International Journal of Theoretical & Applied Sciences, 10(1): 21-26(2018)
Special Issue on Environmental Contaminants and Management

ISSN No. (Print): 0975-1718
ISSN No. (Online): 2249-3247

Mortality of Painted Stork *Mycteria leucocephala* by H5N8 Avain Viral Strain in Gandhi Zoological Park in Gwalior, Madhya Pradesh (India)

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(Received 12 November, 2017 accepted 12 December, 2017)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: H5N8 Influenza is one of the most dreadful diseases that have caused great damage to birds all over the world. The objective of the present study is to assess the gravity of damage caused by H5N8 in Gandhi Zoological Park in Gwalior. The study used observation methods and carried out a desktop analysis to know the causes and future implications of this emerging deadly disease. Further, water quality was assessed to study the physic-chemical and biological parameters of drinking water for the birds in zoo. The study identified overcrowding, migration of birds and climatic factors as main causes of its emergence that has caused 100% mortality of the painted stork in Gandhi Zoological Park in Gwalior. The current study stressed the need that overcrowding of birds in the zoo and such places must be regulated in a systematic and scientific manner along with regional weather monitoring with bird flu surveillance system collaboration with countries who have proper control over such epidemics must be initiated to respond to the potential threats of pandemic avian influenza.

Keywords: H5N8; mortality; painted stork; overcrowding; Migration

I. INTRODUCTION

H5N8 is considered as the newly emerging infectious diseases. And was for the first time reported in China [1] and South Korea [2]. This viral strain is responsible for large number of outbreaks all over the world and at different places in India since it showed its previous exposure [3]. There is Chaos over the possibility of the cross- species infection that may occur between migratory birds, poultry and humans [1]. Avian Influenza is found among large number of birds in India [4]. Changing climate has great role in the outbreaks of avian influenza because it shows seasonality and behavior of population as the indoor crowding during winter season [5]. Mostly the outbreak occurs during winters because humid and warm climate may increase the chance of transmission of virus while hot and dried conductions may decrease. Migratory birds act as the base line source in the spread of these diseases [6]. Asia is having great diversity of habitats for water birds including rivers, tundra, estuaries, lakes, Marshes, coastal marshes, beaches, mudflats, atolls, mangroves and costal reefs. According to the Wetlands International, water birds in Asia are facing a long-term threat for conservation in changing habitat due to human utilization practices. Habitat loss has direct impact on the spread of bird flu in various ways due to increase in population there is directly increase in urbanization in the undeveloped areas that will lead to contacts between wild birds and humans and possibility of bird to human transmission of avian influenza may increase. Migratory birds have inheritance potential as they are extremely loyal in their migration and also as well as in their stopover to replenish their fat for onward journey. Forests and wetlands acts as the stopover points during the course of migration due to the destruction of these stopover points. Migratory birds are forced to rest and refuel at some other places like Farms, cities, zoos. These stopover points increase the contact between wild and domestic bird population and increase the chance for transmission of infection between wild and domestic bird population then to humans. In densely population there is a great chance of virus to spread quickly through respiratory droplets. Mixing farming is a process of rearing of different species in a common farm and is practiced in Asia were both poultry and pigs are reared in vary close association.

Pigs are susceptible to both avian and human influenza strains so they may act as a possible host for viral Re-assortment and may lead to spread infection through feces or saliva. Unhygienic conductions also increase the chance to spread infection in Asia as the open markets are responsible for the spreading of zoonotic diseases to people. Animals are transported to large distance from their habitat along with large number of microorganism and theses new markets provides a best environment for these new microorganisms to get flourish and to infect new species including humans SARS is to be believed to have infected humans from civet cats which were sold in open markets. Recently an outbreak took place at Gandhi Zoological Park, Gwalior M.P India in which 28 painted Strok birds were died within seven days, and the causative agent was confirmed as H5N8 viral strain of Avian Influenza.

II. METHODOLOGY

A. Study area

The current study was carried out in Gandhi Zoological Park Gwalior. Situated with the geographical co-ordinates of 26°12'34.9"N latitude and 78°10' 09. 5"E longitude at the center of city (Fig. 1). The zoo is inhabited by different types of animals that comprised of 27 species of birds, 17 species of mammals and 10 species of reptiles with a total of 316, 106 and 79 birds, mammals and reptiles, respectively. 159 endangered animals are also present in the zoo as shown in table 1.

