Toxins in the food chain

The decline of the bald eagle parallels that of the Indian vulture

S ANANTHANARAYANAN

The Indian vulture, descendant of the legendary *Jatayu* of the Ramayana, faces extinction because of a popular veterinary medicine, which is toxic to vultures that feed on animal carcasses. The bald eagle, found in North America, and some other bird, amphibian, reptile and fish species, are now in danger because of a similar reason, a substance used as a weed killer in large water bodies.

Steffen Breinlinger, Tabitha J Phillips, Brigette N Haram, Jan Mareš, José A Martínez Yerena, Pavel Hrouzek, Roman Sobotka, W Matthew Henderson, Peter Schmieder, Susan M Williams, James D Lauderdale, H Dayton Wilde, Wesley Gerrin, Andreja Kust, John W Washington, Christoph Wagner, Benedikt Geier, Manuel Liebeke, Heike Enke, Timo H J Niedermeyer, Susan B Wilde, from Martin-Luther-University Halle-Wittenberg, Leibniz-Forschungsinstitut für Molekulare Pharmakologie, Berlin, and Planck Institute for Marine Microbiology, Bremen, Germany, the Czech Academy of Sciences and the University of South Bohemia, Czech Republic, University of Georgia, US and the US Environmental Protection Agency, describe in the journal, *Science*, the course of detecting the agent behind the loss of eagles in North America.

Over the winter of 1994 to 1995, the paper says, there was unprecedented mortality of bald eagles at DeGray Lake in Arkansas, US. The cause of eagle deaths was found to be a neuro-degenerative disease, avian vacuolar myelinopathy, which destroyed brain tissue and led to birds losing control over their bodies. The birds would "overfly perches and collide with rock walls", says a publication. In the three years that followed, the same disease was confirmed in five other states and in many other avian species. The outbreaks were during the fall and winter and in or near man-made water bodies which had abundant aquatic vegetation. Good numbers of fish and water birds in the winter months attract bald eagles. The water birds were affected by AVM and were easy targets, but they passed the disease on to the predators.

It was found that wild coots and mallards developed AVM within five weeks of being released into a lake which had the disease. Chemical analyses of bird carcasses, however, revealed no traces of chemicals that could be responsible. The material from affected lakes and ponds did not induce disease and the disease did not transfer from one bird to another. The source of the disease was elusive.

Then, it was found that all the disease-affected water bodies contained rapidly spreading, or invasive, underwater vegetation, mainly *Hydrilla verticillata*, or waterthyme, with the leaves heavily colonised by a previously unidentified form of blue-green algae. And it was shown that AVM was induced in water birds when they fed on *Hydrilla verticillata* which had the algae. The algae have been named *Aetokthonos hydrillicola* – Greek for "eagle-killer that resides on hydrilla". And then, as AVM was also found to affect amphibians, reptiles and fish, the letter, "A" has been dropped from the acronym, leaving it as "VM".

As blue-green algae, also known as cyanobacteria, are known to participate in production of toxins and other specialised chemicals, it was possible that the *A. hydricillicola* on the leaves was responsible for the outbreak of VM. The paper states that H. verticillate, colonised by *A. hydricillicola*, was found to be widespread in major water bodies throughout southeastern US. The team hence collected samples of the plant and its coloniser, to isolate and culture the cyanobacterium, and then to track down the neurotoxin.

The process took two years, the paper says, but *A. hydricillicola* was successfully cultured. And the culture was also confirmed as *A. hydricillicola* by testing for a specific genetic component. Administering the substance to experimental chickens, however, did not result in VM. The idea that *A. hydricillicola* produced the toxin was hence not borne out. Could it be that the cyanobacterium produces the suspected toxin only when it grows on *H. ventricillata*, and not when cultured in the lab?

To test the possibility, the team collected more A. hydrillicola, not from cultures, but as growing on H. verticillate from water bodies that were known to host VM. The colonies of cyanobacteria were then analysed using a complex laboratory technique, which could identify individual compounds in a mixture of many. The procedure detected a molecule that contained, like many organic substances, atoms of carbon, hydrogen and nitrogen, and in addition, a good number of atoms of bromine. Significantly, this molecule was not there in the cyanobacteria grown in the lab. It was apparent that the molecule was a new one that arose when the cyanobacterium colonised the water plants and could be the reason for VM. The paper also notes that compounds containing bromine or chlorine are known to produce VM-like brain lesions.

Control trials were conducted by adding bromine sources to the laboratory cultures. The result, sure enough, was that the cultures then led to VM – which showed that it was bromine that made the difference. Further trials could show how varying the level of bromine affected the production of the toxin. And more important, that the environmental stress of lower temperatures, as found in the fall and winter, led to the greatest production.

