AN INVESTIGATION ON THE CAUSES OF MORTALITY IN CAPTIVE GHARIAL HATCHLINGS AT THE CHITWAN NATIONAL PARK, NEPAL

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While working in Chitwan National Park, staff often used to seek help for the sick gharials, which I always avoided, but this often touched me deeply for not being able to help them. Quite recently, when I joined the M.V.Sc. program at IAAS, I had the opportunity to study gharials, an unexplored species. Thus, I stepped forward to learn more about this endangered species in Nepal.

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Kamal P. Gairhe

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ACRONYMS

%	Per cent
˚C	Degree Celsius
A. hydrophila	*Aeromonas hydrophila*
ACT	Australian Capital Territory
Apr	April
Aug	August
cm	Centimeters
CNP	Chitwan National Park
DNPWC	Department of National Parks and Wildlife Conservation
E. coli	*Escherichia coli*
Feb	February
Fig.	Figure
GBC	Gharial Breeding Center
gm	Gram
IAAS	Institute of Agriculture and Animal Science
Jan	January
Kleb.	Klebsiella
KOH	Potassium hydroxide
LCB	Lactophenol cotton blue
m	Meter
M. morganii	*Morganella morgani*
M.V.Sc.	Master of Veterinary Science
m^2	Square Meter
Mar	March
Max.	Maximum
Min.	Minimum
mm	Millimeter
N	Number of samples
ND	Not Detected
NE	Not Examined
No.	Number
Nov	November
Oct	October
P	Probability Value
Path.	Pathological
PCR	Polymerase Chain Reaction
r	Correlation Coefficient
r^2	Pearson's Coefficient of Determination
S. albus	*Staphylococcus albus*
S. aureus	*Staphylococcus aureus*
SD	Standard Deviation
Sep	September
spp./sp.	Species
Temp.	Temperature
UK	United Kingdom
USA	United States of America
VDC	Village Development Committee
VTH	Veterinary Teaching Hospital
WWF	World Wildlife Fund
$\chi 2$	Chi Square

ABSTRACT

Name: Kamal Prasad Gairhe
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Gharial is one of the endangered species among two species of crocodiles found in Nepal. The status is endangered globally and hence, given the protected status in Nepal. They were brought back from extinction by means of captive rearing and release program started in Chitwan since 1978. The mortality of young gharial is high in the wild as well as in captivity particularly under the age of one year. Therefore, this study was carried out to find out the involvement of pathogenic agents such as bacteria, fungi and internal parasites, if any, in the mortality episodes as causative agents. In this study, 137 dead gharial hatchlings in the age between 6 to 42 weeks were collected from Gharial Breeding Center (GBC), autopsied and examined. General examination of the carcasses revealed 12 gross pathologic conditions: fungal lesions in the skin (18.97%), retention of intact or decomposed fish in the oesophagus (37.95%), drawn in muscle (19.70%, cysts in body membranes (15.32%), yolk sac retention (10.94%), nodular lesions in the intestinal wall (6.56%), ascites (17.51%), septicemic lesions (27%), snout deformities (2.18%), vent prolapse (1.45%), enteritis (24%) and anaemic visceral organs (21%).

Examination of the stomach and intestinal contents revealed single type of trematode (29.9%), as well as nematode ova (12.40%) and coccidial oocysts (21.90%). Hatchlings were found infected with a typical “Surahi fluke” (Exotidendrium spp., Digenia: Exotidendriidae) and a characteristic lesion around the rectocloacal opening, newly termed as “Sphincter Cap”. Immature forms of the fluke were found in the hatchlings aged 6 to 14 weeks where as adults were found in older ones. This fluke was suspected as causing irritation to the mucosa opening a venue for the bacteria to cause a granulomatous reaction, forming a scab around the opening, most often closing the rectocloacal orifice. The fluke was found in 40.95% of the animals and the distinct "Sphincter Cap" lesions were found in 26.6% of the hatchlings examined.

Four organs of 102 hatchlings subjected to aerobic bacterial cultures identified Gram-positive bacteria and Gram-negative rods. Gram positive organism isolated were *Staphylococcus aureus*, Streptococcus spp., Actinomyces spp., Bacillus spp. and Clostridium spp. Gram negative organisms were *Citrobacter freundii*, *Escherichia coli*, *Providencia rettgeri*, Pseudomonas spp., *Proteus vulgaris*, *P. mirabilis*, Aeromonas spp., *Klebsiella oxytoca*, *Morganella morganii*, Salmonella spp. and Shigella spp. Fourty samples (hatchlings infected with Surahi fluke and having a distinct "Sphincter Cap") from the rectocloacal area were cultured aerobically. It resulted in the isolation of two organisms (*Bacillus subtilis* and *Strep. viridans*) of the Gram-positive group and nine organisms of the Gram-negative group. The dominant isolates were *Citrobacter freundii* (30%) and *E. coli* (20%). One hundred and forty eight culture positive samples based upon monthly mortality were analyzed and it revealed 25% infection in September, 16.89% in August, and less than 14% infection in other months.

Bacterial infections in different organs detected on 148 culture positive gharial hatchlings were found to be 27.03% in liver, 42.57% in lungs 15.54% in kidney and 14.86% in heart. Spectrum of bacteria on 90 culture positive gharial hatchlings were only one species in 70%, two species in 21.11% and three or more bacterial species in 8.89% of the carcasses.

Presence of infection in particular age group of hatchlings led to the finding that the highest percentage of infection(75%) was recorded in the 30-34 weeks old hatchlings and in an average 38.64% hatchlings had been infected by one or more bacteria. Out of 102 hatchlings subjected to bacterial culture, 12 hatchlings (11.76%) were found negative to cultures. Culture of skin scrapings from suspected skin

lesions led to the isolation of six environmental fungus without a higher prevalence of any particular species.

Histopathological studies of 79 hatchlings revealed coccidial parasites (58.22% in liver and spleen, and 68% in colon mucosa), fatty degeneration of hepatocytes (27.85%), hemosiderosis (44.3%), general or focal mononuclear cell infiltration in liver, lung heart or spleen (46.84%), oedema and haemorrhage (51.8%) in liver, lung heart or kidney and septal thickening (19%) in the lung.

It is concluded that higher mortality of gharial hatchlings in Chitwan was multifactorial. Bacteria, fungi, coccidia and parasites were significantly associated to these deaths, but environmental factors such as extreme temperatures and managemental deficiencies could not be ignored.

_____	_____
(Dr. Ishwari Prasad Dhakal)	(Kamal P. Gairhe)
Major Advisor	Author

1. INTRODUCTION

Among the crocodilians, only two species of crocodiles are found in Nepal: the gharial (*Gavialis gangeticus*) and the mugger (*Crocodylus palustris*). The former inhabits the fresh water river and the later prefers the stagnant water bodies. The gharial, *Gavialis gangeticus* is a large crocodilian with a long, slender snout living in fresh water rivers of Nepal and India (Maskey, 1989). Gharial is the oldest representative of the Order Crocodylia and is the only species of the family Gavialidae still surviving (Sahi, 1974). The gharials were abundant in all the major rivers of Indian sub continent until 1960. Habitat loss and disturbances, poaching (for eggs, ghara and hide), entrapment in fishing net, restricted movement through dams were pointed out as various factors responsible for the decline of the gharial population (Maskey, 1989). Thus, gharial has become one of the rarest and most endangered crocodilian in the world. Of all living crocodilians, this species is the most closely bound to its aquatic environment because of its weak legs not well suited to walk on land. It only hauls itself out of the water on exposed sand banks to bask, to build its nest and to lay its eggs (Cadi and Maskey, 2005). It is typically a resident of deep, fast flowing rivers, preferring areas where the water current is low (Whitaker and Basu, 1983).

The species was literally brought back from the brink of extinction by restocking programs initiated in India (1975) and in Nepal (1978). This program strategically protected gharial nest in the wild, collected eggs, raised the hatchlings in captivity and released them back into the main rivers (Cadi and Maskey, 2005). Over 3,000 juveniles had been released in the Ganges (Chambal, Ramganga, Girwa and Sharada rivers in India) drainage (Ross, 1998). The current wild population is estimated to be more than 1,500 individuals in India (Rao and Singh, 1994) and a minimum of 105 individuals in Nepal (Maskey and Percival, 1994). Narayani, Kali Gandaki, Karnali and Babai rivers are the major gharial habitat in Nepal. Data shows that a total of 9849 eggs collected from the wild produced 4992 hatchlings in Nepal in the period 1977-2005; however, only 691 young gharials raised at GBC have been released back in the wild since 1981 till date (Nepal, 2004-5).

Gharials contribute to the health and biodiversity of the river ecosystem. They are the top predator and thus are an essential part of the biodiversity of fresh water habitats. They predate on the slow moving predatory and scaleless fish, thus help to maintain healthy and greater population of valuable fishes removing the diseased individuals. On the other hand, scaleless fishes generally feed on fish species preferred by people. Thus, gharial has a controlling role on the river ecosystems (Mishra, 2002).

Gharial is a native crocodilian species of Nepal and India. It can grow up to 6.75 m in length and matures at an age of 13-16 years. The individuals in the population are categorized by the whole body length such as individuals having less than 60 cm length are classed as hatchlings, 60-90 cm are considered as yearlings, 90 to 270 cm as sub adults and animals beyond this length are regarded as adults (Hussain, 1999). India and Nepal, as the range countries for this species, have the great responsibility for the protection, conservation and judicial utilization of gharials.

Crocodiles do not have sex chromosomes. Instead, the sex of the embryo is determined by the incubation temperature (Ferguson, 1982). Low incubation temperature favours the development of ovaries, while at high temperatures testes are produced. They are exothermic reptiles, unable to maintain a constant internal body temperature independently of the environment. However, they try to achieve and then maintain their temperature with in a preferred range by the use of thermogradients in the environment.

All species reproduce by laying eggs, follicles normally ovulate together over a period of few hours and eggs in the clutch are deposited into the nest at the same time. Most species lay only one clutch of eggs per year, the Mugger (*Crocodylus palustris*) being the exception, with two cycles per year occurring

regularly (Whitaker and Whitaker, 1984). Gharials naturally lay eggs on fine sand banks of river usually 3-7 meters above the water level and 5-20 meter away from the water. The egg laying and incubation are synchronized with high temperature and humidity and hatching with high water levels. Nesting in the early part of the wet season provides high constant temperature and humidity ensuring better embryonic survival, development and growth within the egg chamber; bypassing the nest flooding and young are ready to hatch during the period of high water levels which allow them to disperse into small tributaries containing small fishes facilitating the survival (Maskey, 1989). Despite such a natural phenomenon, less than one per cent of naturally hatched gharials reach a length of 2 meter, a length at which they are generally secure from natural predation. The huge numbers of eggs laid by breeding females are generally destroyed by predators or stolen by poachers or suffer the damaging effect of flooding. If all the eggs laid by crocodilians hatch and grow into mature crocodile, there would be far too many of them in a particular habitat; however, predators destroy eggs and hatchlings and maintain a balance between different animals (Sahi, 1974).

Crocodiles are farmed for production of hide and meat. The farming is increasingly popular in many tropical countries. Several species of crocodiles have been farmed intensively and the industry is growing. The most farmed species are Nile crocodile (*C*. *niloticus*), salt-water crocodile (*C*. *porosus*), Australian fresh water crocodile (*C*. *johnstoni*) and American alligator (*C*. *mississippiensis*).

The promotion of crocodile products and the concern of tourists have greatly enhanced the value of crocodilians. This probably has helped in the preservation of wetland habitats and enabled the collection of eggs or hatchlings in a sustainable way (Ladds *et al*., 1995).

Although considerable research work on crocodiles has been published, the topics have been mainly limited to the behaviour and population demography. Early days, zoo veterinarians were the only concerned about husbandry and diseases of crocodiles. Now, farming and business of the species has taken a dramatic turn upwards worth hundreds of millions of dollars (Messel, 2003).

Government of Nepal has recently designed a working policy granting permissions for commercial wildlife farming thereby opening a venue for gharial farming though it is an endangered species (Nepal, 2003); however, information on the establishment of such farms as yet in Nepal is lacking.

Predation by fishes, birds, jackal, civets and monitor lizards significantly reduces the survivability of the young gharial hatchlings in the wild limiting the survival rate to adulthood just to about one per cent in natural conditions (Dhungel, 1987); likewise, the mortality rate of hatchlings in captivity exceeding 30 per cent during the first year of life have become a major constraint in crocodile farming (Ladds *et al*., 1995).

The GBC at Chitwan National Park was thus established with the objectives to protect gharial nesting sites, collect and incubate eggs and rear hatchlings to a length of two meters for restocking in the Narayani and Rapti rivers.

Problems of parasites, bacterial septicaemia and fungal infections in crocodile hatchlings on farms have been reduced in recent years (Ariel *et al*., 1997a). An attempt to improve the husbandry practice of captive gharials in Chitwan National Park is underway each year; however, significant reduction in the mortality rate of young hatchlings has not been yet achieved.

Maskey (1989) stated that 25 to 80% of the gharial hatchlings die with in the first year of their life in captive breeding facilities because of skin disease, neurological disorders, retention and infection of the yolk sac and prolapse of the rectum; where as only about 10% yearlings die in the second year mostly

due to bacterial and mycotic infections arising from poor quality water and extreme humidity of the Monsoon season.

The mortality rate of gharial hatchlings (hatching to 1-year age) at GBC has ranged between 42.95 to 97.20%. The causes behind such high mortality include occasional predation (by mongooses), pilling up and suffocation, ant attacks, infection of skin and teeth by fungus, etc. This emphasized a need of a thorough investigation work to reveal out the major errors in management and husbandry along with the biological agents involved in the death of young hatchlings for increasing the survival rate which in turn may efficiently help in restoring and augmenting wild gharial populations.

Investigation on the causes of high mortality of captive gharial hatchlings aimed at the following general and specific objectives:

1. General
    - To investigate the causes of mortality of young captive gharial hatchlings to improve their viability in captivity in Chitwan National Park.
2. Specific
    - To assess the field survey on existing mortality patterns of gharial hatchlings and its relation with season,
    - To identify pathogens (bacterial, fungal and parasitic) responsible for high mortality of the young gharials in captivity,
    - To document and analyze histo-pathologic changes in the tissues of the carcasses due to bacteria, fungus and parasites, and
    - To recommend further diagnostic, managemental and curative measures to improve health and husbandry of captive gharial hatchlings.

