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Cover: Dorsal view of Mantis Shrimp *Cloridina ichneumon* (Fabricius, 1798) & *Gonodactylus demanii* (Henderson, 1893). © Fisheries Research Station, Junagadh Agricultural University, Sikka.



Drought may severely reduce the ability of wild Asian Elephants *Elephas maximus* (Mammalia: Proboscidea: Elephantidae) to resist opportunistic infections

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Abstract: The present study was conducted to assess the microbial quality of water in forest waterholes in different seasons and its possible impact on wild animals, at Bandipur and Nagarhole Tiger Reserve forests in the state of Karnataka, India, during the year 2012 which evidenced drought, and the year 2014 which witnessed normal rainfall in these forests. The forests recorded the death of 39 wild elephants during April and May of 2012. One ailing elephant was confirmed to have high fever, diarrhoea, leucocytosis, and symptoms of colic. Water samples collected from major waterholes during the peak drought showed higher numbers of coliforms and several species of opportunistic bacteria including species of *Vibrio* and *Campylobacter*. In the year 2014–15, with normal rainfall, the death of less than 10 wild elephants was documented during April to May, 2015. We collected water samples from 20 major waterholes every month from June 2014 to May 2015 and assessed the water quality. We found that the microbial water quality improved in rainy season (June–September), started deterioration in winter (October–January) and became poor in summer (February–May). Though, the water during the summer of 2014–15 was equally of poor microbial quality as seen during peaks of droughts, the elephant deaths were relatively lower, signifying the role of normal rainfall in forests which provides the availability of fodder and water, which determines the general body condition and ability to resist opportunistic infections. We discuss the measures suggested and implemented from this study and their utilities at ground level.

Keywords: *Campylobacter*, Coliforms, forest waterholes, microbial quality, rainfall, *Vibrio*, water, wildlife.

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INTRODUCTION

Concern about climate change has intensified interest in understanding how climatic variability affects animal life. Despite such effects being potentially most dramatic in long-lived, slow reproducing, large terrestrial mammals, little is known of the effects of climatic variation on survival in such species. A series of complex climatic changes affecting the equatorial Pacific region causes reversal of wind patterns in the Pacific Ocean and leads to consecutive droughts in Australia and Asia (Wenju et al. 2014; Chris 2015).

Water is essential for living. Wild animals depend on rainwater that accumulates in waterholes in forests. The rainwater that accumulates in waterholes remains throughout the year and is prone to microbial contaminations arising out of various sources, of which faecal contaminations from humans and wild animals are most important (Obi et al. 2002). Unpredictable chronic droughts lead to acute shortage of drinking water, forcing wild animals either to depend on limited water available in waterholes or they get no water at all (Durham et al. 2008). It has been extensively studied and reported that the microbial diarrhoeal diseases are a major public health problem from ingestion of water contaminated with human and/or animal faeces (Seas et al. 2000; Cabral 2010). However, no studies have been undertaken to correlate wild animal mortality with droughts and water quality in the wild.

The present study attempts to assess possible factors contributing to the deterioration of microbial quality of water during different seasons of a year, and its possible impact on wild animals. We used elephant mortality as evidence to compare the water quality and its impact during chronic droughts, in comparison to seasons of normal rainfall (Figure 2). Though microbial quality is not the only reason for animal deaths, this study analyses how the microbial quality of water in waterholes in forests could predispose elephants to mortality during extended droughts. This study was carried out in the Bandipur Tiger Reserve (also known as Bandipur National Park) and Nagarahole Tiger Reserve (also known as Nagarahole/Rajiv Gandhi National Park) forests in the state of Karnataka, India, during 2012 which witnessed severe drought in these forests, and in the year 2014–15 which had normal rainfall. The study is of significance since recurrent droughts could be a common feature in times to come, owing to severely disrupted global weather patterns and we need to know its impact on wildlife.

MATERIALS AND METHODS

Study area

The Bandipur Tiger Reserve with an area of 874.20 km² and the Nagarahole Tiger Reserve with an area of 643 km², are important components of the 5,500 km² 'Nilgiri Biosphere Reserve' which is one of the largest conservation areas in the world (Chandranaike et al. 2016, 2017) (Figure 1). The forests are a large chunk of dry deciduous forest which receives heavy pre-monsoon showers in late May. The south-west monsoon starts by mid-June and lasts until September. These two forests are one of the richest wildlife areas in India, being noted for their assemblage of seven large ungulate species—Muntjac *Muntiacus muntjak*, Chital *Axis axis*, Sambar *Rusa unicolor*, Chousingha *Tetracerus quadricornis*, Gaur *Bos gaurus*, Wild Pig *Sus scrofa cristatus*, & Asian Elephant *Elephas maximus* and three major carnivores—Tiger *Panthera tigris*, Leopard *Panthera pardus*, & Dhole *Cuon alpinus*. The forest supports a high ratio of predator and prey species.