Sources of bromine

The presence of bromine in the water bodies may be partly natural, and partly the chemicals used by power plants or inflows like fungicides, gasoline additives, that flow in. Chemicals used to control the rapid increase of underwater plants, including *H. ventricillata*, contain bromine, the paper says. The paper mentions a biological means of controlling weeds, a species of fish that could deal with excess plants. This is a solution that has been used to eliminate *H. ventricillata* in places where there were eagle deaths, the paper says. There is reluctance on the part of fisheries managers, the authors say, but the concerns need to be assessed and benefits of using chemicals reviewed.

That there is a study and steps have been taken to regulate the use of bromine-containing substances suggests that inadvertent damage to an important part of biodiversity would now be prevented. In the case of the Indian vulture, rapid decline in numbers, due to kidney disease, was noticed in the 1990s. In 2003, the Peregrine Fund, which had helped save the peregrine falcon in the US, identified the cause as diclofenac, a drug used to relieve joint pain and swelling. A veterinary preparation was also widely used with cattle. When vultures, which forage in flocks, fed on carcasses of the cattle, they ingested diclofenac and started dying in numbers.

Despite lobbying by wildlife conservation groups, Indian authorities w ere slow to ban the veterinary drug. Even after the ban, dairy farmers use, not an alternative that is available, but the human version of the drug with diclofenac, which does not help the Indian vulture. The bird is now scarcely seen and is said to be there in captive breeding.

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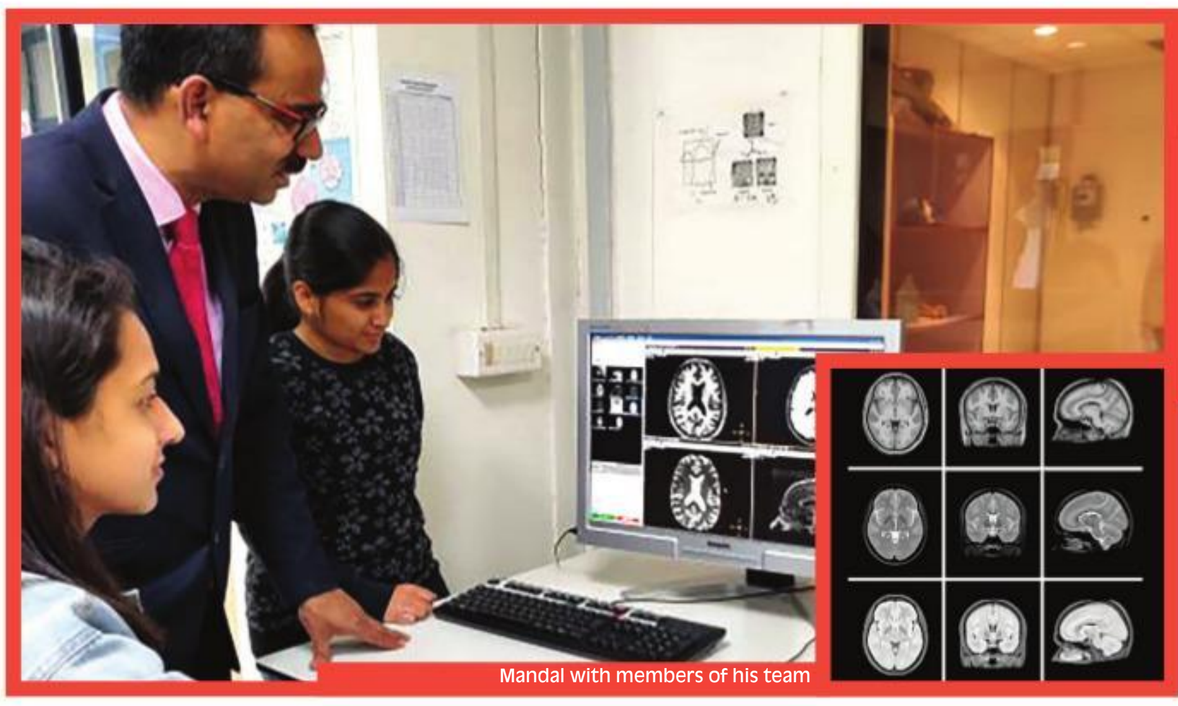
The vulture in Europe

In 2016, the journal *Nature* cited a current paper in the *Journal of Applied Ecology*, which said diclofenac, approved in Spain, endangers thousands of that country's Eurasian griffon vultures.

"In Europe, diclofenac has been approved for veterinary use since 1993. In 2014, the European Medicines Agency acknowledged that vultures are at risk of consuming residues of the drug in dead livestock but did not recommend banning it. In 2015, the European Commission decided to follow the EMA's recommendation, leaving it up to EU member states to prevent diclofenac-laced carcasses from entering the food chain," the news item in *Nature* said.