2. LITERATURE REVIEW

Literature pertaining to the gharial hatchling mortality is scarce. Rigorous work is still important to know the involvement of the pathological agents in higher percentage of hatchling mortally. The causes have not been explored well due to the difficulty in obtaining gharial specimens from the wild or because of the inaccessibility of the gharial breeding centers to the research institutions. The difficulty is further increased by the protected status of the species requiring permissions from the respective governments for activities related to scientific research and study. Several studies related to gharial ecology, habitat and movement were carried out in the 1980's; however, studies concerning the diseases, its effect on mortality and ways to increase their viability through improved health, hygiene and veterinary interventions, were less than a handful of scientific work.

2.1 Bacterial infections

Captive crocodiles are highly susceptible to opportunistic gram-negative bacterial infections, especially under stressful conditions. Changes in diet, overcrowding, fighting and changes in the environment are believed to be predisposing factors for opportunistic bacterial infections in captive crocodilians (Lane, 1996). They are also highly sensitive to stress under farming conditions and thus are easily exposed to stress septicaemia. Stress septicaemia in conjunction with temporary immune suppression probably is the most important mechanism for bacterial infections in crocodiles. According to Huchzermeyer (2000) a separate phenomenon comes to play under conditions of severe stress where intestinal bacteria apparently enter the bloodstream more easily, although the exact mechanism is still unclear. When the animal recovers from the stress, normal immune functions eliminate these bacteria again. However, if the immune functions remain suppressed, either by continuing or repeated stress or during severe cold, the bacteria can carry on multiplying and eventually will settle in various organs and tissues.

Mihsra *et al*. (1993) investigated the probable cause of death of gharial hatchlings at the Gharial Research and Conservation Center, Tikapura, Orrisa where heavy mortality occurred in 1986 and isolated Clostridium spp. from the oedematous fluid taken from the swollen limbs of the diseased hatchlings. They also observed critically low level of water in one of the hatchling ponds as well as found water without replacement for a long time. Water samples from this pond led to the isolation of *E*. *coli* suggesting that clean water and sound management practice are required to get disease free hatchlings. They also evaluated the resident bacteria and isolated Citrobacter spp., *Aeromonas hydrophila*, *Edwardsiella tarda*, *Haffnia alvei* and *Escherichia coli* from the cloaca of the apparently healthy gharial hatchlings.

Mishra *et al*. (1996) examined the gharial hatchlings aged between four and seven weeks, which were dead during a heavy mortality episode that occurred in 1993 in Nandakanan Zoological Gardens, Orrisa, India. In these examinations, Pseudomonas spp. were predominantly found responsible for hatchling deaths. Ten out of 24 hatchlings were found infected with *Pseudomonas aeruginosa* and only one sample was found positive for Mycoplasma where as 13 putrefied hatchlings were unqualified for further study. They found congestion and oedema in the major internal organs of gharial hatchlings as predominant lesions mostly without much cellular reaction in histopathological examination.

Maskey *et al*. (1998) evaluated the disastrous impact of intestinal infection in captive bred gharial hatchlings. Mehrotra *et al*. (2000) reported mortality of ten days to four months old gharial hatchlings at Jaipur zoo in 1998 and 1999 exhibiting sudden death but with out any significant symptoms. Examination of most of these hatchlings revealed hemorrhages at umbilical region and

had severe congestion and petechial haemorrhages in all vital organs. There were also cases of retention of yolk sac with foul smelling fluid accumulated in the abdominal cavity. All these hatchlings were suffering from septicaemia and a multitude of organisms were isolated from 17 visceral samples. These included *E. coli*, *Pseudomonas aeruginosa*, Staphylococcus, Corynebacterium spp., Bacillus spp. and some fungal agents. These bacterial agents were found responsible for major septicaemic condition and death of these captive gharial hatchlings. Concurrently, examination of soil and water samples proved the presence of similar organisms emphasizing the need of fresh running water for gharials instead of stagnant ponds.

Similar observations and isolation of *Corynebacterium bovis*, *Pseudomonas aeruginosa* and *E. coli* from digestive contents of dead gharial hatchlings had been made by Arora and Kumar (1985-89).

Bacterial septicaemia with particular involvement of certain organs, notably the liver, is a frequent cause of illness and death in crocodilian hatchlings (Ladds *et al*., 1996). Gram-negative bacteria were most often involved in these septicaemic conditions, the most frequent being Aeromonas and Salmonella spp. and less often *Escherichia coli*, *Edwardsiella tarda*, *Providencia rettgeri*, Pseudomonas, Klebsiella and Pasteurella spp. (Shotts, 1981; Foggin, 1987; Ladds and Sims, 1990; Buenviaje *et al*., 1994; Huchzermeyer *et al*., 1994). Shotts (1981) has emphasized that disease resulting from infection with these organisms is usually, if not always, of a secondary or opportunistic nature. Such disease, therefore, signals inappropriate husbandry particularly in relation to temperature, feed or stocking density.

Aeromonas spp. is reported to cause a variety of conditions ranging from enteritis to meningitis in humans; the infection is most severe in poikilothermic animals which are mostly triggered by stresses such as low dissolved oxygen concentrations in the water, abrupt temperature changes and spawning (Shotts, 1981).

Sinha *et al*. (1988) recorded *Escherichia coli* outbreak in Mugger crocodiles (*Crocodylus palustris*) at a Crocodile Breeding Center in Muta (Ranchi, India). About 25% of the three to four years old mugger crocodiles manifested signs of anorexia, dullness, vomition, emaciation and died within three to four days. Severe haemorrhagic and catarrhal enteritis were noted in 18% of the dead crocodiles in necropsy examination and *E. coli* was isolated from heart blood, pericardial fluid and intestine indicating septicaemia. Histopathological examination revealed desquamation of lining epithelial cells and severe infiltration of laminae with macrophages and focal haemorrhage in the intestinal mucosa.

Intestinal contents of normal wild-caught African dwarf crocodiles (*Osteolaemus tetraspis*) cultured aerobically revealed Salmonella (three isolates), *Escherichia coli* (eight isolates), Klebsiella (four isolates) and some members of the family Enterobacteriaceae and some intestinal fungi (Huchzermeyer *et al*., 2000).

Providencia rettgeri was isolated in pure culture from the brain and liver of four months old *Crocodylus porosus* hatchlings during winter months for three consecutive years (Ladds *et al*., 1996). All the hatchlings were of an approximate length of 200 to 400 mm. All were reported showing nervous signs (swaying, swimming in circle) but without any gross lesions other than generalized visceral congestion and oedema in the lungs. Acute meningitis with marked thickening of the meninges by heterophil infiltration was noted in histopathological findings. Suboptimal temperatures at nights or during the period of pond filling and an overcrowding of animals compared to Goudie's (1989) recommendation (0.01m^2/hatchling) were suspected as contributing

factors. *Providencia rettgeri* was found associated with high mortalities in farmed American alligator (*Alligator mississippiensis*) reared under intensive commercial aquaculture facility (Camus and Hawke, 2002). The outbreak was precipitated by severe temperature stress in the pens that occurred due to human error. Hatchlings looked lethargic and exhibited neurological signs including head tilting and circling. Heterophilic meningoencephalitis, pneumonia, hepatitis and splenitis were histological findings observed. *P*. *rettgeri* was also isolated from a stressed adult *Crocodylus porosus* which was transferred to captivity from a wild state; afterwards it had enteritis (Sinha *et al*., 1987) and required treatment with tetracycline and it was concluded that changes in habitat and food type in captivity upset the digestion process and led to toxaemia. The organism has also been recovered from human clinical specimens (O'Hara *et al*., 2000).

There are various reports of isolation of *Morganella morganii* from crocodiles and the genus Morganella consists of a single species. *M*. *morganii* is a part of normal flora and is classified as an opportunistic pathogen to cause infection in humans and animals (Quinn *et al*., 1994). This bacterium was isolated from multiple tissues and organs of an anorectic West African dwarf crocodile suffering from bilateral limb paresis (Heard *et al*., 1988). Severe anemia, multiple skin wounds, osteomylitis and bacteremia were reported in this crocodile. *Morganella morganii* was also isolated from juvenile *Crocodylus porosus* (Hibberd *et al*., 1996) and from the joints and internal organs of stressed Nile crocodile hatchlings (Huchzermeyer, 2000). It was also reported as the cause of suppurative bronchopneumonia and death in a captive Jaguar (*Panthera onca onca*) and the bacteria was isolated in pure culture from lungs, spleen and heart blood (Choi *et al*., 2002).

Chakraborty *et al*. (1988) reported the death of a 2-year-old crocodile in Calcutta Zoological Garden as a result of septicemia due to Proteus spp. They found consolidation of lung tissue, congestion of kidneys and spleen and pale whitish liver as gross lesions in this crocodile. Focal haemorrhage and severe pneumonic changes characterized by serofribinous exudation and aggregation of mononuclear cells and giant cells were reported found in lung sections. *Proteus mirabilis* were also isolated from Nile crocodiles (Roggendroff and Muller, 1976) and from severely stressed African dwarf crocodiles (Huchzermeyer and Agnagna, 1994). *Proteus vulgaris* was found as an oral flora of the American alligators (Flandry *et al*., 1989) and was also isolated from captive Nile crocodiles (Roggendroff and Muller, 1976). There are reports that Proteus spp. can cause meingo-encephalitis, empyema, osteomylitis and septicaemia in human neonates (O'Hara *et al*., 2000).

Klebsiella pneumoniae and *K*. *oxytoca* are opportunistic pathogens found in the environment and in mammalian mucosal surfaces. *K*. *oxytoca* have been isolated from severely stressed African dwarf crocodiles (Huchzermeyer and Agnagna, 1994) as well as from the American alligators (Flandry *et al*., 1989).

Aeromonas hydrophila is reported as emerging potential pathogen in the immuno-compromised peoples (Chang *et al*., 1997) and has caused gastroenteritis in children (Rathinasamy *et al*., 2006). Gorden *et al*. (1979) reported isolation of *A*. *hydrophila* from the internal organs of adult alligators (*Alligator mississippiensis*). They reported that aeromonads are ubiquitous in the natural habitats of reptilians apparently not causing any clinical disease. However, factors stressing the animals such as trapping, handling and an increase in water temperature may be conducive to the rapid proliferation of the bacteria, there by facilitating disease. Turutoglu *et al*. (2005) reported the death of a captive male crocodile (*Crocodylus niloticus*) without any signs of disease but with septicaemic and skin lesions associated with *A*. *hydrophila*. Brown or red-spotted skin lesions of

varying size, mostly scattered over the abdomen were noted on gross examinations. At necropsy, numerous white, multifocal and randomly distributed areas were seen on the liver. Gram-negative bacilli arranged in clusters were found in the skin and liver lesions and pure cultures of *A*. *hydrophila* were obtained from skin, internal organs and blood and it was concluded that *A*. *hydrophila* may cause skin lesions and even death due to septicaemia in crocodiles. *A*. *hydrophila* is one of the most common bacteria associated with the aquatic environment; however, limited data are available regarding infection in crocodilians. *Aeromonas hydrophila* and Flavobacterium spp. have massively killed immunosuppressed wild common carp (*Cyprinus carpio carpio*) in Canada where the infection were induced by physiologic (spawning) and environmental (high temperatures and low water levels) stressors (Shotts *et al*., 1972; Monette *et al*., 2006).

Stenotrophomonas maltophilia had caused acute septicaemia and death of a West African dwarf crocodile (*Osteolaemus tetraspis*) and it was proved through histopathologic examination and bacteriologic culture (Harris and Rogers, 2001). The organism was isolated from the lungs, liver and kidney in pure culture. The bacterium has recently gained importance as a nosocomial pathogen in humans where it causes septicemia, endocarditis, meningitis, pneumonia, urocystitis and wound infections.

Novak and Seigel (1986) reported gram-negative septicaemia in American alligators (*A*. *mississippiensis*) by six species of bacteria previously not reported as disease agents in crocodiles. They found that other than *A*. *hydrophila*, *Citrobacter freundii*, *Enterobacter agglomerans*, Proteus spp., *Morganella morganii*, *Serratia marcescens* and *Klebsiella oxytoca* were also responsible for septicemia in crocodiles; the genus Aeromonas being the most commonly reported to cause disease in crocodilians.

Huchzermeyer (2000) reported stress septicemia induced by repeated handling in young farmed Nile crocodiles. The stressed crocodiles developed paralysis and polyarthritis due to *M*. *morganii* infection; the organism being a normal gut inhabitant penetrated the joints and internal organs in stressed conditions.

The prevalence of Salmonella in captive *Crocodylus johnstoni* and *C*. *porosus* were investigated by Manolis *et al*. (1991) by taking cloacal and fecal swabs. Van der Walt *et al*. (1997) studied the reptilian Salmonella and found 145 isolates from farmed Nile crocodiles and three isolates from wild caught African dwarf crocodiles but did not find any pathological conditions in the host. Huchzermeyer *et al*. (2000) also found three isolates of Salmonella in the wild caught African dwarf crocodiles (*O*. *tetraspis*) as normal intestinal flora. Obwolo and Zwart (1993) reported salmonella as normal flora in the intestinal tracts of farm reared Nile crocodiles but they also reported that Salmonella septicemias in crocodile and other reptiles could be precipitated by stress. A severe outbreak of salmonellosis in Nile crocodiles (*C*. *niloticus*) was reported by Huchzermeyer (1991) in South African farm producing crocodile leather and reported it as a common disease despite very few documented reports of salmonellosis in crocodiles. Poor hygiene, contamination of feed, water stagnation and overcrowding resulting in high bacterial concentrations in the ponds were suspected as predisposing factors for salmonellosis.

Citrobacter spp. was isolated as bacterial intestinal flora of captive gharials (Mishra *et al*., 1993), as oral bacterial flora from the American alligators (Flandry *et al*., 1989), from the faeces of captive Nile crocodiles (Roggendorf and Muller, 1976) and from the intestines of severely stressed wild African dwarf crocodiles (Huchzermeyer and Agnagna, 1994). *Citrobacter freundii* has caused a serious pathological condition in turtles, called septicaemic cutaneous ulcerative disease (SCUD)

(Ebani and Fratini, 2005); various other Citrobacter isolates have been found responsible for meningitis (Gross *et al*., 1973; Ross, 1979) and septicaemia (Altmann *et al*., 1976); George, 1973) in human neonates.

Escherichia coli forms a part of normal intestinal flora and may cause urinary tract infections, neonatal meningitis, gastroenteritis and septicemia in man and animals (Chakraborty, 1995). Poor hygiene, intensive husbandry practices and lower age are common predisposing factors for *E. coli* infection in animals (Quinn *et al*., 1994). *E*. *coli* was isolated from captive gharial hatchlings as a normal intestinal flora (Mishra *et al*., 1993), but was also found responsible for septicaemic death in gharial hatchlings (Mehrotra *et al*., 2000). It was also recovered from severely stressed African dwarf crocodiles (Huchzermeyer and Agnagna, 1994), from an outbreak in Mugger crocodiles (Sinha *et al*., 1988) and also from Nile crocodiles (Roggendorf and Muller, 1976). Xuesong *et al*. (2002) found endogenous spread of *E. coli* infection inYangtze crocodile and reported 90% prevalance rate and 30% mortality.

