhabited by Maasai pastoral community (Fig. 1).

Clinicopathological examination

Affected flamingos were observed from a distance using binoculars and fresh dead birds were assessed for the general condition and necropsied to examine for pathological lesions and the significant pathological changes were recorded. The age of affected birds was estimated in two categories as either juvenile or adult basing on the colour of the feathers.

Sample collection

Dead flamingos and water samples were collected from Empakaai Crater and Lake Natron in 2000 and 2002 respectively and from Lake Manyara in 2004 (Fig. 2). The dead flamingos were grossly examined and some fresh carcasses necropsied. About 10 g of tissue samples from each organ were collected in 50 ml glass bottles containing 98% methanol (analytical grade) for cyanotoxins analysis. Thin slices of tissues (1x 0.5cm thick) for histopathological analysis were collected and preserved in sampling bottles containing 10% buffered formalin.

Laboratory analyses

Local laboratories

Tissue samples (spleen, fat, intestines, lungs, liver and kidneys) from fresh carcasses from Empakaai

Crater and Lake Natron were submitted to the Government Chief Chemist in Dar es Salaam, where Lassaigne's test was used to analyze the tissues for the acidic fraction and confirmation was done by Fourier Transformed Infra Red method. Presence of organophosphorus or organochlorine pesticides in water samples were determined using High Performance Liquid Chromatography (HPLC) method with precolumn derivatization and spectrophotometric detector as previously described (Tsui and Chu, 2004). Formalin fixed specimens were submitted to the Central Veterinary Laboratory (CVL) in Dar es Salaam and at the Faculty of Veterinary Medicine of the Sokoine University of Agriculture (SUA) for histopathological examination.

International laboratories

Duplicate tissue samples from Empakaai Crater, Lake Manyara and Lake Natron were submitted to the Leibniz Institute for Fresh Water Ecology and Inland Fisheries, Berlin, and the Institute for Molecular Pharmacology, Berlin Germany. Twelve tissue samples were subjected for toxicological assay and were analyzed using HPLC with Photodiode Array detection (HPLC- PDA), and the samples were screened for microcystins and anatoxins. Preparation and analysis of samples including type of column, running conditions, mobile phase used, linearity for the standards and detection limits were performed as previously described (Ballot et al., 2004).

Data analysis

Data were processed and analyzed using the JMP® Software for Statistical Visualization (SAS Institute Inc), version 6. Descriptive statistics were used to determine means and standard error of the mean (SEM). Because of small sample size and variation of type of samples of flamingos from each of the two lakes, test for statistical significance was carried out to compare levels of cyanotoxins in liver and kidneys only.

Results

Algae species identified in soda lakes of Tanzania

A number of algae species have been identified in some of the soda lakes of Tanzania as described by Lugomela et al. (2006), and soda lakes are mostly dominated by *Arthrospira* sp. which is the main feed for lesser flamingos (Tuite, 2000; Metcalf et al. 2013).

Clinicopathological findings in affected birds

Juvenile and adult birds were affected; however, more adult carcasses were encountered than juveniles. Before death some of the birds exhibited symptoms of staggering movement, convulsions and bending of the neck backwards, which is a neurological symptom. In addition, a number of water ducks were among the water birds that died in the lake Manyara die-off. In general it was estimated that over 1,000 lesser flamingos died in Empakaai Crater in 2000 and in Lake Natron in 2002 and more than 15,000 birds died in Lake Manyara in 2004. The significant pathological lesions observed in fresh carcasses included edema in lungs, enlarged liver, hemorrhages in liver with multiple necrotic foci, hemorrhages in kidneys, hemorrhages in intestines with erosion of mucosa. These pathological changes resemble the features produced by fowl cholera (*Pasteurella multocida*) and colibacillosis (*Escherichia coli*) in poultry (Aguirre et al., 1991). It is unfortunate that samples were not taken for bacterial culture due to lack of cold chain and transport media at the time of mass-die off.

Histopathological examination

Histopathological examination revealed hyperemia, edema and extensive areas of hemorrhages in liver with multifocal mononuclear cellular infiltration in the portal triad areas and severe hepatic degeneration suggesting chronic hepatitis and/or microgranulomas. In kidneys, there were edema, hyperemia, hemorrhages with focal mononuclear cellular infiltration and inflammatory reaction in interstitium suggestive of extensive interstitial nephritis. The intestines had chronic inflammatory reaction suggesting chronic enteritis. Similarly, the spleen had chronic inflammatory reaction. The lungs had edema and infiltration of neutrophils and mononuclear cells suggestive of pneumonia from bacterial infection and the pathological changes in brain tissue indicated myelin degeneration.