As per the 2012 elephant census, Bandipur forest has a population of 1,697 elephants and Nagarahole forest has 1,320 elephants, constituting 27.9 % and 21.8 % of the total 6,072 elephants in Karnataka state, respectively (Varma & Sukumar 2012).

Ten major waterholes each in Bandipur forest and Nagarahole forest were selected for the purpose of monitoring the quality of water during this study period. Ten major waterholes selected in Bandipur forest included; Moolapurakere (Range: Bandipur), Kharapurakere (Range: Kundkere), Tavarekattekere (Range: Bandipur), Hirikere (Range: G.S. Betta), Natkalkere (Range: Maddur), Madrakatte (Range: Moolehale), Nataraja Kolachi (Range: A.M. Gudi), Hidgalpanchi (Range: Muliur), Chikkamauthige Kolachi (Range: N. Begur), and South Kere (Range: Omarkar).

Ten major waterholes selected in Nagarahole forest for the purpose of monitoring the quality of water included; Kambapurakere (Range: Anechoukur), Maralakandakere (Range: Anechoukur), Kallahalla (Range: Kallahalla), Doddahallakere (Range: Nagarahole), Marappanakere (Range: Nagarahole), Bisilawadikere (Range: Antharasanthe), Bidirukattekere (Range: Veeranahosahalli), Rajegowdanakatte (Range: Veeranahosahalli), Holerahundikere (Range: Metikuppe), and Seegurukere (Range: D.B. Kuppe).

For the purpose of this study we have considered the months from June to September as rainy season; October to January as winter season, and February to May as summer season.

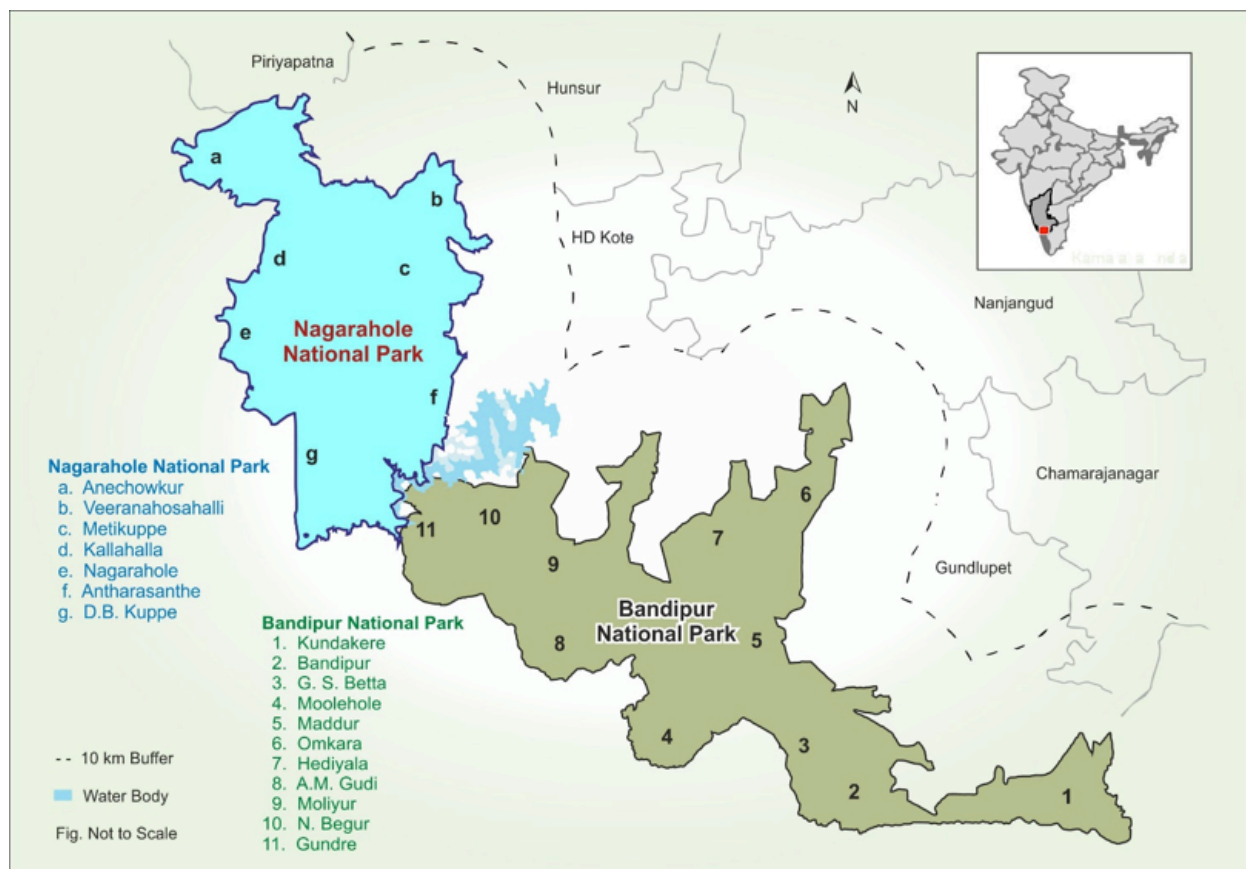


Figure 1. Map of the study areas.