Staphylococcus is ubiquitous organism primarily found on mammalian skin and mucosal surfaces. *Staphylococcus aureus* is associated with most supporative lesions; however, it is the common cause of bacteremia, the infection reaching the blood through lungs, gastrointestinal tract, urinary tract and skin abrasions. It was isolated from gharial mortality cases (Mehrotra *et al*., 2000), as an intestinal aerobic flora of gharial hatchlings (Mishra *et al*., 1993) and from stressed African dwarf crocodiles (Huchzermeyer and Agnagna, 1994).

Mycobacteria may be commonly recovered from healthy reptiles but they may cause disease in compromised hosts. Ariel *et al*. (1997b) reported the case of Mycobacteriosis in twelve young fresh water crocodile (*C*. *johnstoni*) hatchlings weighing 186-390 gm and having a length of 42-53 cm. Post mortem examination revealed protruding pale nodules, one to four millimeter in diameter in several organs including the lungs, liver, spleen and kidney with caseous material. Well-demarcated single small or larger granulomas surrounded by multinucleate giant cells were observed in microscopic studies.

Dermatophilosis (Brown spot disease) caused by filamentous and branching gram-positive bacteria resembling *Dermatophilus congolensis* has been described as the most prevalent skin disease with serious impact on the quality of the crocodile skins in Australian farms (Buenviaje *et al*., 1997; 1998; 2000; Summers, 2000).

Mohan *et al*. (1995) recorded outbreaks of mycoplasma-associated polyarthritis for the first time in farmed yearling Nile crocodiles in Zimbabwe, which exhibited swelling of the limb joints, progressive lameness and paresis as prominent signs. An epidemic of fatal pneumonia with fibrinous polyserositis and multifocal arthritis in captive adult American alligators (*A*. *mississippiensis*) were reported by Brown *et al*. (2001a); the causal agent *Mycoplasma alligatoris* was isolated from peripheral blood, synovial fluid and cerebrospinal fluid of affected alligators. Mild interstitial and peribronchiolar pneumonia to fibronecrotic pneumonia with extrapulmonary complications such as pericarditis, myocarditis and multifocal arthritis were reported as gross findings at necropsy. The findings were regarded as remarkable because mycoplasmosis is rarely fatal in animals. Antibodies of the pathogenic Mycoplasma in American alligators (*Alligator mississippiensis*), Broad-nosed caimans (*Caiman latirostris*) and Siamese crocodiles (*Crocodylus siamensis*) were also detected by Brown *et al*. (2001b).

Mishra *et al*. (1996) while analyzing the etio-pathological agents of dead gharial hatchlings aged between four and seven weeks during a heavy mortality episode in the Nandakanan Zoological Gardens, Orrisa, India, also isolated Mycoplasma spp. from one hatchling.

High mortality in two to five months old farmed salt-water crocodile (*C. porosus*) hatchlings due to Chlamydia was reported from Australia killing over 3000 hatchlings in a period of three months (Australia, 2006). The affected animals were reported as having fibrinous pharyngitis and conjunctivitis often causing laryngeal obstruction and the disease was diagnosed by using PCR technique.

Mohan *et al*. (2005) described the isolation of a new Chlamydophila (Chlamydia) spp. causing chlamydiosis in Nile crocodiles (*C. niloticus*) farmed in Zimbabwe. Huchzermeyer *et al*. (1994) while investigating acute mortality in farmed Nile crocodile (*C. niloticus*) hatchlings aged five months measuring 35-54 centimeter in length, isolated *Chlamydia psittaci* as the causal agent of the disease from the liver of affected animals. The prominent pathological findings were acute hepatitis with intracellular chlamydial colonies and generalized oedema.

2.2 Mycotic diseases

Fungi are common inhabitants of soil and can cause opportunistic diseases in reptiles. Fungi can enter through wounds, cuts and abrasions. Trapping and handling, temperature changes, overcrowding and transport are just some of the stressors of reptiles that can allow opportunistic pathogens to cause disease (Migaki *et al*., 1984). Dermatophytes are the predominant cause of cutaneous disease in mammals but these fungi are rarely implicated in reptile disease (Pare *et al*., 1997). Fungal infections in crocodile hatchlings are mostly precipitated by sub-optimal water and or pen temperatures (Ladds, 2003). Pulmonary infection by *Fusarium moniliforme* in a captive alligator (*Alligator mississippiensis*), which was in excellent nutritional condition, was reported with severe necrotizing bronchitis and bronchiectasis at necropsy. Histological examination revealed numerous branched, septate, hyaline hyphae within the necrotic debris lining the bronchi and rarely infiltrating into the adjacent stroma (Frelier *et al*., 1985).

The captive salt-water crocodile hatchling (*Crocodylus porosus*) exhibiting sudden death was reported infected with *Paecilomyces lilacinus*. Granuloma-like lesions were seen in the liver, lung and spleen and branching, septate fungal hyphae were observed in sections of liver and spleen. This was apparently the first report of the isolation of this fungus from a reptile in Australia (Maslen *et al*., 1988).

Hibberd and Harrower (1993) described an unacceptably high mortality (50%) in farmed *Crocodylus porosus* hatchlings due to *Fusarium solani*, an ubiquitous saprophytic fungus and were demonstrated in liver, lungs and small intestine samples stained with Periodic Acid Schiff method. The conidia of this opportunistic fungus were believed to have entry through small wounds on the skin of the hatchlings. The fungus was isolated, both superficially and systemically, from diseased juvenile hatchlings and various internal tissues contained granulomatous inflammations and cultures of these samples yielded the same pathogen. Asymptomatic tissues were also infected. *Paecilomyces lilacinus,* Cladosporium spp. and Aspergillus spp. were also isolated from these hatchlings but in a lower frequency. Environmental samples showed that the pathogen was widely distributed in the farm environment. Contamination of eggs by the fungi was determined as the probable primary cause of infection along with subsequent physical trauma in juveniles. Natural nesting material was implicated as a major source of egg contamination. Changes made in the

artificial incubation techniques used at the farm prevented the infection of eggs and a significant increase in hatchability was achieved (Hibberd, 1994; 1996).

Thomas *et al*. (2002) reported the death of 48 salt-water crocodiles (*C. porosus*) hatchlings from the infection of Chrysosporium anamorph of *Nannizziopsis vriesii*. This fungus was recently identified as the cause of cutaneous infections in chameleons and brown tree snakes (Montali *et al*., 1975).

The entomopathogenic fungus, *Beauveria bassiana*, had been found responsible for the death of an American alligator (*Alligator mississippiensis*) and the fungus was isolated from pulmonary lesions. Colonies of the fungus, which had sporulated in vivo, were found in the thoracic air spaces. Septate, branching hyphae and fungal spores were seen in stained histologic sections of pleura and the lungs. Dissemination to other viscera had not occurred. This case indicated that *B. bassiana*, a rare vertebrate pathogen, might be a fatal mycotic agent in captive reptiles (Fromtling *et al*., 1979).

Lal (1982) reported rotting of snout bones and falling of teeth in gharial hatchlings due to fungal infection as well as observed mycotic skin lesions in arm pits, limb joints and over the back which subsequently shed off. Maskey (1989) stated that 60 per cent of the gharial hatchlings died of mycotic infections particularly in Monsoon season. He noted mycotic lesions around the cervical region and margin of the jaws. He further realized that poor water quality and extreme humidity favored the high incidence of mycotic infections.

Fusarium spp., Penicillium spp., Trichoderma spp. and Curvularia spp. were reported to occur as oral flora of American alligator and intestinal flora of African dwarf crocodiles (Huchzermeyer, 2003). Foreyt and Leathers (1985) recorded generalized growth of Trichoderma sp. in an alligator hatchling and considered it pathogenic to juvenile animals under malnutrition or other debilitating illness.

Curvularia spp. has been found associated with peritonitis in elderly human patients undergoing peritoneal dialysis (Guarner *et al*., 1989; Pimentel *et al*., 2005) though the infections are uncommon despite the ubiquity of the organism in the environment. Rhizopus spp. (Family-Mucoraceae) has caused acute and rapidly fatal infections in humans by invading major blood vessels, with ensuing ischemia, necrosis and infarction of adjacent tissues, resulting in the production of black pus (Vazquez, 2006). They are ubiquitous and generally saprophytic, but rarely cause disease in immunocompromised patients. Cutaneous infection may occur through local trauma and the fungal hyphae may be demonstrated with Grocott methenamine-silver stain or periodic acid-Schiff (PAS) staining.

Pseudallescheria boydii, a low-virulence fungus, has been found as the main causative agent of post-traumatic mycetoma in human patients (Nonaka, 2002). The hyphae would have a random, haphazard pattern of branching rather than the progressive, arborizing pattern of the branching characteristics of Aspergillus infections. Also, hyphae of *P. boydii* do not have the fragile, ‘crumpled,’’ or ‘‘twisted ribbon’’ appearance of Zygomycetes, which could also have rare septation.

Mycoses are still a common problem in gharial hatchlings in Chitwan; both oral mycoses as well as cutaneous mycoses are reported (Personal communication, B. B. Khadka, 2006) but the prevalence has significantly been lowered by the use of chemicals.

2.3 Parasitic infections

Neodiplostomum gavialis, a trematode, was reported to occur in gharials by Narain (1930); *Exotidendrium gharialii* was reported to occur in gharials by Mehra (1935); and *Acanthostomum elongatum* and *A*. *atae* were reported to occur in Philippine crocodile (*Crocodylus mindorensis*) by Tubangui and Masilungan (1936). Deblock *et al*. (1965) reported *Exotidendrium gharialii*, *Pseudoneodiplostomum bifurcatum* and *P*. *thomasi* from the *Crocodylus niloticus* in Madagascar. Blair *et al*. (1989) reported *Renivermis crocodyli* from the kidneys of *C*. *porosus* in northern Australia; the genus Renivermis is grouped with Exotidendrium and Simhatrema in the family Exotidendriidae. *Acanthostomum americanum*, *Pseudoneodiplostomum groschafty*, *Pelazia loosi* and Telorchis spp. were reported to occur in *Crocodylus moreletii* in Mexico (Moravec, 2001). Blair *et al*. (1988) reported the occurrence of *Timoniella absita* in the small intestine of the salt-water crocodile (*C*. *porosus*) in Australia. Four species of trematodes viz *Polycotyle ornata*, *Acanthostomum coronarium*, *Archaediplostomum acetabulum* and *Pseudocrocodicola americaiense* were reported to occur in American alligator by Hazen *et al*. (1978). Sebekiosis caused by *Sebekia oxycephala* has been reported in captive Alligator (*A*. *mississippiensis*) hatchlings aged four weeks (Boyce *et al*., 1984). These inhabited the lungs and were transmitted from mosquito fish (*Gambusia affinis*) fed to them (Boyce *et al*., 1984). *Micropleura viviparous*, a filarial nematode occurring freely in the abdominal cavity was reported by Linstow (1906) and *Typhlophoros lamellaris* and *Procephalus indicus* were reported to occurr in gharials (Ladds, 2003).

Telford and Campbell (1981) state that cestodes are unknown in crocodilians, despite their abundance in most other reptiles but Ladds and Sims (1990) found a solitary cestode larva and claimed only indication of cestodiasis in crocodiles.

2.4 Protozoan diseases

Intestinal protozoans of the group Coccidia cause diseases in many animals including domestic rabbits and poultry. The parasite lives inside the cells of its host and commonly it is the lining of the gut, which is affected. *Eimeria crocodyli* in *Crocodylus acutus*, *Eimeria kermoganti* from the spleen of gharials and *Eimeria pintoi* in Caiman spp. were previously reported (Gardiner, 1986; Levine, 1987; Aquino-Shuster and Duszynski, 1989; McAllister and Upton, 1990). Coccidiosis was noted as a major disease of crocodiles in Zimbabwean farms affecting mostly small weak hatchlings (Foggin, 1987). Jacobson (1982) noted that most free-ranging reptiles were parasitized by Coccidia without any sign of illness but young animals, especially, were suspected to fall ill after stress. Diarrhoea, often with blood, was usual symptom of infection with coccidia. Positive diagnosis required post mortem microscopic examination of the gut and other parts, notably the gall bladder and bile duct (Foggin, 1987). According to Huchzermeyer (2002), several coccidial species from crocodiles have been described but those associated with outbreaks of coccidiosis have not been identified. It has been suggested that the organisms responsible may belong to the genus Goussia. Ladds (2003) has also described that Goussisa-like coccidial organisms appears to be an important cause of ill-thrift and mortality in crocodile hatchlings. The oocyst of the pathogenic coccidia are very fragile and usually only the sporocysts are found, often trapped in the mucosal crypts by exudates and also transported by lymph and blood to other organs and causing cases of generalized coccidiosis, which have been reported in Nile crocodiles in addition to *C*. *porosus* and *C*. *novaeguinae* including cases of transovarian transmission in Spectacled caimans. Intra-abdominal sporulation is typical of this type of crocodilian coccidiosis.

Crocodiles affected with coccidiosis are reported to become listless and may take a long time to die. On post mortem examination, fibrinous enteritis is usually seen, often occluding the intestine;

infection cannot be diagnosed by means of analyses of intestinal smears because of highly fragile oocysts, but it can be detected only by histopathological examination (Huchzermeyer, 2003).

2.5 Metabolic and nutritional diseases

The disorders associated with unbalanced nutrition in crocodiles are in increasing trend. Gout is one of the common problems reported in crocodiles. Several factors such as nephritis, administration of nephrotoxic drugs, high protein diet, deficiency of vitamin A and severe dehydration are implicated for the induction of gout. Ariel *et al*. (1997a) reported gout due to deficiency of vitamin A in the diet in hatchling *Crocodylus johnstoni* and *C*. *porosus*. They observed white granular material throughout the kidney and in the knee joints as well as they found similar flaky white material on the serosa of liver, gastric mucosa, peritoneum and in the pericardium at post mortem examination. They had enough evidence of hyperplasia and tophy formation in the tissues. They analyzed the feed and found it composed of 69% protein and 125 IU/Kg vitamin A on the dry matter basis, strongly suggesting the root cause of gout in hatchlings. Similar findings were reported by Buenviaje *et al*. (1994) in 22-150 gm 18-43 cm long *C. johnstoni* hatchlings fed with day old chicks; eosinophilic amorphous or crystalline deposits in either renal tubules or collecting ducts were detected on histopathological examination. Thiamine deficiency have been observed in *Crocodylus porosus* hatchlings due to feeding of frozen fishes or perhaps due to sulphides in preserved meat. In such cases, hatchlings lost righting reflex and thus were found floating or lying on their sides with the jaws open (Jubb, 1992). Osteomalacia is frequently seen and is characterized by "rubber jaws" and glassy teeth in hatchlings; steatites or necrosis of fat is believed to occur in older crocodiles due to inadequacy of vitamin E in the diet (Huchzermeyer, 1986).