Table 1: Number of species and their status.

Animal Name	Species	Number	Endangered
Birds	27	316	40
Mammals	17	106	66
Reptiles	10	79	53

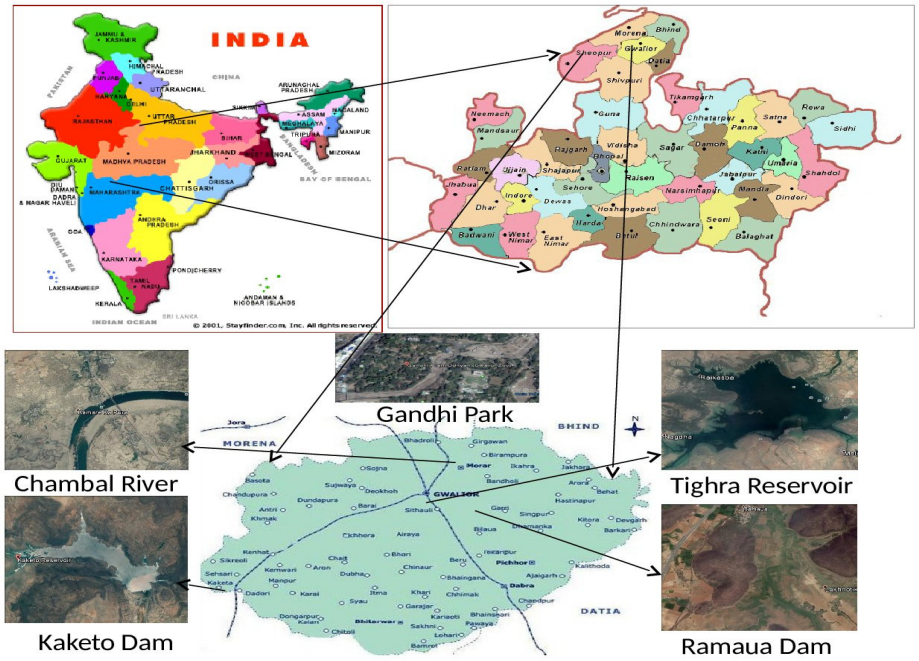

Fig. 1. Map of Gandhi Zoological Park Gwalior.

A survey was conducted in the Gandhi Zoological Park, to observe both healthy and infected birds. During the survey group discussions were conducted at regular interval with the experts and in- charge zoo veterinary officer. Nikon digital cameras was used to capture photo of both affected (dead) and live birds.

B. Monitoring of weather parameters

The data of weather parameters like temperature, humidity, precipitation were collected from metrological weather station Thathipur Gwalior, for the period of one year, (2016).

C. Analysis of water quality

The water quality parameters *viz*., pH, Electrical conductivity, Total dissolved solids, Calcium, Magnesium, Alkalinity, bicarbonates, Total hardness, chlorides, sodium, Dissolved oxygen, copper, iron, sulfate Total coliform and Standard plate count were analyzed by following the standard methods of APHA, 2012 [7].

III. RESULTS

During the period of observation it was observed that (Painted stork) and (Pelicans) were in mixed farming in an area of 3500 square feet's in Gandhi Zoological park, both the species depends upon the same source of food and water, mostly on fish includes major carps including *Catla Catla, Lebo Rohta, cirrus Magalia* and the source of water is ground water, Pelicans were 03 in number and all of them are at post breeding stage i.e 30-40 years and alive, while as there was 100% impact on painted stock. There was 28 birds of Painted Stork *M. Leucocephala* in zoo and all of them died within a week, after autopsy, the samples were analyzed in National Institute High Security Animal disease, Bhopal as per reports it was confirmed that the strain responsible for the cause of death of painted stork was H5N8 (Table 2, Fig. 2).

Table 2: Date wise deaths of Painted Storks at Gandhi Zoological Park Gwalior.