Sample Collections

i) During droughts of 2012

Clinical samples from ailing and dead elephants.

Thirty-nine wild elephants died during the months of April–May, 2012. An ailing elephant was examined on the banks of the dried-up Kabini River in Bandipur forest and blood samples were collected for laboratory examination. The elephant was treated symptomatically with fluids and antibiotics but the animal did not survive. Post-mortem examination was conducted on the fresh elephant carcass.

In most other cases of elephant deaths it was very difficult to get fresh carcasses for post-mortem examination and hence, alternatively, bone marrow samples from femur bones were aseptically collected from 12 near putrefied elephant carcasses in Bandipur and Nagarahole forests during April–May, 2012.

Water samples

Water samples from the waterholes were aseptically collected during April–May, 2012, as per the procedure described previously (Obi et al. 2002) and transported on ice to the Institute of Animal Health and Veterinary

Biologicals, Bengaluru, India, for microbiological and parasitological investigations.

ii) During normal rain fall year of 2014–15

Water samples were collected from each of the above 20 major waterholes every month, starting from June 2014 (beginning of rainy season) to May 2015 (end of the summer season) for microbial and parasitological analysis. Samples were collected as described previously and transported to laboratory under cold chain conditions.

Microbiological Analysis

(i) Water samples: Microbiological analysis of water samples were performed as described previously (Standard Methods 1998; Nevodo & Cloete 1999; Obi et al. 2002; Quinn et al. 2011). Briefly, for heterotrophic bacteria, the spread-plate method was done on nutrient agar and plates were incubated at 37 °C for 48 hours. The total Coli forms and *E. coli* counts were enumerated using USFDA and WHO approved petri-films procured from 3M Company, USA, as per previously described methods (Jordano et al. 1995). All the bacteria that were

isolated under the present study were confirmed by specific biochemical tests as prescribed previously (Obi et al. 2002; Quinn et al. 2011).

(ii) Organ samples: Blood samples collected from an ailing elephant, organ samples collected at post-mortem, and bone marrow samples collected from putrefied carcasses were subject to microbiological culture as per previously described procedures (Quinn et al. 2011; Chandranaike et al. 2015, 2016).

Parasitological quality assessment

Presence of parasitic worms and/or their eggs in water samples was done by floatation and sedimentation techniques as previously described by Soulsby (1982).

Polymerase Chain reaction

For DNA extraction, five milliliters of nutrient broth inoculated with bacteria from a single colony of the isolate, and culture was incubated overnight with shaking. Bacterial cells were harvested by centrifugation at 1,000 X g for 15 min. Genomic DNA was extracted from the disrupted cells using DNA extraction kit procured from Amnion Biotech Pvt. Ltd. Bengaluru, Karnataka, India, following the protocols provided by the manufacturer. The previously described primers and the protocols were used for PCR confirmation of *Escherichia*, *Compylobacter*, and *Vibrio* species (Hollond et al. 2000; Soren & Katharina 2005; Cheryl et al. 2007).

RESULTS

Drought of 2012

Out of 20 major waterholes in forest area under the study, nine had dried up by April–May of 2012 (3b,c in Image 1). The other eleven waterholes under the study had very little water, which appeared muddy, greenish, with heaps of dried-up as well as fresh elephant dung (Supplementary Image 1). It is important to note that these two forests received less than the normal rainfall in the year 2011 (Figure 2), and the drought of 2012 was an extended period of dry spell (Figure 2). Water samples collected in all the waterholes had high microbial contamination with an average total coli form counts of 6.7×10^5 cfu/ml, mean total *E. coli* counts of 9.2×10^3 cfu/ml (Table 1). On petri films, the coli formed red colonies and *E. coli* formed blue colonies (Supplementary). The water samples collected from the 11 waterholes which contained water at the time of collection during April–May, 2012, yielded growth of *Vibrio cholerae*, *V. parahaemolyticus*, and species of *Salmonella*, *Klebsiella*,

Shigella, *Staphylococcus*, *Streptococcus*, *Bacillus*, and *Campylobacter* (Supplementary Image 3). These bacterial isolates were confirmed by biochemical tests, viz., Indole, citrate, catalase, nitrate, urease, oxidase, methyl red, voges-prausker, ornithine-decarboxylase, nitrate reduction, lysine decarboxylase and arginine hydrolase test. *Escherichia*, *Vibrio*, and *Campylobacter* were additionally confirmed by polymerase chain reaction.