MARKING A FIRST

Professor Pravat K Mandal and his team at the National Brain Research Centre in Haryana have developed the Indian Brain template to aid neuro-imaging analysis



Mandal with members of his team

BIJU DHARMAPALAN

The "Brahma" project, started in 2018 and supported by the Union government's department of science and technology, envisaged the development of a brain template for the Indian population. Like a geographical map that helps to explore real world places, a brain template provides a standard reference coordinate system for brain structure analysis. A specific anatomical presentation depicting finer details, doctors need it for planning a brain surgery.

Till recently, most clinicians in India used the brain template developed by the Montreal Neurological Institute, Canada. But it is based on data from Caucasian populations. Overall brain features – brain volume, shape, and size – vary across populations due to phenotypic, genetic, developmental, and environmental factors. Many countries have created brain templates based on their populations but unfortunately, India didn't have one.

That struck professor Pravat K Mandal, director-in-charge of the National Brain Research Centre at Manesar, Haryana in 2011. He mooted the need for an Indian Brain database in a paper published in 2012. Within two and a half years of the "Brahma" project's inception, commendable results have been gathered from every state in the country.

The Indian Brain database created by Mandal's group is the first-of-its-kind across the world as it provides functional as well as anatomical aspects of the brain. More importantly, the database is freely downloadable and can be assessed by the public. Such an Indian population-specific template is also particularly relevant for accurate neuro-imaging data analysis in clinical conditions.

Mandal used a unique cost-effective model for data collection. Instead of going to different parts of India, he collected data from volunteers provided by State Houses stationed in New Delhi. The critical data was generated at NBRC using state-of-the-art magnetic resonance imaging scans using a 3T Philips scanner. Phase I of the project collected data from 140 people below the age of 50, phase II consisted of 90 healthy senior citizens above the age of 60, and phase III, which is under progress, focuses on the unique template from Alzheimer's disease patients.

The use of the "Brahma" template for analysis of structural and functional neuro-imaging data from Indian participants provides improved accuracy with statistically significant results over that obtained using the International Consortium for Brain Mapping-152 template. The availability of different image contrasts, tissue maps and segmentation atlases make the "Brahma" template a comprehensive tool for multimodal image analysis in laboratory and clinical settings.

Mandal's team uses functional magnetic resonance imaging methods to investigate a variety of aspects of human cognition, and understand how and why cognition changes as a result of healthy aging and Alzheimer's disease. Recent neurophysiological and imaging studies have also revealed that in addition to memory deficits, changes in functional voltage-sensing phosphatases are also pervasive in the early stages of Alzheimer's disease. The team uses FMRI to define the role of different brain regions involved in VSP and investigates the functional re-organisation of VSP networks in early Alzheimer's disease.

The Neuroimaging and Neurospectroscopy Laboratory at the NBRC has developed a technology that uses the brain's stress levels and pH for early identification of brain disorders such as Alzheimer's disease, Parkinson's disease, depression and schizophrenia. Mandal's group has also discovered the role of glutathione – abbreviated as GSH – in maintaining a healthy environment in the brain. It is a major endogenous enzyme-catalysed antioxidant that plays a fundamental role in detoxification of reactive oxygen species and regulates the intra-cellular redox environment.

One of the emerging causative factors associated with Alzheimer's disease pathology is oxidative stress. The increase in oxidative stress has been attributed to decreased levels of GSH. GSH is also known to help repair cell damage and reduced concentrations of the molecule in specific brain regions may help predict Alzheimer's disease. Deficiency of GSH also causes various psychiatric illnesses.

GSH exists in two forms and different brain regions have different amounts of them. The good form is always more than the other one. There may be two people of the same age, but one may get Alzheimer's disease and other may not. That is because micro-environments of their brains are not same, and they have different amounts of protective GSH.

Mandal works in a trans-disciplinary group consisting of engineers, computer scientists, clinicians, psychologists, basic science researchers and social scientists. It has developed various software tools for large-scale data analysis using brain imaging where metabolic and neuro-psychological scores provide information about disease progression and identify early diagnostic biomarkers. One such is "Bharat" or the big data analytic model for early diagnostic biomarker of Alzheimer's disease. With data of Alzheimer's disease and Parkinson's patients having increased to the tune of thousands, there is a need to check the purity of data especially those that emerge from brain chemical databases. For that, the group has recently made a software called "Gangotri" that could be used to check the quality of data.