2.6 Skeletal deformities

Maskey (1989) recorded three gharial hatchlings with snout deformities in 1979-80. In these, the upper jaw crossed over the lower jaw and projected to the side. Also reported were four hatchlings devoid of upper snouts in the year 1987.

Singh *et al*. (2001) noted that rubbery snout and hunch back condition could occur in captive mugger crocodiles (*Crocodylus palustris*). In rubbery snout, jaws, snout and head could be bent from with the pressure of two fingers. Similar pathological conditions were reported by Huchzermeyer (1986) in young captive Nile crocodiles (*C*. *niloticus*). Osteomalacia in one-year-old crocodiles were manifested as kyphoscoliosis, glassy teeth, rubber jaws and extreme weakness. High doses of oral calcium produced slow improvement but deformity of the vertebral column did not resolve. Youngprapakorn *et al*. (1994) has described varieties of skeletal deformities in the Indopacific crocodile (*C*. *siamensis*) hatchlings.

2.7 Management and husbandry practices

Buenviaje *et al*. (1994) investigated husbandry disease associations in farmed crocodiles in Australia and showed relations between husbandry practices and the occurrence of disease in crocodiles. This study examined the structure of crocodile pens, water supply, food and supplements and the effect of temperature on mortality. They observed that 47% overall mortality in *Crocodylus porosus* were reduced to 28.6% just with improvements in husbandry and housing conditions. The higher mortality of hatchlings in winter months were reduced by the use of thermostatically controlled heating system maintaining a temperature of 30˚C. Improved housing and management significantly restricted the spread of parasitism as well as the introduction of concrete indoor pens and the use of bore water-prevented occurrence of coccidiosis.

Ladds *et al.* (1996) have mentioned that the group of *C. porosus* hatchlings in Australian farms had been given a floor space of 0.06-0.04 m^2/hatchling but showed up with *P. rettgeri* meningitis and septicaemia. Goudie (1989) suggests that the area needed by crocodiles depends upon body weight of the animals and he recommended roughly 0.1 m^2 for *Crocodilus porosus* weighing about four kilograms.

2.8 Miscellaneous observations

Lal (1982) reported that gharial hatchlings under one year of age die from a number of causes or diseases; the mean survival rate at this age often reaching only 55%. A significant number of hatchlings were reported dead due to deformity (bend neck and weakness). Obvious relation between the death pattern and seasonal variances were not found as the hatchling death occurred every month. He argued that the cause of death of hatchlings, at least to some degree, could not be ascertained because of poor knowledge of diseases affecting gharials. Mouth canker, skin diseases and gastric troubles were grossly speculated as the cause of death in hatchlings in Kukrail Gharial Rehabilitation Center in India. Fungal infections were indicated by rutting of the snout bones and teeth leading to difficulty in fish catching and feeding. Fungal lesions also were found in the armpits, limb joints and over the back, which caused discoloration and consequently flecking up of the skin. However, laboratory confirmation was lacking. Sudden death in the hatchlings was also observed with antimortem signs of abdominal distention, sluggishness and shivering; such signs were associated with gastric trouble. However, laboratory examination of a few hatchlings could not find any specific cause other than scarification, congestion of lungs and absence of clostridial toxin in the intestinal contents. Jumping activity of the juveniles in empty rearing ponds causing internal injuries were blamed as the cause of death.

Giant cell enteritis was reported in *C. porosus* hatchlings aged about a year. Thickening of the proximal intestine along with presence of large number of multinucleate giant cells in the lamina propria was principle findings (Ladds *et al*., 1994).

An interesting hatchling disease known as interdigital subcutaneous emphysema or "bubble foot" has been reported in *Crocodylus porosus* hatchlings, the clinical abnormality consisting of loss of body weight and gaseous inter-digital swellings involving one or more limbs making them unable to swim and compete for food (Turton *et al*., 1996); the cause of the disease is still unknown but may be comparable to gas bubble disease in frogs (Colt *et al*., 1984). Radial *et al*. (1998) is of the opinion that infections by gram negative bacteria together with heavy spirorchid cardiovascular flukes or other internal parasites be considered as the cause of systemic illness and death in green sea turtles (*Chelonia mydas*). Their study was focused on mortality associated with a high prevalence of parasitism or some other undetected debilitating conditions. Tissues from all examined turtles contained evidence of systemic Gram-negative bacterial infection; the isolates in this case (*E. coli*, *C. freundii*, Salmonella and Moraxella) were probably opportunistic environmental pathogens.

Piling up at one corner of the enclosure or on top of each other is regarded as a normal reaction of the hatchlings to fear (Huchzermeyer, 2003); the frightened hatchlings prefer deep water than shallow water as a suitable refuge for them. The reaction may contribute to death by suffocation or may cause scratches on the skin permitting infectious agents (Huchzermeyer, 2003). At least these stressful conditions can trigger the effect of other factors for mortality of hatchlings.

Plate 1. Piling up of gharial hatchlings in one corner of the hatchling pen

3. MATERIAL AND METHODS

3.1 Location of study site

The study was carried out in Veterinary Teaching Hospital (VTH), Institute of Agriculture and Animal Science (IAAS), Rampur, Chitwan (August, 2006-April, 2007). Dead gharial hatchlings that hatched from June 15-28, 2006 were collected from Gharial Breeding Center (GBC), Chitwan National Park, Kasara (Fig.1).

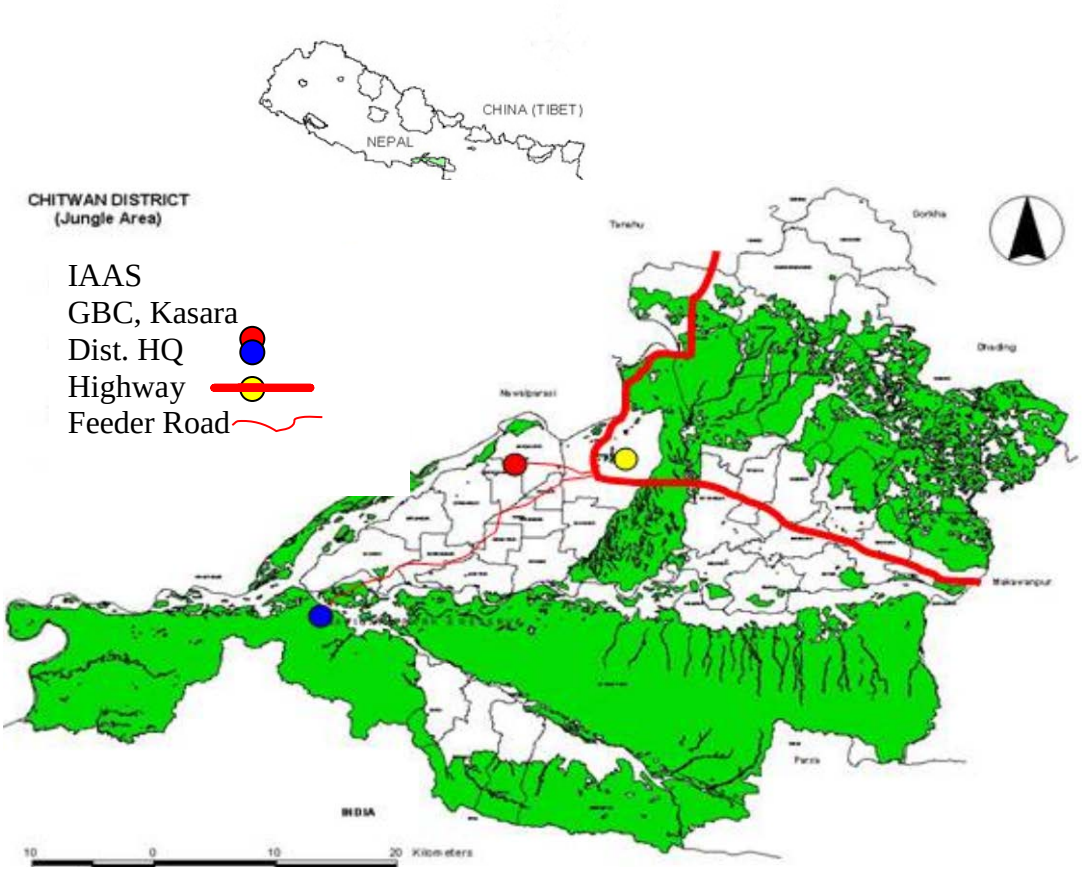


Figure 1. Map of Chitwan district showing study area

3.2 Acquisition of specimens

Dead gharial hatchlings that were raised at GBC at Kasara (Batch of 2006), were collected post death every morning. A total of 137 (six to 42 weeks old) dead hatchlings constituted the study population. These carcasses in whole were transported to IAAS Veterinary Teaching Hospital (VTH) in ice, examined immediately or wrapped individually in a plastic bag, labeled with indelible ink and stored again in the freezer (-20ºC) until necropsy.

3.3 Necropsy examination

Before necropsy, each carcass was taken in a 41.5 x 30.5 x 6.5 cm plastic tray, washed thoroughly with tap water to remove all the dirt and sand particles. The length of the hatchlings was recorded from the anterior end of the snout to the tip of the tail. Weight was taken using electronic scales (Model AL-300, Denver Instrument Company, USA). The hatchlings were then thoroughly washed with distilled water and soaked for five minutes in 70% dehydrated alcohol (Ethanol®, B. P., Bengal Chemicals and Pharmaceuticals Ltd., Calcutta, India). The alcohol was drained thoroughly and every carcass was assigned a label written in a clean paper with dates of death, necropsy examination and its weight and length measurements recorded and photographed.

Before opening the carcass, the head and skin were examined including the eyes, nostrils and mouth. The skin was checked for injuries, ulcers, swellings, abrasions under the chins and soles of the feet and any other visible abnormalities.

Every hatchling was placed in a plastic container over the table with its belly up and the skin incised across the lower neck, just caudal to the fore limbs as described by Huchzermeyer (2003). From there, the skin and the body wall were cut along both sides of the body, first through the coracoid and then through the cartilaginous part of the ribs.

Plate 2. Hatchling in a plastic tray

Plate 3. Cleaning

Plate 4. Weighing

Plate 5. Measurement

Plate 6. Dissection (common method)

Plate 7. Dissection (Huchzermeyer, 2003)

The lateral cuts were continued beyond the ribs through the abdominal wall, taking care not to cut into any of the abdominal organs, particularly into the stomach, which is attached to the left body wall. The pubic plates protruding cranially from the pelvic girdle were removed by cutting through the pelvic symphysis exposing colon, cloacal sac and parts of the kidney. The skin of the ventral body was then lifted and all attachments were carefully dissected until the whole piece could be lifted off, exposing the lungs, heart, liver and intestine.

The thoracic part of the trachea was inspected first. The pericardial sac was opened and the heart was taken out, then the membranes covering the intestine, liver and lungs were examined.

The duodenum was pulled from the pyloric antrum of the stomach and the whole intestine was gently pulled loose from the mesentery and lifted out to the left side of the body. This allowed seeing pancreas and spleen; however, the fat body was never found in these observations. The stomach was severed from its attachment to the left body wall and then lifted out with parts of the oesophagus. It was then opened and inspected for normal contents, foreign bodies, parasites and ulcers. The two lobes of the liver together with the gall bladder were removed and examined. The intestines were severed caudally after the sphincter and examined by incising longitudinally. Brain and spinal cord were not examined. All the lesions were noted in a specified necropsy protocol (Appendix I). Using all sterile tools, dissection was also carried out through ventral midline, stretching the abdominal wall sidewise and finally removing it by cutting. A horizontal cut was made before the cloaca by giving lateral incisions to avoid cloacal contamination. The lesions observed were noted with a particular case number, as well as the liver, lungs, heart, spleen and kidneys were collected for bacteriological inoculation. Small parts of these organs were also preserved in 10% buffered neutral formalin for histo-pathological study.

3.4 Microbiological examination

Liver, lungs, kidneys, heart or heart blood and swabs from sphincter and colon were collected aseptically. These samples were inoculated immediately in nutrient, McConkey and blood agar and incubated at 37ºC for 24-48 hours and the colony characters were recorded. The colonies obtained on the agar surface were stained with Gram's stain for morphological studies and subjected to conventional biochemical tests for identification of the organisms (Barrow and Feltham, 2004). The specific single colony were also inoculated in 1 ml of nutrient broth (HiMedia Laboratories Pvt. Ltd., Mumbai, India) in a 5 ml sterilized screw capped glass vial and incubated till development of cloudiness to which was then added 1 ml of 40% glycerol (Qualigens Fine Chemicals, Glaxo Smithkline Pharmaceuticals Ltd., Mumbai, India). These were stored in a freezer and dispatched to Health Research Laboratory, Institute of Medicine, Maharajgunj, Kathmandu, in ice for further confirmatory results. Skin scrapings were treated with 10% KOH and stained with Lactophenol cotton blue (LCB) for observation of fungal elements. The samples were inoculated in Sabouraud's dextrose agar and potato dextrose agar (HiMedia Laboratories Pvt. Ltd., Mumbai, India) and identified through slide culture and respective morphology of the spores and hyphae.

Plate 8. Microbiological work

Plate 9. Preserving bacterial isolates

3.5 Parasitological examination

The digestive tract was opened and the oesophageal canal was observed attentively for the retained fish in it. The stomach and intestine were thoroughly incised and contents washed in saline, then sedimented and examined under low power microscope (Model SD-1LK, Olympus Co. Ltd., Japan) for adult and immature nematode, trematode and their eggs. Together with the parasites, the lesion produced by them in the tissues were also examined and recorded.

3.6 Histopathological examination

After postmortem, the appropriate tissue samples were removed and marked appropriately. The marked tissue samples were fixed into 10% neutral bufferred formalin. It was then deformolized in tap water for over night. Then, these tissues were dehydrated by passing on ascending series of graded alcohol and were cleared by xylene followed by impregnation into melted paraffin wax at a temperature of 56˚C. Then, samples were transferred to an embedding mold for block making. The blocks were then trimmed and sections were cut at 5 µm thickness on a microtome. The cut sections were transferred to microscopic slides and fixed with gentle heat. After that, routine staining was done with hematoxylin and eosin stains. The prepared slides were then examined for microscopic changes with the help of compound microscope. Gharial tissues for histopathological studies were processed at the Veterinary Disease Investigation Laboratory (Nepal Agriculture Research Council, NARC, Khumaltar, Lalitpur) and Avian Disease Investigation Laboratory (Bharatpur, Chitwan).