The water samples contained eggs of gastrointestinal parasites of Strongyles, Amphistomes and Fasciola flukes (A, B, C in Supplementary Image 4). During the drought, the forests witnessed recurrent massive forest fires (Supplementary Image 5) destroying minimally available fodder to larger mammals, and killing several smaller wild animals which could not escape the raging forest fire.

The ailing elephant that was examined on the banks of Kabini River at the peak of drought conditions had a high fever of 104 °F. Blood samples revealed elevated liver enzyme SGOT at 219 IU/μl (Normal value: 5–55 IU/μl), total leukocyte counts at 18,000/μl (Normal value: less than 12, 000/μl) (Miller & Fowler 2012). The ailing elephant finally succumbed to acute colic symptoms. Post mortem revealed lesions of severe enteritis, empty bowels, heavy worm loads (Image 2), and hepatitis. Out of 12 bone marrow samples collected from elephant carcasses in late decomposition, nine yielded growth of mixed cultures of *E. coli*, *Salmonella* sp., *Shigella* sp., and *Klebsiella* sp. The study recorded death of 39 elephants during April–May, 2012.

Normal rainfall year of 2014–15

During this study, it was observed that the quantity of water in waterholes started increasing from June through the rainy season in August and reached the maximum levels by November. The water level started depleting from December and reached minimum levels by April to mid-May (1a,b,c in Image 1). Total coli form counts and *E. coli* counts were lowest during rainy season which gradually increased during late winter and the counts reached highest number during summer months (Table 1). Water samples collected during the months of June, July, August, September, October, November, and December yielded growth of *Escherichia*, *Aeromonas*, *Psuedomonas*, *Staphylococcus*, *Salmonella*, *Streptococcus*, *Bacillus*, *Klebsiella*, and *Shigella* bacterial species. Water samples collected during January, March, April, and May in addition to the above bacterial species yielded growth of *Vibrio cholerae*, *V. haemolyticus*, and species of *Campylobacter* (Table 2)

Table 1. Bacterial counts observed during different seasons in major waterholes of Bandipur and Nagarhole Tiger Reserve forests.

Parameter	During normal rainfall year of 2014–15			During the drought year 2012
	Rainy season	Winter season	Summer season	April and May, 2012
Coli form count	Mean: 2.4×10^2 S.D: 2.1×10^2	Mean: 1.8×10^3 S.D: 2.45×10^2	Mean: 4.3×10^5 S.D: 3.2×10^5	Mean: 6.7×10^5 S.D: 4.2×10^5
<i>E.coli</i> count	Mean: 3.7×10^2 SD: 3.1×10^1	Mean: 2.7×10^2 SD: 2.7×10^2	Mean: 6.2×10^3 SD: 4.2×10^3	Mean: 9.2×10^3 SD: 5.1×10^3

**Image 1. Water levels in waterholes: 1a, 2a, 3a—Completely filled up waterholes in rainy season | 1b, 2b, 3b—Waterholes in winter | 1c, 2c, 3c—Dried up waterholes in summer. © Authors.**

Water samples collected during all the months (June 2014–May 2015) revealed the presence of eggs of *Fasciola*, *Amphistomes*, *Strongyles*, *Taenia* and *Coccidian* oocysts (Table 2). The study found that the habit of wild animals to defecate while consuming water (as observed in several instances during this study while collecting water samples) had possibly resulted in an abundance of faecal droppings in the water holes, especially at the fringes of the waterholes where they stand and drink water (D, E, F, G in Supplementary Image 4). Abundant numbers of different types of snails which act as intermediate hosts for trematode flukes (*Fasciola* and *Amphistomes*) were observed near the waterholes (H, I,

in Supplementary Image 4). The monthly average rainfall data in the study area during 2011, 2012, and 2014 is depicted in Figure 2. Forests witnessed the death of less than 10 elephants in April–May, 2015.

DISCUSSION

Bandipur and Nagarhole Tiger Reserves witnessed an extended drought during 2012. Most of the findings that are described in this study are the first time reports in elephants; hence, we have discussed our results in comparisons with available reports in domestic animals

and humans.

Drought of 2012

The major waterholes had either completely dried up or were left with little water which was highly contaminated. There was an acute shortage of fodder to elephants as the green vegetation had dried-up in the forest. Also, the dried-up grass, shrubs, and trees had been destroyed by recurrent forest fires. These factors lead the elephants to chronic starvation and dehydration; gradually contributing to poor nutrition, poor body condition, and consequent immunosuppression.

In the absence of any other water sources, elephants had to drink the contaminated water available in the waterholes, which were the source of heavy loads of different types of opportunistic pathogens especially the coli forms. Under natural conditions when the elephants are healthy with good nutrition and immunity, they can withstand most opportunistic pathogens including coliforms and the gastrointestinal parasitic infestations (Quinn et al. 2011; Miller & Fowler 2012). However, under severe drought conditions, the immune compromised wild animals are susceptible to opportunistic and/or acute bacterial infections/septicemia (Quinn et al. 2011; Chandranaik et al. 2015) which cause high fever, hepatitis, pancreatitis, acute enteritis, dehydration, and other systemic disorders. Hepatitis, pancreatitis, and enteritis are highly painful conditions which cause colic and struggling, as observed in most of the elephant deaths in the present investigation.