Water and tissue analysis

The analysis of water samples did not detect any traces of pesticides, however, in tissues traces of organochlorine and organophosphate (endosulphan and malathion respectively) were detected but it is unfortunate that the pesticides levels were not quantified in parts per million (ppm) due to little tissues submitted for analyses.

HPLC-PDA assay in Berlin

The analysis revealed presence of neurotoxin (Anatoxin-a) and hepatotoxins (Microcystins LR, RR). Anatoxin-a was detected but the amount of tissue submitted for analysis was not sufficient to determine toxin concentration (anatoxin-a ng /g tissue) in tissues. Analysis showed in most of the organs two different toxin congeners and no toxins were detected in lung tissues. The results for microcystins indicating levels of hepatotoxins in different tissues are shown in Table 1. Tissue samples that were collected in Empakaai Crater and Lake Natron revealed higher concentration in liver

Table 1: Laboratory results from lesser flamingos collected from Empakai Crater, Lake Natron and Lake Manyara

Location	Year	Organ	Microcystin-LR		Microcystin-RR	
			Mean ± SEM	Range	Mean ± SEM	Range
Empakaai Crater & Lake Natron	2002	Liver	0.167±0.002(5)	0.162-0.172	0.159±0.002(5)	0.152-0.165
		Spleen	0.048±0.001(5)	0.045-0.051	0.086±0.004(5)	0.074-0.097
		Lung	0	-	0	-
		Kidney	0.013±0.004(5)	BDL-0.02	0.02±0.003(5)	0.014-0.026
		Fat	0.022 ± 0.004(5)	0.009-0.031	0.05± 0.01(5)	0.031-0.085
		Intestine	0.001±0.0002(5)	BDL-0.012	0.014±0.001(5)	0.011-0.016
Lake Manyara	2004	Liver	1.13 ± 0.01 (7)	BDL– 1.16	0.2 ± 0.16 (7)	BDL–0.84
		Kidney	0.03 ± 0.01 (7)	BDL – 0.08	0.03 ± 0.02 (7)	BDL-0.08

BDL = below detection limit; (n) = number of samples from different birds

than in other organs. Concentration of microcystin LR was significantly (P=0.0003) higher in liver than in kidney for the samples that were collected in Lake Manyara in 2004. Although levels of microcystin RR were higher in liver than in kidney samples, the difference was statistically insignificant (P=0.3). Similarly, the differences on levels of microcystins in liver and kidneys from lake Manyara and those from lake Natron and Empakaai Crater were statistically insignificant (P=0.075).

Discussion

The timing of flamingo deaths suggested that poisoning from a bloom of toxic algae might have been responsible for increased levels of mortality, as cyanotoxin is known to rapidly multiply during periods of low precipitation. Furthermore, Cyanobacterial toxins were found in tissue samples from lesser flamingos that died at Lake Bogoria and Lake Nakuru, Kenya (Codd et al., 2003; Krienitz et al., 2003; Metcalf et al., 2013). In Lake Manyara, a number of ducks and Egyptian geese were observed to have died at the time of mass mortality of lesser flamingos, possibly from the same toxicant and this corroborate with the reports of other researchers (Creekmore, 2001; Metcalf et al., 2008). Partially digested filaments of cyanobacterium *Arthrospira fusiformis* have been recently identified in guts and faeces of lesser flamingos concluding that probably these birds feed mostly on this algal species (Lugomela et al., 2006; Metcalf et al., 2013). At the time, when mass mortalities were experienced, other studies using mice inoculation test revealed high toxicity of crude microalgal extract dominated by *A. fusiformis* (Lugomela et al., 2006). Inoculated mice became lethargic, with loss of balance, uncoordinated movements, intermittent tremors, dyspnoea with gasping, respiratory arrest and finally death. Observations from the current study, in addition to findings from others (Lugomela et al., 2006; Metcalf et al., 2013) give circumstantial evidence that cyanobacterial toxins and secondary bacterial infection were the likely causes of mortalities of lesser flamingos (Aguirre at al., 1991).