3.7 Management study of gharial hatchlings

During the study, GBC was visited frequently and various aspects of hatchling management were studied. It was found that the new nursery pool built in the year 2006 was stocked for the first time with new batch of hatchlings in July 2006. The hatchlings were allowed to stay about a week with breeding females; the later excavated and released them from the nests. The near approach of hatching time was naturally indicated by calls of the hatchlings within the eggs in the nest; the old practice of digging the nest and releasing hatchlings manually were rather abandoned.

The nursery pool consists of 10.5 m wide and 18.5 m long walled and netted area. This contains a one-meter wide path in the center longitudinally and there are equally spaced six ponds on each side of the path of the size two meters wide and four meters long and with a maximum depth of 50 cm in the center. The pond has gentle slopes of about 30º at both the ends. Each pond on either side of the central path has a 75 cm netted barrier from the surface to control the movement from one pen to another. The remaining area is filled with sand for basking. The total

space available was 194.25 m^2 which permitted an average of 0.5 m^2 areas for every hatchling in the year 2006.

The pool was supplied with water drawn from a nearby creek using a water pump in the northern aspect. The creek flows through the GBC and is originated in the park. The water of the creek is out of approach of domestic livestock.

Water in the pools was changed daily with the exception during malfunctioning and repairs of the pumping machine. The cemented wall and floor were generally scrubbed after draining of the water in the morning hours. The hatchlings were force fed with tiny fresh fishes for almost three months before starting to eat themselves. The fishes were collected from the Rapti river and fed generally in the afternoon. Occasionally, when appropriate size fishes could not be collected due to flood in the river, hatchlings were not fed.

Hiding boards and shades were installed along the wall and the sand filled area of the hatchling nursery but the number was not enough as piling up of hatchlings were frequently seen.

The new hatchlings were hand fed daily small fishes just one month after hatching until mid December (2006), in alternate days until mid January (2007) and left to eat on their own completely afterwards. The whole rearing pool was covered with plastic sheet (January to mid March) to protect hatchlings from cold nights.

3.8 Statistical analysis

Data obtained were analyzed by Microsoft Excel 2000 and SPSS 11.5 for windows program. The relationship between body mass and length of hatchlings were tested by Pearson's correlation coefficient to verify the significance of the findings. The association between the Surahi fluke (Exotidendrium spp.) and occurrence of a distinct "Sphincter Cap" in the hatchlings were tested by using Pearson's Chi-square test.

4. RESULTS

The dead gharial hatchlings were thoroughly examined at IAAS Veterinary Teaching Hospital. Observations were made on various aspects of the carcass such as lesions on the skin, status of neck and tail muscles, presence of intact or decomposed fish in the oesophagus, retention of yolk sac, enteritis, septicaemic lesions, skeletal deformities, ascites, cysts in body membranes and prolapse of the vent. In addition, intestinal contents were examined for helminth parasites, coccidial oocysts and the internal organs particularly the liver, lung, kidney and heart blood were subjected to microbiological studies. The result of each speciality is presented on separate headings.

4.1 Mortality patterns in hatchlings

4.1.1 Annual trends of mortality

A retrospective data of the past three years (year 2003, 2004 and 2005) and current year (2006) was collected first hand from the GBC at Kasara to know the pattern of gharial mortality during the first 10 months (July-April) after hatching (Fig. 2, Appendix VI)). The data showed that 179, 298, 333 and 262 gharial hatchlings were obtained in the years 2003, 2004, 2005 and 2006 respectively. Only five hatchlings (2.8%) survived in the year 2003, 170 (57%) in 2004, 77 (23%) in 2005 and 101 (38%) in the first 10 months after hatching (2006-2007).

4.1.2 Monthly trends of mortality

Monthwise mortality pattern of gharial hatchlings during four years are presented in Fig. 3. The mean mortality numbers of four years revealed that a maximum mortality occurred in August (37) followed by September (28) and March (22). The least mortality number was recorded in December (8).

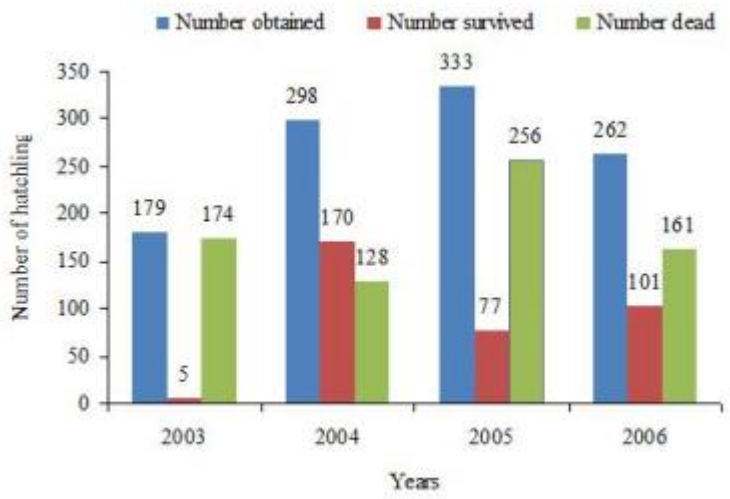


Figure 2. Mortality and survivability of gharial hatchlings during first 10 months after hatching

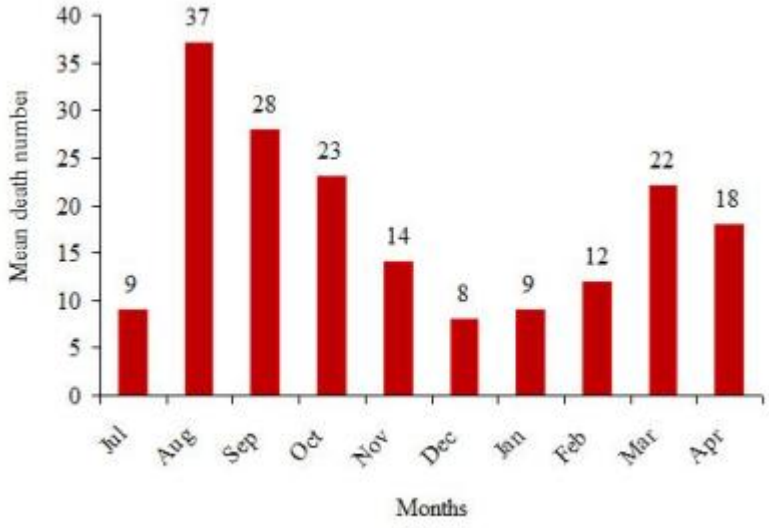


Figure 3. Monthly mean mortality patterns of gharial hatchlings (2003-2006)

Table 1. Agewise mortality pattern of gharial hatchlings in the first 10 months after hatching (2006)

Age (Weeks)	Number obtained	Deaths		Total death %
		No.	%	
4-6		11	4.20	
6-10		46	17.56	
10-14		34	12.98	
14-18		20	7.63	
18-22		5	1.91	
22-26	262	6	2.29	61.45
26-30		14	5.34	
30-34		4	1.53	
34-38		15	5.73	
38-42		6	2.29	

Hatchling death record of the year 2006 was collected and analyzed for the calculation of mortality pattern among certain age groups. Maximum death (17.56% and 12.98%) of hatchlings was found in the age group six to ten and 10-14 weeks old respectively. The results are presented in Table 1.

4.2 Body weight and length analysis

One hundred and thirty seven dead hatchlings were weighed and their measurement taken. The mean weight and length of the dead hatchlings are presented in Table 2. The snout to tail tip length was found proportionately increasing during all the months except those dead in the month of November, where as the mean weight dwindled after February. This could be due to prolonged illness among the dead hatchlings or reduced food intake during colder months.

Table 2. Length and body weight of dead hatchlings

Months	N	Length (cm)			Weight (gm)		
		Mean	SD(σ)	Range	Mean	SD(σ)	Range
August	46	42.21	1.68 ±0.25	37.2-46.00	88.25	9.51 ±01.40	66.60-110.90
September	34	43.59	1.72 ±0.30	41.0-47.50	96.12	9.37 ±01.61	69.47-106.60
October	7	45.33	2.56 ±0.97	43.0-49.00	113.72	32.12 ±12.14	71.05-163.39
November	5	44.34	3.48 ±1.55	40.5-50.00	95.48	27.13 ±12.13	73.58-136.75
December	6	46.50	2.85 ±1.16	43.5-51.00	133.57	37.83 ±15.44	89.10-172.76
January	14	46.36	3.55 ±0.95	40.0-53.50	141.36	45.74 ±12.22	86.00-253.40
February	4	46.40	0.82 ±0.41	45.6-47.50	124.48	23.69 ±11.85	90.20-143.30
March	15	46.51	3.27 ±0.84	38.0-51.60	114.57	21.64 ± 25.51	85.30-149.50
April	6	47.55	2.23 ±0.91	44.0-50.00	111.27	29.34 ±11.98	80.10-158.20

Relationship of body weight and whole body length was calculated by Pearson's coefficient of correlation - R. Strong correlation between body mass and length was found in the hatchlings that died in October, November, December and January (r^2=0.84); medium in the August, March and April (r^2=0.49); and poor in the September and February (r^2=0.16).

Chitwan experiences a gradual drop in the ambient temperature from November until March; then again temperature is gradually elevated (Table 3). Gharial hatchlings grow better during mid-summer and least in spring and fall. The growth rate is a product of food intake and bioenergetic

needs of gharial hatchlings both of which are influenced by temperature; however, diseases also play an important role. The mean atmospheric temperature was highest (35.4ºC.) in April and lowest (22.3ºC.) in January likewise mean rainfall was highest in August and not at all in January. The mean relative humidity was lowest in August while it was 100% in December. Temperature in January (coldest month) ranges between 4 and 28°C. and in July (warmest month) ranges between 18-44°C. (Maskey *et al*., 1995).

Table 3. Mean temperature, rainfall and relative humidity (June 2006-April, 2007), Chitwan*

Month	Mean temperature (°C.)		Rainfall	Relative humidity
	Max.	Min.	(mm.)	(%)
June (2006)	34.65	24.86	387.10	79.30
July	34.31	26.22	352.30	83.97
August	34.70	25.50	405.40	80.20
September	33.40	24.08	362.00	85.50
October	32.90	19.90	60.60	83.50
November	28.50	14.60	2.10	95.10
December	24.30	10.76	19.00	100.00
January (2007)	22.30	7.80	0.00	99.60
February	24.40	11.70	80.30	96.90
March	30.10	13.40	47.60	86.20
April	35.40	19.80	3.40	69.50

*Data recorded at Rampur, 15 kilometers northwest of GBC, Kasara, Chitwan

4.3 General pathological conditions

The general pathological conditions observed in the dead hatchlings were identified and recorded. These included cutaneous fungal lesions, atrophy of the neck and tail musculature, retention of intact or decomposed fish in the oesophagus, retention of yolk sac, occurrence of cysts in body cavities and membranes, nodular lesions in intestinal wall, enteritis, excess of serous fluid in body cavities, anaemic visceral organs, vent prolapse, skeletal deformities and septicaemic lesions. The details of frequencies of such conditions are presented in Table 4.

Table 4. General pathological conditions observed in 137 dead gharial hatchlings

Pathological conditions	No. of positive cases	Percentage
Anemic visceral organs	29	21.00
Ascites	24	17.51
Cysts in serous membranes	12	8.76
Drawn in neck and tail muscle	27	19.70
Enteritis	33	24.00
Fungal lesions on skin	26	18.97
Nodular lesions in the intestinal wall	9	6.60
Retention of the fish in the oesophagus	52	37.95
Retention of yolk sac	15	10.94
Septicaemic lesions	37	27.00
Snout deformity	3	2.18
Vent prolapse	2	1.45

4.3.1 Retention of fish in the oesophagus

During necropsy examination, special attention was paid towards the observation of an intact or decomposed fish retained in the oesophagial lumen. In this study, 52% of the hatchlings that died in August and 61.76% in September had intact, decomposed or relatively large size fish in their oesophagial lumen (Fig. 4). Fishes that were force fed to hatchlings were found lodged at the lower part of the oesophagus mostly at the junction of tracheal bifurcation and inlet of the heart. A few hatchlings with retained fishes had congested peripheral tissues.