Potential pathogenic and/or opportunistic bacterial species of *Escherichia*, *Vibrio*, *Aeromonas*, *Shigella*, *Klebsiella*, *Salmonella*, *Bacillus*, *Pseudomonas*, and *Campylobacter* were isolated from all the water sources studied during drought. The presence of these bacteria in water sources is in agreement with previous reports (Cabral 2010). These enteric bacteria have been reported to act as the causative agents of various diseases and their complications such as diarrhoea/dysentery, septicaemia, dehydration, hypovolaemic shock, acidosis, and haemo-concentration (Ongunsanya et al. 1994; Seas et al. 2000; Cabral 2010).

Vibrio cholerae can grow at 40°C with pH 9–10. The growth is stimulated by the presence of sodium chloride which is available as a result of rapid evaporation of water in waterholes due to heat of the summer. There are more than 200 serovars of *V. cholera*, characterized based on the structure of the lipopolysaccharide. Only two serovarieties named O1 and O139 are involved in causing true cholera. However, other serovarieties can cause gastroenteritis, but not cholera. The severity of

Table 2. Bacterial isolates and parasitic eggs/cysts recovered from the water samples collected during this study.

<i>Escherichia</i> spp.	
<i>Vibrio</i> spp.	
<i>Salmonella</i> spp.	
<i>Klebsiella</i> spp.	Fasciola
<i>Campylobacter</i> spp.	Amphistomes
<i>Pseudomonas</i> spp.	Strongyles
<i>Streptococcus</i> spp.	Taenia
<i>Staphylococcus</i> spp.	Coccidia
<i>Shigella</i> spp.	
<i>Bacillus</i> spp.	
<i>Aeromonas</i> spp.	



A



B



C

Image 2. Post mortem lesions in elephants died during drought: A—Lesions of severe enteritis | B, C—Heavy helminth worm load.

the disease depends on several factors, and importantly on the individual's immunity and the inoculum (Sack et al. 2004; Todar 2009). *Vibrio parahaemolyticus* is a well-documented causal agent of acute food-borne gastroenteritis (Sack et al. 2004; Quinn et al. 2011).

The principal habitat of *Salmonella* is the intestinal tract of humans and animals including wild animals. Food-borne *Salmonella* gastroenteritis is frequently caused by ubiquitous *Salmonella* serovars (Quinn et al. 2011). *Shigella* is typically an inhabitant of the intestinal tract of humans and other primates. It is primarily spread by fecal-contaminated drinking water causing bacillary dysentery (Kapperud et al. 1995; Farque et al. 2002; Tetteh & Beuchat 2003).

E. coli strains have been grouped into several groups of which enterotoxigenic, enterohemorrhagic and enteroinvasive (Cabral 2010; Quinn et al. 2011) serotypes are of significant importance and can be transmitted through contaminated water. Disease caused by *E. coli* follows ingestion of contaminated food or water and is characterized by acute abdominal pain, profuse watery diarrhoea lasting for several days that often leads to dehydration. Outbreaks involving consumption of drinking water contaminated with human sewage or cattle feces have been documented in human dwellings. An increasing number of outbreaks are associated with the consumption of fruits and vegetables (e.g., sprouts, lettuce) contaminated with feces from domestic or wild animals at some stage of growth. EHEC has also been isolated from water bodies (ponds, streams), wells and water troughs, and has been found to survive for months in manure and water-trough sediments (Scheutz & Strockbine 2005).

Possible sources of contamination of the water bodies in forests include animal faeces or introduction of micro-organisms by birds and insects (Paul et al. 1995; Nevodo & Cloete 1999; Obi et al. 2002; Cabral 2010). Higher bacterial levels could also be due to heightened ecological activities (Strockbine & Maurelli 2005). The habits of wild animals to defecate and urinate in the waterholes as they drink water could be important sources of faecal contamination with coli forms, the parasitic eggs and other opportunistic pathogens isolated during this study. The flow of water into waterholes from adjacent (surrounding) villages with human habitations where open defecation is practiced by their populace could also be another significant source of coli forms and parasitic eggs/cysts noticed in the waterholes. It should, however, be noted that the presence of faecal coli forms in the water sources may not be definitive for a faecal origin of the bacteria (Paul

et al. 1995). Investigators have reported the presence of faecal coli forms in tropical environments in the absence of any source of fecal contamination (Hardina & Fujioka 1991; Palupi et al. 1995; Hazen 1998; Fernandez et al. 2000).