Mandal gives the credit for his success to mother Kalpana, and teacher Chandicharan Mukherjee, who taught him at the Dhulai Ramkumar Mrinmoyee Vidyamandir in the Bankura district of West Bengal. Mukherjee used to give late night tuition and as a boy, Mandal had to sleep in the school. After studying for a physical chemistry degree at the University of Burdwan, he completed his PhD in computational chemistry from the Indian Institute of Technology-Madras. He pursued postdoctoral studies at the University of California, Davis, US and moved on to the Johannes Kepler University Linz, Austria as a guest scientist. He later joined the department of psychiatry, School of Medicine, University of Pittsburgh, US as an expert in in vivo brain spectroscopy. In 2008, he joined the NBRC.

A person who has transformed from a physicist to a chemist and now, a neurologist, Mandal says, "If we understand the Indian brain thoroughly, then it is equivalent to understanding the brains of the whole world. That is because India is a rich and diverse country with different social, cultural, racial and geographical differences. All of those also make our brains complex. The brains of people from an Indian state are equivalent to those in the whole of the US or any other country. So, the information we gather would unravel a lot of secrets and prove beneficial to humanity."

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PLUS POINTS

Covid-19 & heart disease

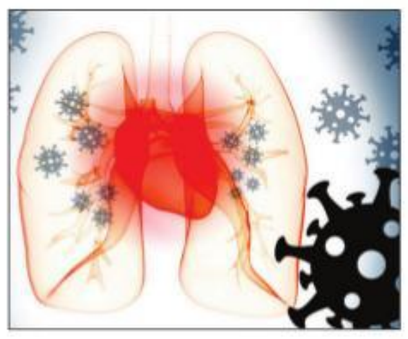
The coronavirus disease has affirmed itself as a special clinical challenge with unpredictable symptomatology. Co-morbidities, mainly pre-existing heart diseases, have spelt poor prognoses alongside a higher risk of intensive care mortality. Attention to the coexisting conditions is being brought to the forefront by the research contributions of Dr Namrata Singhania, who is based out of Mount Carmel East Hospital in Columbus, Ohio, US.

Dr Singhania, a medical graduate from New Delhi, currently focuses her practice on holistic aspects of chronic medical conditions. More specifically, she has alerted people that in the setting of chronic heart failure, Sars-CoV-2 can cause significant heart muscle injury and acute decompensation of chronic heart failure. She has also made major strides in emphasising continuation and adherence to heart failure pharmacotherapy, especially during hospital admissions.

Chronic heart failure affects around 10 per cent of the population over 70 years of age in developed countries. This known epidemic has significantly accentuated the current coronavirus pandemic. Dr Singhania's expert clinical therapeutic advice of continuation of angiotensin converting enzyme inhibitors and angiotensin receptor blockers during hospital admission is critical in preventing patient mortality. Two of the main determinants of heart failure, arterial hypertension and coronary artery disease, pose additional risks in the infected population. Importantly, Dr Singhania's recommendations have also been adapted in the Kidney Disease Improving Global Outcomes 2020 Clinical Practice Guidelines for diabetes management in chronic kidney disease.

Recent global clinical observations do suggest that chronic heart diseases including CHF are critical variables that promote a severe Covid-19 phenotype. Dr Singhania pointed out that Sars-CoV-2 infection may lead to increased cardiac arrhythmia (heart rhythm disturbances) and ischemia, enhancing the risk of sudden cardiac death. Added to those are inflammatory cytokines that raise the viscosity of blood and coagulability, thus increasing the risk for ischemia and heart attacks.

The respiratory failure caused by the viral infection aggravates the imbalance between scarce oxygen supply and raised energy demand of the heart muscles, leading to myocardial dysfunction. Use of a mechanical ventilator in the ICU elevates the workload of the right side of the heart, leading to further reduction of cardiac output in the backdrop of a failing heart. It creates a complex situation of heart and lung dysfunction, which may be compared to the unfortunate combination of failing events that occur during an airplane crash.



The functional receptor for Sars-CoV-2, human ACE2 or the angiotensin converting enzyme, is overly expressed in patients with heart diseases. Thus, viral infection of the heart cells sets off direct heart muscle damage. Sustained work by Dr Singhania has suggested the deleterious effect of upregulation of renin-angiotensin-aldosterone system. She also pointed out that though there is a correlation between ACE inhibitors and the ACE2 enzyme, evidence regarding whether ACE inhibitors raise the ACE2 enzyme expression is conflicting. Dr Singhania particularly recommended against the interruption of such drugs like ACEIs/ARBs for management of acute and chronic heart failure. In fact, losartan, an ARB is being shown to play a protective role on lung injury.

Experimental anti-retroviral medications like hydroxychloroquine diminish clearance of heart failure medication like digoxin. Other medications like azithromycin can pose increased risk of heart rhythm abnormalities. Dr Singhania said that structural heart diseases, fluid balance, electrolyte disturbances, liver and kidney failure all trigger a deadly scenario in chronic heart failure patients affected by the Sars-CoV-2 virus, significantly raising morbidity and mortality.

–SUBHENDU MAITI