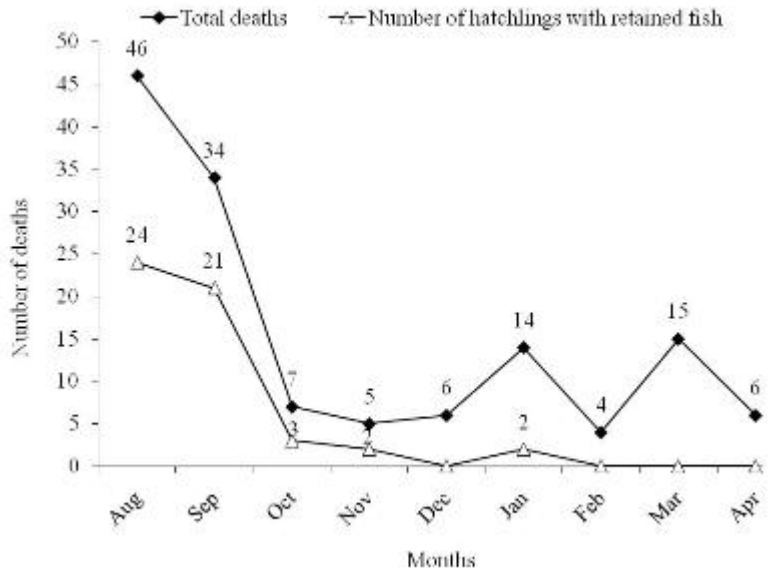


Figure 4. Carcasses with intact or decomposed fish in the oesophagus

4.3.2 Ascites, cysts and nodular lesions

Hatchlings with an excessive amount of fluid in the body cavities were noted (Fig. 5). Among the carcasses examined 17.51% had this condition. Cysts (8.76%) measuring 0.25-0.50 mm were frequently found in the serous membranes of the body cavities particularly on the covering of the liver, stomach and costal lining (Plate 12). These cysts yielded a coiled cestode larva encased in a whitish membrane. The cestode larvae

Plate 10. Retention of fish

Plate11. Drawn in neck and tail muscles

Plate 12. Cysts in the stomach wall

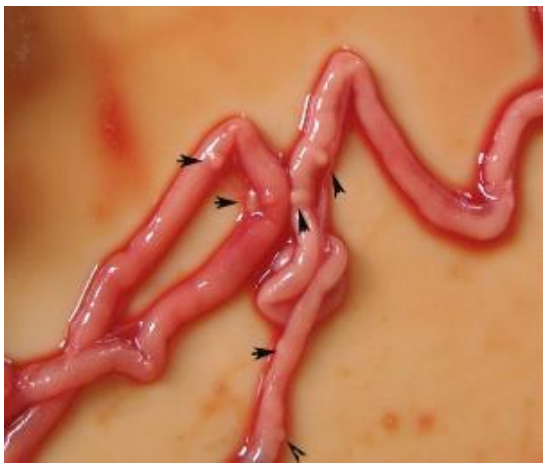


Plate 13. Nodular lesions

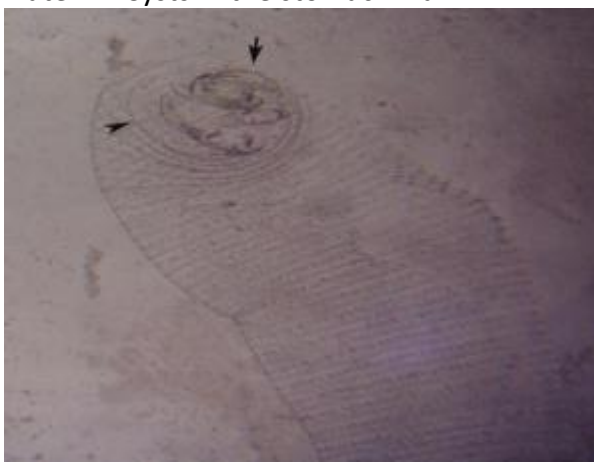
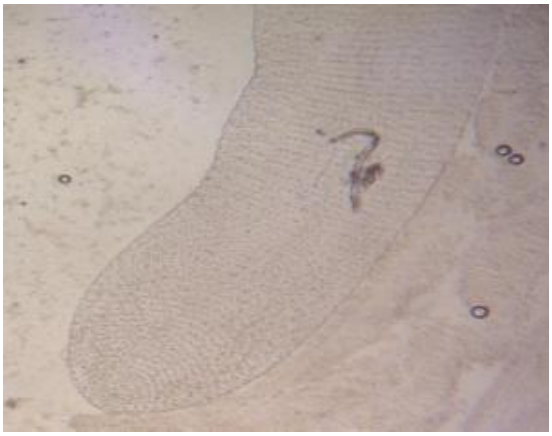


Plate 14a.Anterior part of larval cestode



b. Posterior part

measured 5.30 mm in length and 0.73 mm in width and contained four rostral hooks (Plate14a). Nine hatchlings (6.60%) had nodular lesions in the intestinal serosal layer. These lesions in the intestinal serosa yielded very small amount of whitish fluid when incised. The impression smear produced Gram-positive cocci on Gram's staining.

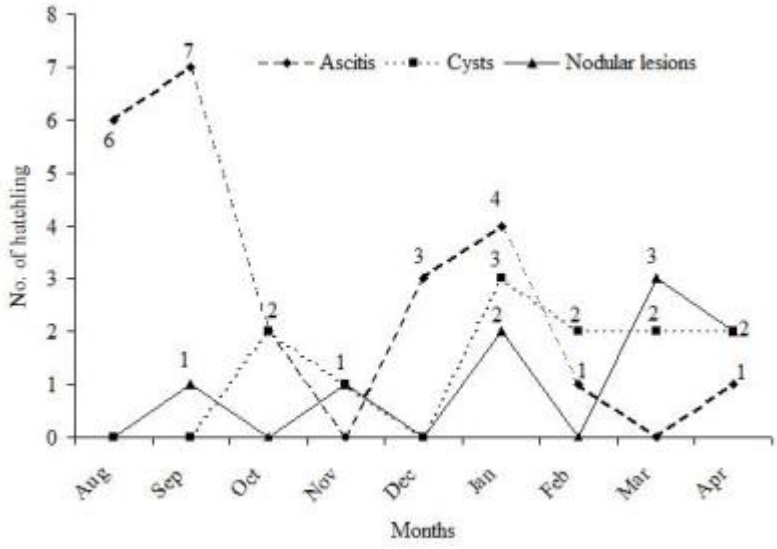


Figure 5. Carcasses with prominent ascites, cysts in the serous membranes and nodular lesions in the intestinal wall

4.3.3 Fungal lesions and drawn in muscles

Carcasses when examined were found to have dramatically drawn in muscles in the neck and tail particularly at the base (Plate11 and 18). The scutes of the posterior third of the tail were drooped sideward in many cases. Fungal lesions were recorded on the head, neck, shoulder, belly and hind legs. The frequency of fungal lesions was 18.90% and the drawn in muscles of neck and tail base was 19.70% in the population examined (Table 4, Fig. 6).

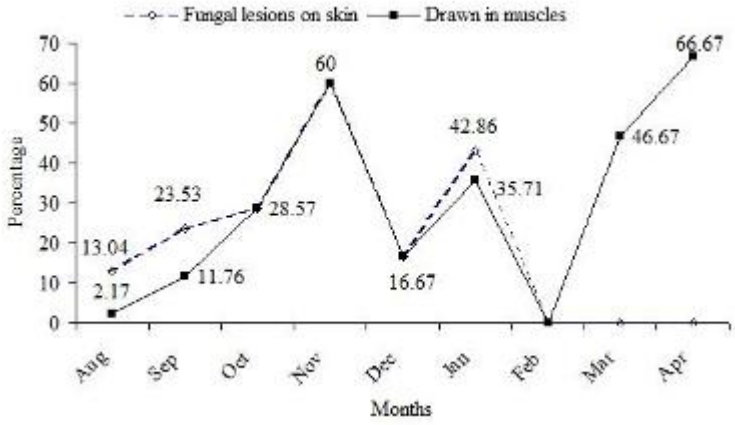


Figure 6. Fungal lesions and drawn in muscles in the hatchlings examined

4.3.4 Yolk sac retention, snout deformities and vent prolapse

Retained yolk sac was seen in 11% of the hatchlings where as vent prolapse and snout deformities were present in 1.46% and 2.19% respectively (Fig. 7). Of the examined hatchling carcasses, 2.18% had snout deformities. Vent prolapse in this study was found in

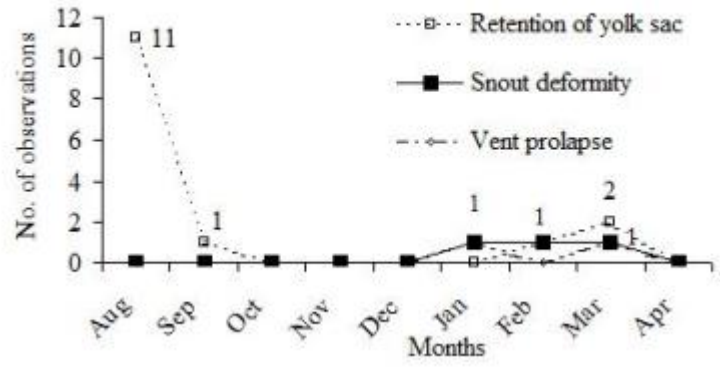
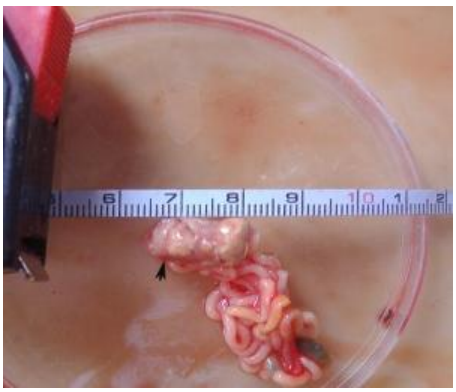


Figure 7. Prevalence of yolk sac retention, skeletal deformity and vent prolapse in gharial Hatchlings.

1.46% of gharial hatchlings. Pseudomonas spp. was isolated from kidney and recto-cloacal sphincter of one case where as *Proteus mirabilis* was isolated from the liver and sphincter of the other hatchling with prolapsed vent.

Plate 15a. Retained yolk sac

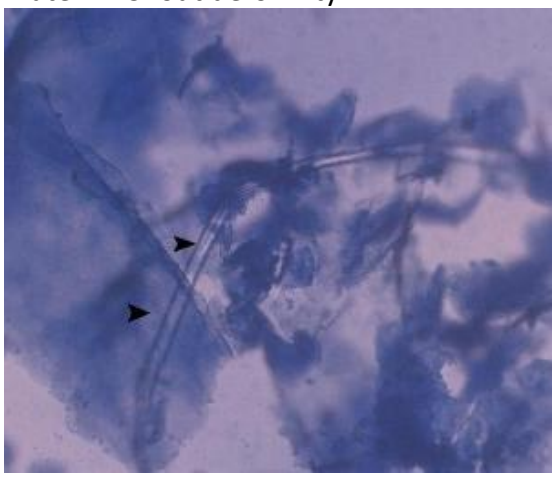


b. Hard yolk sac (fibriscess)

Plate 16. Vent prolapse

Plate 17. Snout deformity

Plate 18a. Cutaneous fungal lesions



b. Fungal elements (KOH mount)

4.3.5 Septicaemic lesions

Carcasses were examined well before as well as after dissections. The prominent signs observed were reddish tinge on the skin of the ventral area of the belly and the chest, hemorrhagic costal musculature, engorgement of the splanchnic vessels, hyperemia of the intestinal serosa and pleural membranes (Plate19). In this study, 27% of the carcasses were found to have such septicaemic lesions (Table 4).

Plate 19. Septicaemic lesions in abdomen

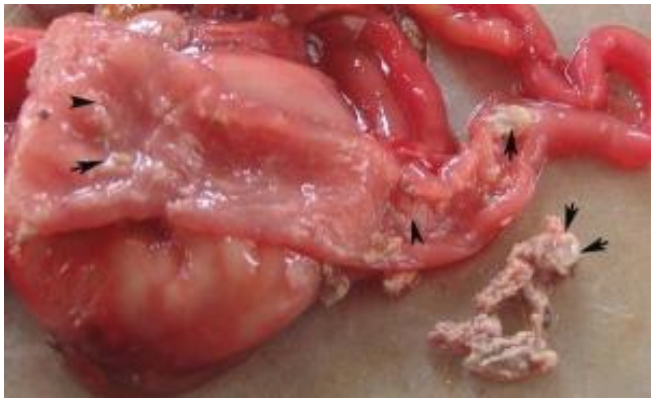
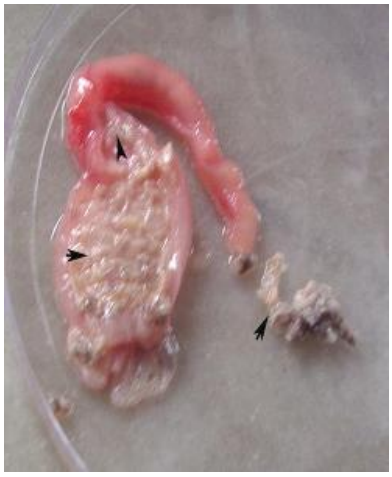


Plate 20a. Fibrinous enteritis



b. Fibrin deposits in colon

4.3.6 Enteritis and anemic visceral organs

Inflammation of the intestinal mucosa recorded with prominent signs of externally reddish look, having fibrinous whitish flakes, pseudomembranes or mucoid or occasionally reddish contents in the duodenal lumen or focal or generalized deposits or white or dirty white fibrin in the mucosa of colorectum. Out of 137 hatchlings examined, 24% (33 animals) were found having these lesions (Table 4). A gross picture at postmortem examination of whitish (anaemic) or yellowish colored intestine was observed in 21% of the carcasses. Interpretation to a diagnosis was not possible.

4.4 Parasitological examination

4.4.1 Helminth eggs, adults, coccidial oocysts and pathogenic lesions

A total of 137 stomach and intestinal contents were examined for the presence of coccidial oocysts, nematode and trematode eggs and their adults. An adult fileroid nematode, 55 mm in length, belonging to the family Micropleurinae was found in the abdominal cavity of only one hatchling where as adult trematodes (other than Surahi fluke) were recorded from 13 hatchlings. Eggs of a single type of nematode as well as those of a trematode was seen quite often. All the trematode ova seen in fecal examination were of the unidentified Paramphistomum-like trematode. Among the adults of trematode parasite, a Proctocaecum sp. and the Paramphistomum-like sp. were found in the intestine and stomach of three hatchlings

respectively. Pseudoneodiplostomum spp. was recorded in one hatchling. A typical tiny trematode found in the colo-rectum has been dealt in separate heading (see below). Coccidial oocysts were observed in 21.90% of the hatchlings. The prevalence of coccidial oocysts, helminth eggs, their adults and related pathogenic lesions are presented in Table 5 and Fig. 8.