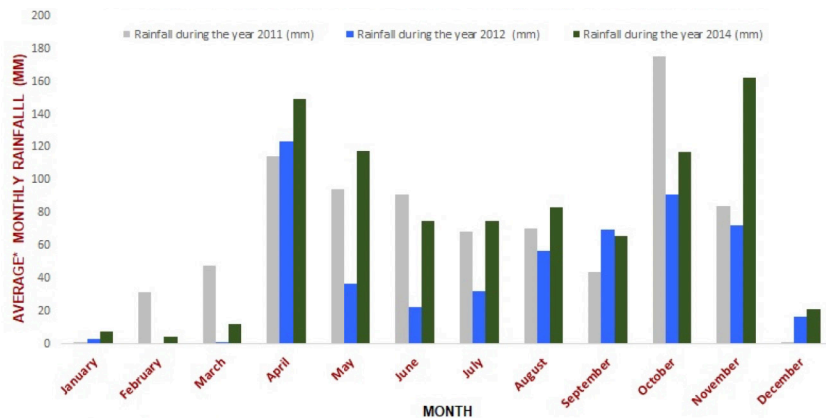
Snails act as intermediate hosts for *Fasciola* and *Amphistome* trematodes (Soulsby 1982), the presence of abundant snails of different species on the shores of waterholes could be a prominent reason for detection of fluke eggs in water samples.

These two forest areas had received lower than normal rains in the year 2011, and the situation worsened in 2012 leading to severe drought conditions (Figure 2). Possibly, as a consequence of all these factors, 39 elephants died during April–May, 2012 in these two forest areas. Most of the elephants had died with symptoms of colic as observed by severe struggling of the animals before death. The blood picture of leucocytosis indicated bacterial infection, and increased liver enzymes indicated toxic changes. The post-mortem examination revealed lesions of severe inflammation of intestines and septicaemic changes in an elephant that was examined on the banks of Kabini River during the peak of drought in 2012. Further, the bone marrow samples of the elephants that died during droughts yielded growth of *E. coli* and other coli forms and these opportunistic pathogens have been reported to be aetiologies for severe enteritis and septicaemia in immunosuppressed animals (Quinn et al. 2011). The post-mortem also revealed the presence of heavy loads of parasites in the gastro-intestinal tract, which correlates with the current findings of parasitic eggs in water samples.

Normal rainfall of 2014–15

After good pre-monsoon and monsoon rains, all the waterholes were full to their brim by November. The quantity of water gradually decreased from the month of December, reached the lowest in the summer months of March, April, and May. Even when the rainfall is normal, the water in waterholes continue to be the source of opportunistic pathogens and various species of gastrointestinal parasites as evidenced by growth of coliforms and presence of eggs /ova in water.

During 2014–15, the forests received normal rainfall but the bacterial counts were very high during the summer season (March–April, 2015) which was almost similar to the counts recorded during the drought conditions of 2012. However, the death of less than ten elephants was noted in April–May, 2015. The normal monsoon rains of 2014–15 had possibly resulted in



* Average of rainfall recorded in rain gauges at Bandipur (Rain gauge code :80201); Begur (Rain gauge code: 80202); Gundlupete (Rain gauge code: 80203); Hunsur (Rain gauge code:220202) during the year 2011, 2012 and 2014

Figure 2. Average monthly rainfall in the study area during the years 2011, 2012, and 2014.



Image 3. Measures suggested from the findings of this study and their implementation: A—Construction of smaller artificial water tanks and fill them with water tankers | B—Removing obstructing bigger shrubs surrounding major waterholes before every rainy season so that more and more water gets accumulated in waterholes | C—Installation of solar powered pumping bore wells near major waterholes at feasible locations in the forest.

sufficient availability of fodder for animals keeping them in good body condition and relatively better immunity, which possibly gave them the ability to resist infections caused by opportunistic pathogens present in the water they consume.

The study records that rainfall directly controls the availability of feed and water in forests; and availability of feed and water determines the general body condition of wild animals and their ability to resist infections. During droughts there is an acute shortage of feed and water leading to poor body condition with total immunosuppression; possibly making them susceptible for opportunistic pathogens present in water they consume leading to colic, diarrhoea, dehydration, septicemia, and death.

El Nino events are a prominent feature of climate variability with global climatic impacts, severely disrupted global weather patterns, affecting ecosystems agriculture, tropical cyclones, drought, bushfires, floods, and other extreme weather events worldwide. Here we present evidence of such changing climate on the survivability of wildlife. Increasing temperatures, combined with changes in rainfall and humidity, may have significant impacts on wildlife, domestic animals, and human health. When combined with expanding human population, these changes could increase demand on limited water resources, leading to more habitat destruction, and provide yet more opportunities for infectious diseases (Hofmeister et al. 2012) and the elimination of wildlife species (McLean 2016). Droughts of the future are likely to be more frequent, severe, and longer lasting than they have been in recent decades (Toby 2020). Through this present study we have attempted to give a glimpse of the future of wildlife in events such as drastic climatic changes.