S.No	Date	No of birds died	Cumulative frequency	Temperature (^{o}C)	Humidity (%)
1	18/10/16	03	-	35	72
2	19/10/16	05	08	33	74
3	20/10/16	07	15	35	77
4	21/10/16	04	19	34	65
5	22/10/16	0	19	34	48
6	23/10/16	03	22	34	71
7	24/10/16	06	28	36	56

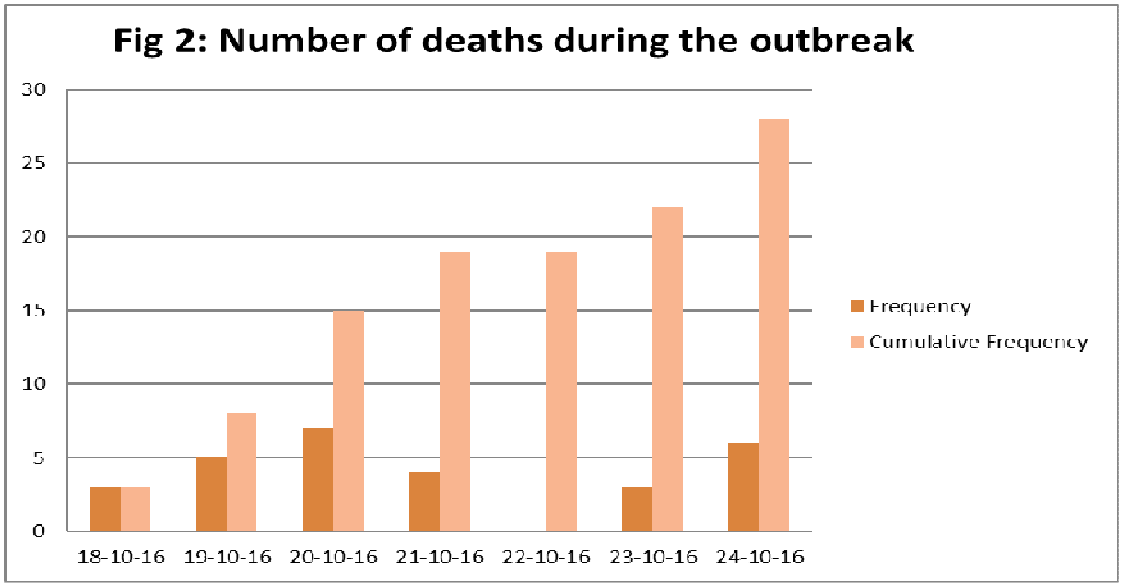

Table 3: Average, minimum, maximum temperature, rainfall and humidity during 2016.

Month	Average Temperature (°C)	Maximum temperature (°C)	Minimum temperature (°C)	Average relative humidity (%)
January	15.8	24.7	8.2	71.5
February	19.3	27.8	11.2	62.7
March	26.1	34.1	17.1	48.6
April	32.4	40	22.4	35
May	34.9	41.8	24.9	40.6
June	35	40.7	27.9	52.9
July	29.7	33.4	26.1	82.1
August	28.6	32.3	25	84.8
September	29.3	34	25	73.1
October	26.6	34.5	19.6	57.9
November	19.9	30.4	12.3	58.3
December	15.8	26	9.1	72.3

Table 4: Physio chemical parameters of water supplied to painted stork.

Parameters	SI	S2	S3	S4	Standard values
Temperature (^{O}C)	23.2	23.5	NE	NE	NE
pH.	8.0	6.5	7	9.2	6.8-7.2
EC (ms)	0.65	0.32	NE	NE	NE
Total dissolved solids, (Max(mg/l)	450	460	400	410	NE
Calcium(mg/l)	30	63	65	65	60 mg/l
Magnesium(mg/l)	54	214	30	150	NE
Alkalinity(mg/l) Bicarbonates	102	120	112	118	NE
Total hardness as $CaCo_3$ (mg/l)	60	330	100	500	60-180
Chloride (mg/l)	110	150	100	105	250 mg/l
Sodium (mg/l)	Nil	06	0	0	32 mg/l
Potassium (mg/l)	0.2	00	0	0	NE
DO (mg/l)	0.8	0.7	0.8	0.8	NE
Copper (mg/l)	0.2	0.02	0.02	0.04	0.6 mg/l
Iron (mg/l)	0.8	1	1.4	0.8	0.3 mg/l
Sulfate (mg/l)	80	75	78	70	250 mg/
Total coliform MPN	09	700	500	600	50 mg/l

Table 5: Suspected migratory birds responsible for bird flu.