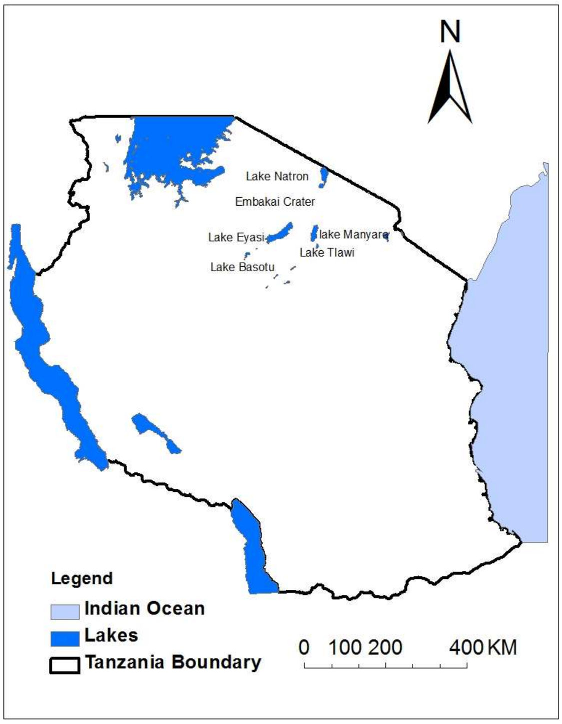

Fig. 1: Soda lakes of Eastern Great Rift Valley in northern Tanzania

Fig. 2: Mass mortality of lesser flamingo at Lake Manyara National Park in August 2004

Anatoxin-a and microcystins were detected in tissues from lesser flamingos collected from Soda lakes in Tanzania and the concentration of hepatotoxins were higher than what has been reported elsewhere in East Africa (Pflugmacher, 2004, unpublished). The toxins have been demonstrated in *A. fusiformis* which is the main food of lesser flamingos suggesting that this algae species can produce lethal toxins during low precipitation (Ballot et al., 2004; Metcalf et al., 2013).

Variation in pH in Soda lakes due to prolonged drought associated with climate change is probably a major factor which trigger toxin production in *Cyanobacteria* (Vonishak and Tomaselli, 2000; Ballot et al., 2004; Araoz et al., 2010; Downing et al., 2011). In 2000, 2002 and 2004, the northern zone of Tanzania was hit by severe drought and the water levels in the Soda lakes receded very dramatically. Such an inclement climatic condition is probably ideal for toxic *Cyanobacteria* bloom. Generally, the algae bloom at higher alkaline pH, which occur during low precipitation or drought. Anthropogenic activities such as use of fertilizer and organic manure, which is eventually drained by rainfall run off into the lakes can stimulate the algae bloom due to increased levels of nitrates and phosphorous (Kotut et al., 2010).

The observed clinical symptoms of neurological features are suggestive of neurotoxin, which could probably result from consumption of cyanotoxins. Presence of hepatotoxic substances in conjunction with secondary bacterial infection probably exacerbated the clinical condition leading to mass mortality (Aguirre et al., 1991; Kock et al., 1999). The clinical and post mortem picture conform to the laboratory findings of the cyanotoxins and bacterial infection. The pesticide residues in tissues in conjunction with secondary bacterial infection can have a synergistic effect in exacerbating the clinical condition leading to mass mortality. However, failure to detect organochlorine and organophosphate pesticides in water samples from the study sites indicate that the agricultural activities in the up streams do not contribute significant amount of pesticides in the soda lakes. Therefore, pesticide residues which were detected in tissue samples were probably a result of long time bioaccumulation from other Soda lakes in East Africa as flamingos undergo extensive movement in the region and the birds live 30-50 years (Simons, 2000; Tuite, 2000). Drainage of pesticide residues from surrounding farmland in the East African soda lakes have been reported (Mavura and Wangila, 2003). The pathological effects of these pesticides are known for instance organochlorine pesticides affect the breeding performance of many organisms including reproductive failure and shell thinning in birds (Lindberg et al., 2004).

Conclusion

A complex array of factors may be causing flamingo mortality including algal toxins, secondary microbial infections and malnutrition from low green-algae crop as a result of drying out or receding of Soda lakes (Simons, 2000; Tuite, 2000; Araoz et al., 2010). Since the mass die-off of lesser flamingos started to occur in recent years in Tanzania, it is difficult to predict the consequences for the sustainability of the species. However, increased environmental degradation from anthropogenic activities and frequent prolonged drought will probably lead to negative impact on the sustainable conservation of these threatened bird species.

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