MANAGEMENT IMPLICATIONS

Measures suggested from the findings of this study and impact of their implementation

1. The study found that the growth of heavy shrubs in and around major waterholes had prevented the flow of water into waterholes. It was suggested to take measures to clear these shrubs before every rainy season so that more water accumulates in waterholes.

2. In absence of water in major waterholes during drought conditions, it was suggested to take measures to provide water in a few major waterholes through water tankers.

3. To help smaller animals in the forest it was suggested to construct small artificial water tanks and fill them with water.

4. It was suggested to install solar powered pumping bore wells at feasible locations in the forest.

All the suggested measures have been implemented (Image 3) at most major waterholes by the Government of Karnataka, possibly helping many wildlife species during summer and drought situations at Bandipur and Nagarhole Tiger Reserves in recent years.

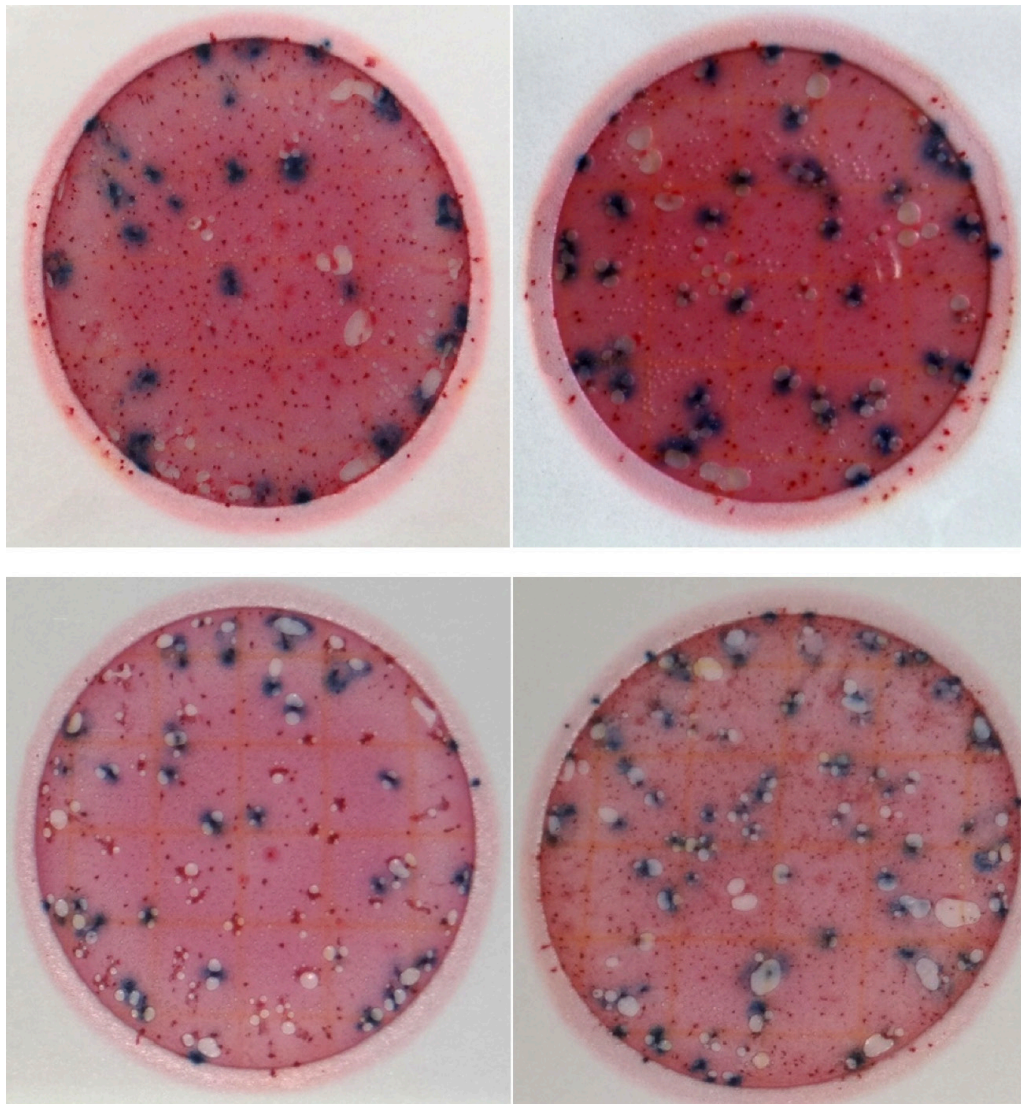
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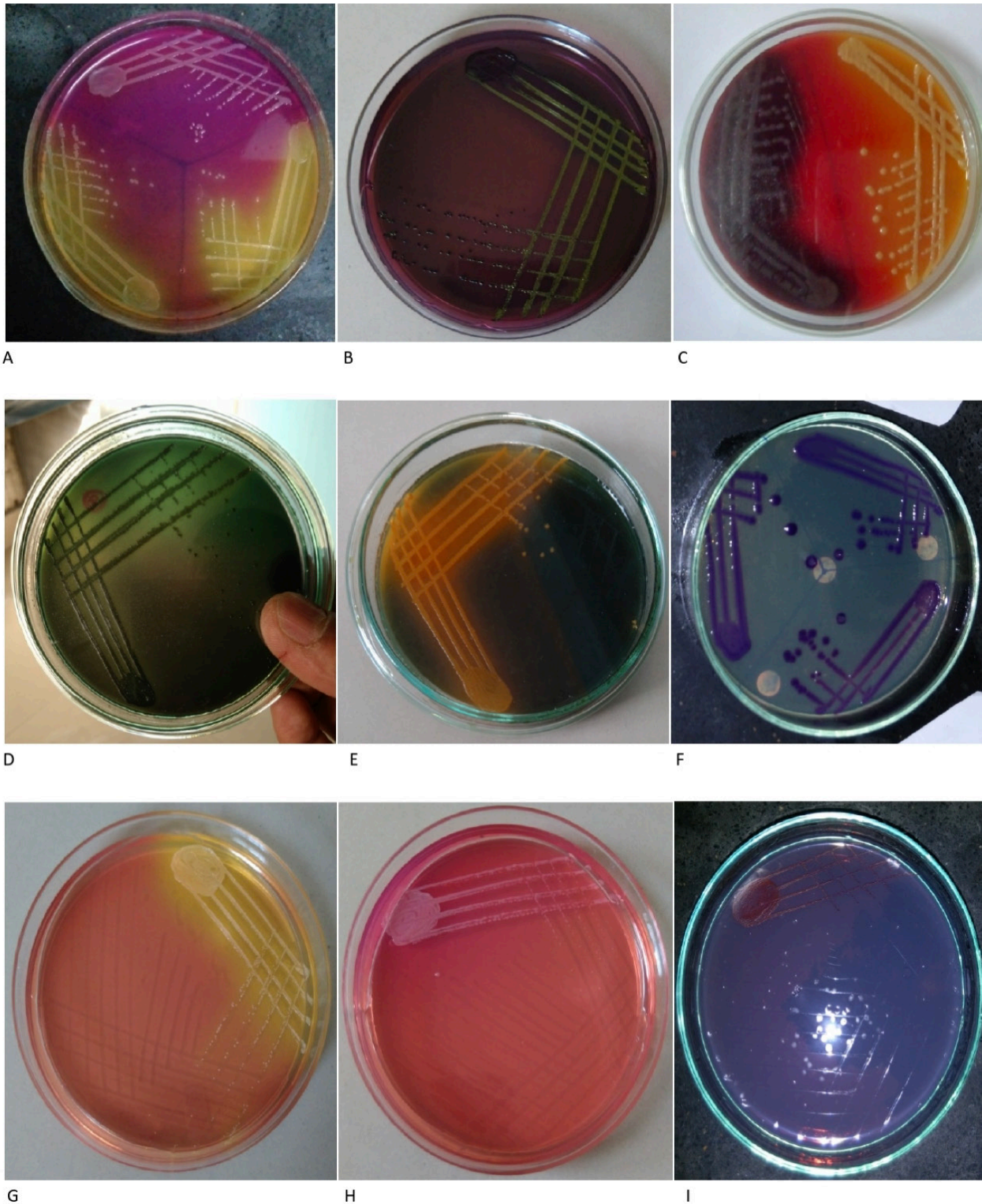
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Supplementary Image 1. The water in waterholes during drought appeared greenish with heaps of elephant dung at periphery.



Supplementary Image 2. Colonies of coli forms and *E. coli* on the petri films.



Supplementary Image 3. Growth of opportunistic pathogens from water samples: A—colonies of *Aeromonas* (yellow) and *Pseudomonas* (white) on *Aeromonas-Pseudomonas* agar | B—*E. coli* on EMB agar | C—*Salmonella E. coli* on XLD agar | D—*Vibrio parahaemolyticus* on TCBS agar | E—*Vibrio cholera* on TCBS agar | F—*Klebsiella* species on *Klebsiella* agar | G—*Staphylococcus aureus* on MSA agar | H—*Staphylococcus intermedius* on MSA agar | I—*Streptococcus* species on KF Streptococcal agar.



Supplementary Image 4. Eggs of gastro intestinal parasites in water samples: A—Strongyle egg | B—Amphistome egg | C—Fasciola egg | D, E, F, G—Dung in the water in the waterholes | H, I—Different species of snails observed in and around the waterholes.



Supplementary Image 5. The forest witnessed massive forest fires during the drought.

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