Common name	Taxonomic name	Status
Coots	*Fulica atra (Linnaeus)*	RM
Grey Heron	*Ardea Cinerea (Linnaeus)*	RM
Large Carmorant	*Phalacocorax carbo*	RM
Phalacrocorax (vieillot)	*Microcarbo nigers*	RM
Redcrested Pochard	*Netta rufina (Pallas)*	M
Small blue Kingfisher	*Aicedo athus (Linnaeus)*	RM

RM: Residential Migrants, M: Migrants

IV. DISCUSSIONS

It is very important to comprehend the different routes of H5N8 transmission and its pathways towards the site of infection. It is observed that transmission occurs principally by inhalation of infectious droplets or airborne droplet nuclei and direct, or possibly indirect fomite, contact followed by transfer to the upper respiratory tract via the nose, mouth or conjunctival mucosa eyes [8].

However, till date no case of human infection has been observed by H5N8 strain only bird to bird transmission has been reported in many places around the world. Other strains of virus may cause Bird-to-human transmission to encompass these routes like ingestion of contaminated water, although evidence is lacking on the mechanisms by which the avian influenza crosses the species barrier from birds to infect humans. Direct contact with poultry or with objects or surfaces contaminated with feces from infected poultry is the primary cause.

Environmental factors on the transmission and persistence of virus have very little impact on these factors. However, in India the outbreak has been observed in the month of October and November when temperature slightly drops to minimum (34.6^{o}C) [Table 3].

The water given to these birds in general was in agreement with the standards (Table 4) however, the presence of excess chloride and sulphate may cause adverse problems to the birds as consuming too much chloride (Cl^{-}) has a detrimental effect on metabolism. Studies have shown that a level of 14 mg/l in drinking water can be detrimental to broilers if combined with 50 mg/l of sodium. High sulphate (SO_4) levels might have caused laxative effects in the birds. Levels as low as 50 mg/l can have a negative effect on performance of either the sodium or magnesium level is 50 mg/l or more. But may not cause death to birds, hence the perception that death may have caused due to poor quality of water can be ruled out.

It is already reported that persistence of AI viruses in water is a function of physical-chemical conditions [9] and were decreases as a function of temperature [9]. Although, up to 2005, the decrease in winter temperatures were repetitively cited as the prime factor for prevalence of infection under laboratory conditions and it is reported that the scenario may be different under field conditions as has been observed in this study. Further, in Indonesia, constantly high temperature and humidity were the main factors in the prevalence of this infection, where HPAI H5N1 virus persists endemically and in Germany the virus has been reported twice (Winter 2006, and mid-summer 2007). The dust storms with rapid temperature decrease may have contributed to the spread of H5N1 in these areas. Avian influenza viruses are also reported to persist for long periods of time in water with lack of evidence.

Maintaining indoor relative humidity >40% will significantly reduce the infectivity of virus as influenza retains maximal infectivity and inactivation of the virus at higher relative humidity. The virus carried on aerosol particles <4 μM may remain suspended in air currents longer and their rapid inactivation at high humidity tempers this [10]. Hence, it can be argued that environmental parameters do not play a direct role in prevalence of this virus and may be linked to different geographic conditions that need to be studied by involving different studies across the globe. Approximately 50-60 poultry farms were present in Gwalior which was found to be one of the main risk factors for the persistence of H5N8 virus. Almost similar observations were reported from Thailand, [11] Indonesia and Vietnam [12] Egypt and Nigeria.

The city of Gwalior has different water bodies that may range from small ponds to big lakes (e.g., Reservoirs, Ponds, Lakes and Rivers) and may be susceptible to avian influenza virus contamination. Open water bodies like Tighra Reservoir, Ramaua Dam, Chambal River, Kaketo Dam, etc. (Fig. 1) are habitat for different migratory birds. Risk to humans will depend on a variety of factors, including the use to which the water is put, the health status of the humans (e.g. immunocompromised versus healthy), and whether or not the water is treated. The biggest change in H5N8 infection results from migration behavior of birds. Fall in temperature and humidity are the precursors of this outbreak in Gwalior. The indirect effects, mainly those occurring as a result of the influence of climate change on water bodies and presence of poultry farms may aggravate the problem. More studies are needed to investigate the optimal weather conditions before the H5N8 outbreaks in each region before weather can be used to better predict outbreaks of this virus. Our findings strongly stress the need that that regional weather monitoring can be used as integral part of a bird flu surveillance system.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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