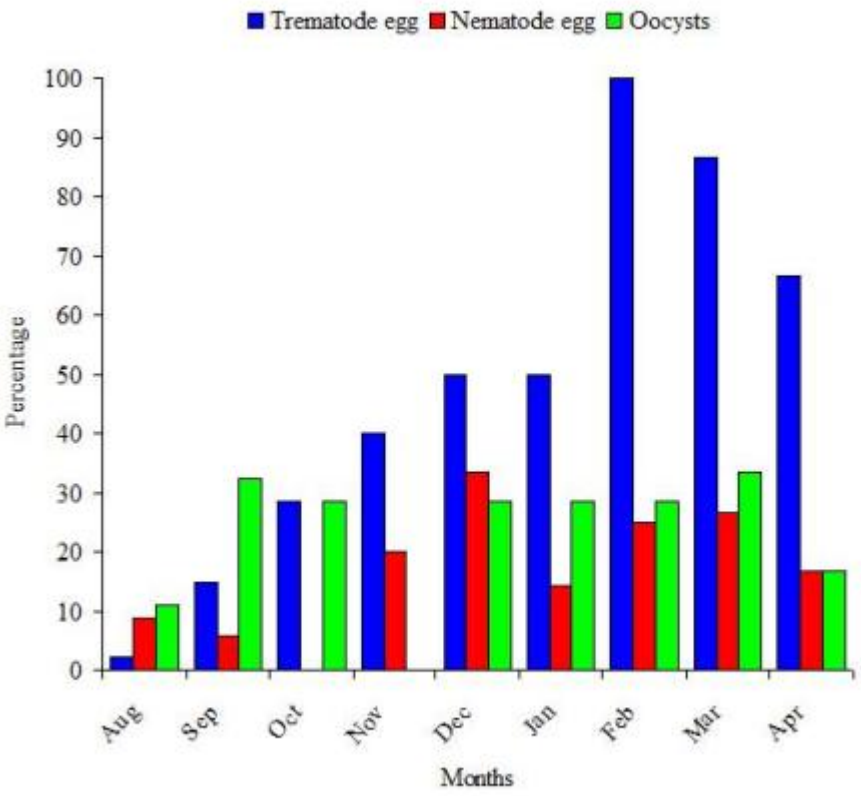


Figure 8. Percentage of helminth eggs and coccidial oocysts observed in gharial hatchlings

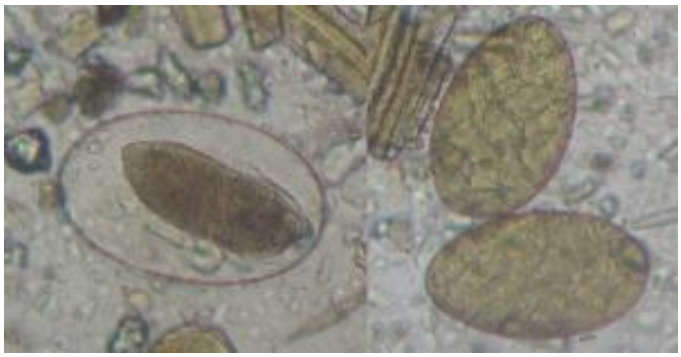


Plate 21. Nematode (left) and trematode ova (right)

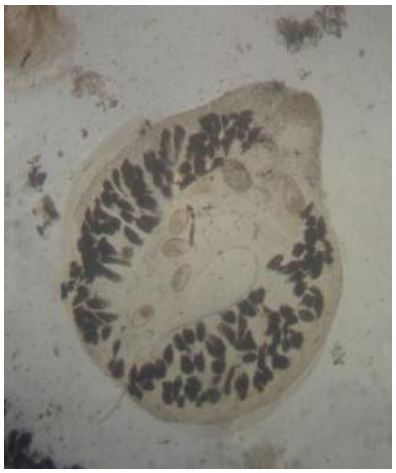


Plate 22. Adult trematode

Table 5. Coccidial oocysts, helminth eggs, their adults and related lesions in dead gharial hatchlings

Coccidial oocysts, helminth eggs, adults and lesions	N	Positive cases	Percentage
Parasitic eggs and oocysts			
Trematode egg	137	41	29.92
Nematode egg	137	17	12.40
Oocysts	137	30	21.90
Adult nematodes			
Micropleura spp.♠	137	1	0.73
Adult trematodes			
Pseudoneodiplostomum spp.♣	137	1	0.73
Paramphistomum like (UI)	137	3	2.19
Proctocaecum sp.♦	137	8	5.84
Larval cestodes	137	12	8.76
Surahi fluke**			
Immature forms	94	31	32.97
Mature forms	94	46	48.93
Lesions			
Distinct "Sphincter Cap"*	94	25	26.60

*Only 94 hatchlings were examined. **Digenea: Exotidendriidae ♠ Nematoda: Micropleurinae ♣Digenea: Proterodiplostomatidae ♦Digenea: Cryptogonimidae, UI= unidentified

The occurrence of these nematode and trematodes could not be guaranteed as the causal agents for the death of the hatchlings. However, in two hatchlings, the enlargement and shortening of the duodenum and the gross thickening of the mucosa at the opening of the bile duct into the duodenum was observed due to the effect of Proctocaecum sp. (Plate 23).

Plate 23. Proctocaecum sp.

4.4.2 Surahi fluke and "Sphincter Cap"

Ninety-four hatchlings were examined for the presence of Surahi fluke and a characteristic lesion "Sphincter Cap" around the rectocloacal orifice opening into the cloaca. Mature forms in older hatchlings and immature forms in the younger hatchlings were recorded. Combined both forms, the prevalence rate was 40.95%. The trematode was identified as Exotidendrium spp. (locally named “Surahi fluke” representing a shape of Nepalese earthen pot for keeping drinking water cool) and it was photographed under low power microscope. They were found in a range of 100-

300 in the colo-rectal lumen and around the sphincter orifice. They were elongated and slender anteriorly (⅔) with a bulbular posterior part. They measured 1.5±0.25 mm in total length. Most of them had cuticular spines in the anterior slender part. The immature forms with a broader anterior part, indistinct posterior bulb and underdeveloped internal organs were found in the hatchlings that died in August and September; adult forms did not occur in these hatchlings. The prevalence rate among the dead hatchlings was 32.97%. The prevalence of adult flukes was found to be 48.93% and the distinct "Sphincter Cap" was formed in 26.6% of the carcasses (Table 6).

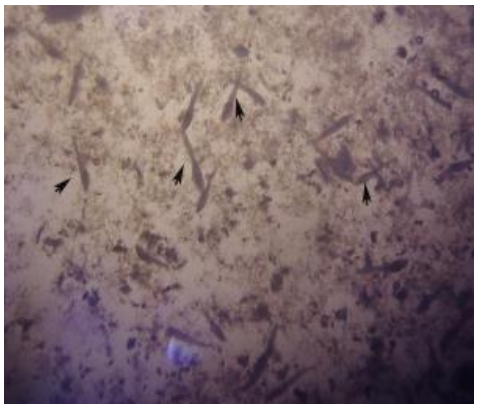
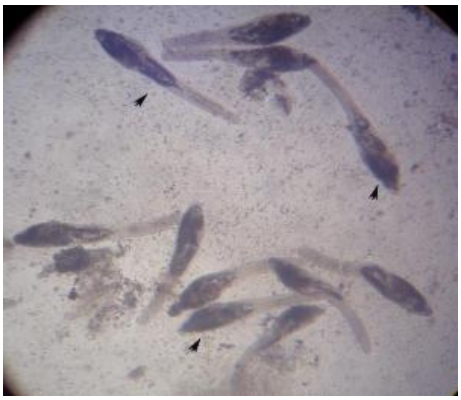


Plate 24a. Surahi flukes x20



b. Surahi flukes x50

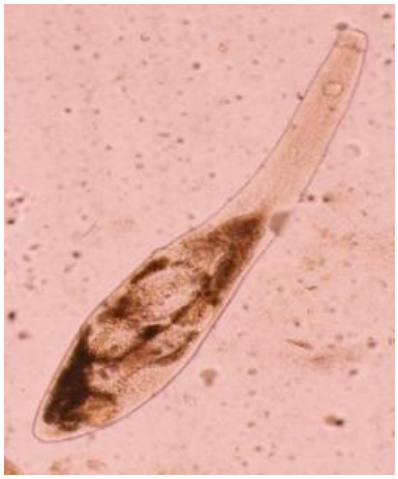


Plate 25. Surahi fluke (Mature form)

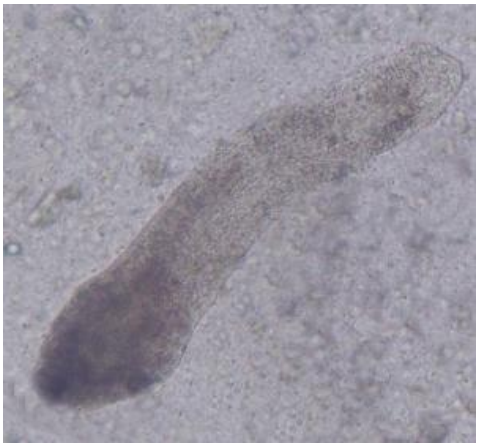


Plate 26. Surahi fluke (Immature form)

A new term “Sphincter Cap” is proposed for a distinct whitish, blackish, yellowish, yellowish green or greenish granulomatous scab formed around the colorectal orifice of the hatchlings. This cap had occluded the orifice eventually in many cases and was composed of bacteria, Surahi fluke, mucus and tissue debris. The cap was often adhered very firmly to the surface of the sphincter but it was loosely attached in majority of cases.

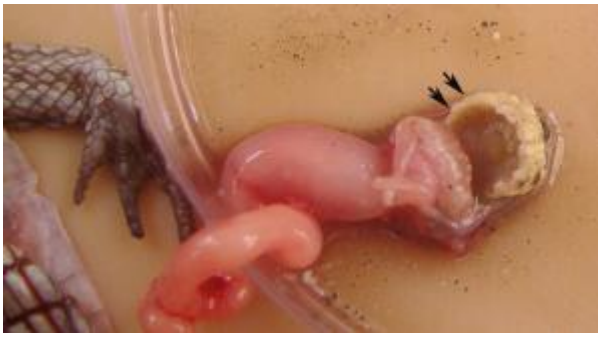


Plate 27. A characteristic “Sphincter Cap”

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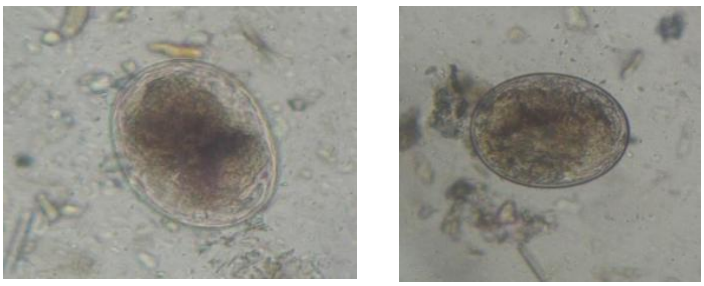

Plate 28. Unsporulated coccidial oocysts

Table 6. Prevalence of immature and mature forms of Surahi fluke and distinct "Sphincter Cap" lesion

Month	No. of cases	Surahi fluke				Distinct "Sphincter Cap"	
		Immature forms		Mature forms			
		Positive	Percentage	Positive	Percentage	Positive	Percentage
August	29	20	68.96	ND	ND	ND	ND
September	19	11	57.80	ND	ND	ND	ND
October	0	NE	NE	NE	NE	NE	NE
November	2	ND	ND	2	100	2	100.0
December	5	ND	ND	5	100	5	100.0
January	14	ND	ND	14	100	8	57.1
February	4	ND	ND	4	100	2	50.0
March	15	ND	ND	15	100	6	40.0
April	6	ND	ND	6	100	2	33.3

ND= Not detected, NE= not examined

The association between the Surahi fluke and the formation of the distinct "Sphincter Cap" were tested using Pearson's Chi Square test and was found highly significant (P=<0.001) as presented in Table 7.

Table 7. Test of significance: Surahi fluke and "Sphincter Cap" lesion in gharial hatchlings

Factors (cause and effect)	No. of positive cases	No. of negative cases	Pearsons $\chi 2$ value	P^{Ψ}
Mature form of Surahi flukes	46	48	9.980^{**}	0.001
Formation of "Sphincter Cap"	25	69		

$^{\Psi}$Probability calculated by Fisher's exact test

4.4.3 Coccidial oocysts

Unsporulated oocysts of coccidial parasites (Plate 28) were observed frequently during the faecal examination of the hatchlings. No special techniques were applied to observe these oocysts; however, they were observed concurrently with the trematode and nematode eggs on simple sedimentation method. The density of oocysts recorded was one to three oocysts per slide. Out of

137 stomach and intestinal contents, 30 hatchlings were positive to coccidial oocysts constituting a prevalence rate of 21.90%.

4.5 Microbiological examination

4.5.1 Bacterial cultures

Internal organs (liver, lungs, kidney and heart or its blood) were subjected to bacterial cultures. Cultures revealed 19 species of bacteria - five genus of Gram positive and 10 genus of the Gram-negative rods. The Gram-positive genuses were Actinomyces, Bacillus, Clostridium, Staphylococcus and Streptococcus where as the Gram-negative rods were Aeromonas, Citrobacter, Escherichia, Klebsiella, Shigella, Morganella, Proteus, Providencia, Pseudomonas and Salmonella. Twelve hatchlings (48 organ samples), however, did not produce any growth of organisms. The frequencies of the isolates obtained from 148 culture positive organs of 102 dead hatchlings are presented in Table 8.

Table 8. Frequency of 148 bacterial isolates obtained from four different organs of 102 dead hatchlings (August 2006-April 2007)

Bacterial isolates	Frequency of isolation	Percentage
Actinomyces spp.	4	2.70
Aeromonas spp.	2	1.35
Bacillus subtilis	2	1.35
Citrobacter freundii	48	32.43
Clostridium spp.	2	1.35
Escherichia coli	18	12.16
Klebsiella oxytoca	11	7.43
Klebsiella pneumoniae	1	0.68
Morganella morganii	6	4.05
Proteus mirabilis	7	4.73
Proteus vulgaris	14	9.46
Providencia rettgeri	4	2.70
Pseudomonas spp.	1	0.68
Salmonella spp.	5	3.38
Shigella dysenteriae	1	0.68
Staphylococcus albus	3	2.03
Staphylococcus aureus	14	9.46
Streptococcus spp.	1	0.68
Streptococcus viridans	4	2.70
Total	148	100.00

Samples from four organs (liver, lungs, kidney and heart) of each hatchling that died in different months were subjected to microbial examinations. A total of 408 organ samples of 102 hatchlings were examined and 148 organ samples (36.27%) gave positive cultures, the highest percentage of infection (25%) were found in September and lowest (3.38%) in December (Table 9).

Table 9. Distribution of infection in different months based on 148 culture positive organs of 102 hatchlings

Month	Samples*	Positive culture	Percentage
August	76	25	16.89
September	104	37	25.00
October	28	11	7.44
November	20	9	6.08
December	24	5	3.38
January	56	21	14.19
February	16	12	8.11
March	60	22	14.86
April	24	6	4.05
Total	408	148	100.00

* Liver, lungs, kidney and heart blood samples were cultured from every hatchling

Samples that were positive for bacterial cultures were analyzed to detect the percentage of infection in the organs. It was found that the lung tissue was infected at highest (42.57%) followed by liver and kidney; and least infection was found in the heart blood (Table 10).

Table 10. Distribution of infections in different organs based on 148 culture positive samples of 102 hatchlings

Organs	Culture positive samples	Percentage
Liver	40	27.03
Lungs	63	42.57
Kidney	23	15.54
Heart (blood)	22	14.86
Total	148	100.00

4.5.1.1 Spectrum of infections

Bacterial culture from organs resulted in the isolation of more than one bacteria from a particular hatchling. The percentage of infection by these organisms was calculated on the basis of total number of samples subjected to culture and isolates obtained. Seventy per cent of the organs harbored a single species of organism among the isolated where as about 9% revealed three or more organisms (Table 11). The details of the species of bacteria recovered are presented in Table 13.

Table 11. Percentage of bacterial isolates obtained from 90 culture positive internal organs of gharial hatchlings

Spectrum of bacterial isolates	Culture positive hatchlings	Percentage
Single isolate	63	70.00
Two isolates	19	21.11
Three or more isolates	8	8.89

4.5.1.2 Bacterial isolates and age of hatchlings

The highest percentage of infection (75%) was found in the 32-34 weeks old hatchlings followed by 18-22 (39.29%) and the least in the 22-26 weeks (20.83%) old group (Table 12). Liver, lungs, kidney and heart samples from 12 hatchlings (two of the age group six to ten weeks and 10-14 weeks, five from 34-38 weeks and one each from other groups excepting 30-34 weeks one) were found negative in bacterial cultures.

Table 12. Percentage of infection in different age groups of hatchlings

Age group (Weeks)	No. of hatchlings examined	Organs cultured	Positive cultures obtained	Percentage of infection
6-10	19	76	25	32.89
10-14	26	104	37	35.58
14-18	7	28	11	39.29
18-22	5	20	9	45.00
22-26	6	24	5	20.83
26-30	14	56	21	37.50
30-34	4	16	12	75.00
34-38	15	60	22	36.67
38-42	6	24	6	25.00
Total	102	408	148	

Out of 12 hatchlings negative to cultures from liver, lungs, kidney and heart, four were not examined well for other problems (two in August and two in September); one each in November and December had both mature forms of Surahi fluke and a distinct "Sphincter Cap"; one hatchling each in January and April was infected by mature Surahi flukes and the later had distinct "Sphincter Cap" too. The two hatchlings that died in March had retained yolk sac and the same flukes; one had only flukes where as the other had both flukes and "Sphincter Cap".

Table 13. Spectrum and percentage of bacterial isolates obtained from gharial hatchlings

Single species (n=63)			Two species (n=19)			Three or more species (n=8)		
Organism	No.	%	Organism	No.	%	Organism	No.	%
Actinomyces spp.	1	1.59	*E. coli* and Salmonella spp.	1	10.53	Citrobacter spp. either with *P. vulgaris*	5	62.5
Aeromonas spp.	1	1.59	*E. coli* and *Kleb. oxytoca*	1	5.26	and *P. mirabilis*; or with *Klebsiella oxytoca* and *Staph. aureus*; or with		
Bacillus subtilis	2	3.17	*E. coli* and *P. vulgaris*	1	5.26	Klebsiella spp. and Morganella spp.; or with *P.vulgaris* and *Staph. albus*; or with		
Citrobacter freundii	21	33.33	*E. coli* and *Citrobcter freundii*	2	5.26	*E. coli* and Morganella spp.; or with		
Clostridium spp.	1	1.59	Citrobacter spp. and Salmonella spp.	2	10.53	*Staph. aureus* and Actinomyces spp.; and *Kleb*. *oxytoca* and *Kleb. pneumoniae* with		
E. coli	8	12.70	Citobacter spp. and *P. vulgaris*	5	26.32	*Staph. albus*		
Klebsiella oxytoca	2	3.17	Citrobacter spp. and *Staph. aureus*	2	10.53			
Morganella morganii	2	3.17	*Kleb. oxytoca* and *Staph. aureus*	2	10.53			
Providencia rettgeri	3	4.76	*Kleb. oxytoca* and *P. vulgaris*	1	5.26			
Proteus mirabilis	4	6.35	*Proteus mirabilis* and *P. vulgaris*	1	5.26	Citrobacter spp. with *E. coli*, Actinomyces spp. and *Strep. viridans*	3	37.5
Proteus vulgaris	4	6.35	Strep. spp. and Actinomyces spp.	1	5.26			
Pseudomonas spp.	1	1.59						
Salmonella spp.	2	3.17						
Shigella dysynteriae	1	1.59						
Staphylococcus albus	1	1.59						
Staphylococcus aureus	6	9.52						
Streptococcus spp.	1	1.59						
Streptococcus viridans	2	3.17						
Total	63	99.99		19	100		8	100

4.5.1.3 Isolates from colon and "Sphincter Cap"

Swabs from colorectal mucosa and sphincter surface of the gharial hatchlings infected with Surahi flukes and having thickened mucosa were subjected to bacteriological culture. The *Citrobacter freundii* was isolated at highest percentage (30%), followed by *E. coli* (20%) and *Proteus vulgaris* (12.5%); Aeromonas spp. and *Srtreptococcus viridans* were isolated in equal magnitude (7.5%). The results are summarized in Table 14.

Table 14. Colon and "Sphincter Cap" isolates from 40 gharial hatchlings

Organisms	No. of isolates*	Percentage
Aeromonas spp.	3	7.50
Bacillus subtilis	1	2.50
Citrobacter freundii	12	30.00
E. coli	8	20.00
Klebsiella oxytoca	2	5.00
Morganella morganii	2	5.00
Proteus mirabilis	2	5.00
Proteus vulgaris	5	12.50
Providencia rettgeri	1	2.50
Pseudomonas spp.	1	2.50
Streptococcus viridans	3	7.50
Total	40	100.00

* Only hatchlings with specific lesion were cultured

4.5.2 Mycological examination

Skin scrapings from six dead hatchlings were subjected to mycological studies. Cultures revealed six different species of saprophytic fungus. The result suggested that the lesions were produced by opportunistic fungal pathogens. Out of 137 hatchlings examined grossly, 26 (18.98%) hatchlings had fungal lesions on the skin. The fungal isolates obtained from the culture of the skin scrapings are presented in Table 15.

Table 15. Fungal isolates obtained from skin scrapings of gharial hatchlings

Age of hatchlings (weeks)	Number	Fungus isolated
13	2	Curvularia spp., Rhizopus spp.
21	1	Trichoderma spp.
22	1	Penicillium spp.
25	1	Fusarium spp.
26	1	*Pseudallescheria boydii*

4.6 Histopathological examination

Histopathological samples constituted the liver, lungs, kidney, heart and spleen of 79 and intestine (a majority of colon sections) of 25 hatchlings. The frequency of particular lesion is presented in Table 16. One or more microscopic lesions were recorded from each hatchling. Respective lesions were photographed (Plate 29-34). The frequency of specific histopathological lesion is presented in Table 16.

4.6.1 Liver: There were fatty degeneration, haemorrhage, hemosiderosis and mononuclear cell infiltration. Coccidial parasites were also present.

4.6.2 Kidney: Hemosiderosis, mononuclear cell infiltration and haemorrhages were observed.

4.6.3 Lungs: The lungs were oedematous and haemorrhagic and interalveolar septa were thickened. Mononuclear cells were infiltrated in few lung samples.

4.6.4 Spleen: There was hemosiderosis and several spleen sections were found to be positive for coccidial parasites.

4.6.5 Intestine: The mucosa, submucosa and occasionally the tunica muscularis were having lesions with necrotic center surrounded by heterophils and coccidial parasites. The mucosal layer of the colon was found thickened.

Table 16. Major microscopic lesions observed in the liver, lung, spleen, kidney and intestinal sections of 79 gharial hatchlings

Microscopic lesions	Positive hatchlings	Percentage
Coccidial parasites in liver and spleen	46	58.22
Coccidia in colon mucosa*	17	68.00
Fatty degeneration in liver	22	27.85
Haemosiderosis	35	44.30
Focal mononuclear cell infiltration in liver, lung, heart or spleen	37	46.84
Oedema and haemorrhage in liver, lung, heart or kidney	41	51.80
Septal thickening in the lung	15	19.00

* Only 25 suspected hatchlings were sectioned

Most significant of the histopathological study was the finding of coccidial parasites in the liver and spleen. Among the lesions recorded, 58.22% of the hatchlings harboured coccidial parasites in their liver and spleen and 68% in the mucosal layer of the colon. The higher percentage of observation of coccidial parasites in the intestinal mucosa was because of processing of only suspected specimens. Coccidia were often massive in numbers, distributed evenly or in bunch particularly in the spleen. There was very little inflammatory reaction against these parasites. This finding is crucial in that massive coccidial infection occurred in the hatchlings and may have contributed to death in association with bacterial and trematode infection.

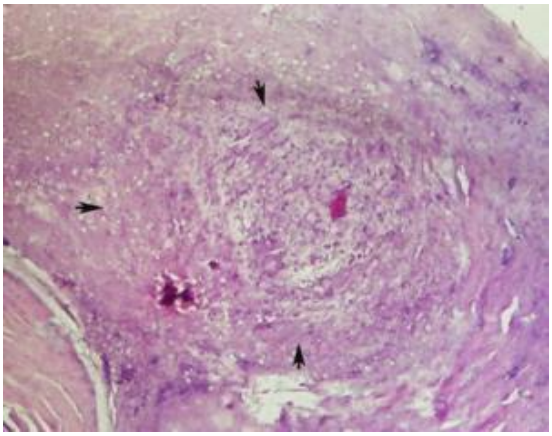

Plate 29. Abscess in the intestinal wall

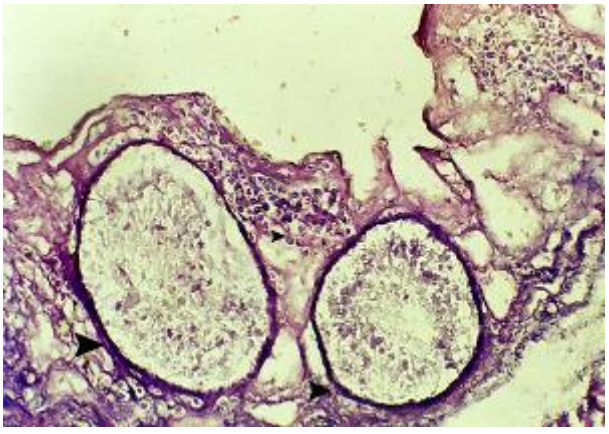

Plate 30. Abscess in the colon mucosa

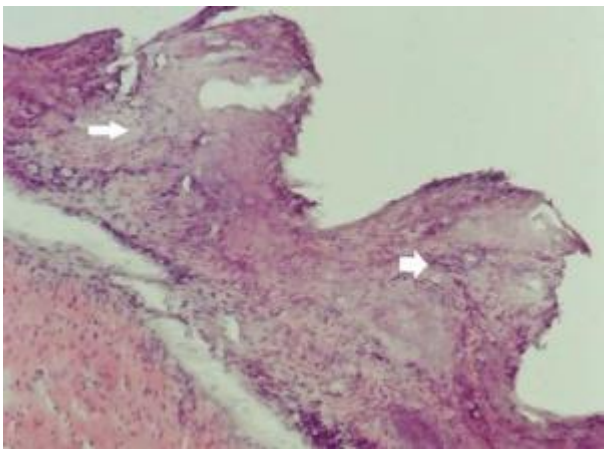

Plate 31. Thickened mucosa in colon

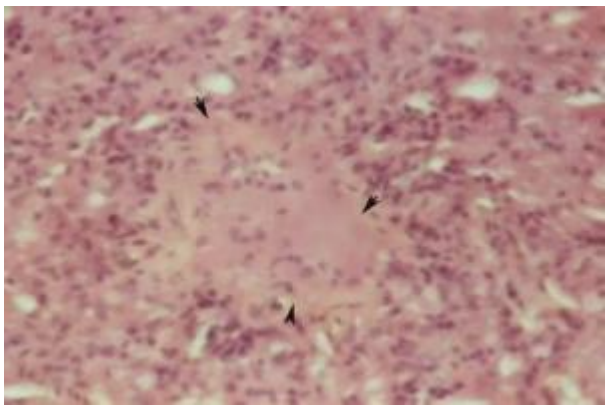

Plate 32. Dissolution of liver parenchyma

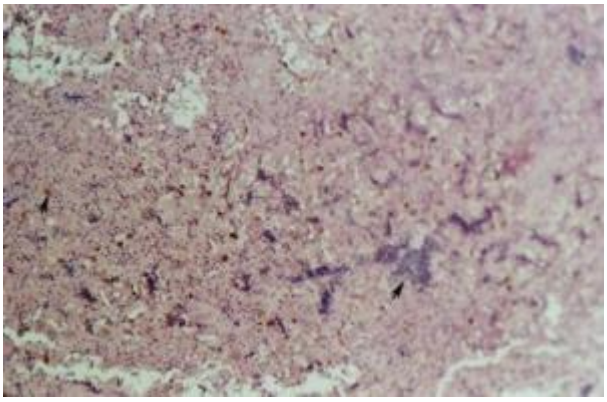

Plate 33. Hemosiderosis and infiltration

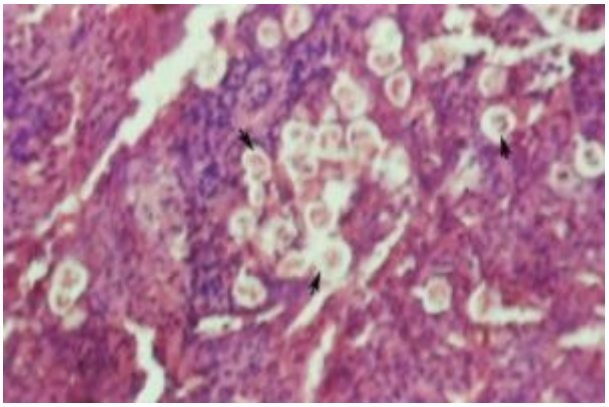

Plate 34. Coccidia in the spleen

5. DISCUSSION

Gharial hatchlings in captivity have been found with high mortality problems. This study aimed at finding the major causes of hatchling death in their first year of life. Data based on four batches of hatchlings revealed 2.79-57.05% hatchling survival rate with a clear indication of high mortality and variations.

Hatchling mortality rates of 97.2, 42.6, 76.9 and 61.5% in the year 2003, 2004, 2005 and 2006 respectively showed large variations. This could be due to the variations in the extent of congenital anomalies, environmental extremes precipitating a particular disease or due to the variation in the degree of care and management of hatchlings.

Monthwise mortality analysis revealed higher mean number of deaths in the month of August (37) followed by September (28), October (23), March (22) and April (18). The former three months simulates with Monsoon season and the later two months corresponds to a post hibernation period. However, mortality occurred in all the months irrespective of season, water quality and environmental temperatures. Lal (1982) also did not find any relation between the death pattern and variances in the season. Similar findings were reported by Maskey (1989) along with the arguments that poor water quality in Monsoon having significant role in hatchling death pattern where as an extreme humidity having role in high incidence of mycotic infections, but neither the previous studies nor the present one assessed the water quality of the hatchling pools.

Gharial hatchlings that died in July were ignored and thus the pathological conditions in the carcasses could not be ascertained. The cause of mortality in most of the hatchlings, if not all, in this age, included accidental pressing under breeding females, overcrowding resulting in piling up and suffocation, ant bites and occasionally killed by predators entering the pen through faulty door or the net. Hatchlings born with congenital anomalies also die within a week or two after hatching.

The data obtained suggest that the high temperature (mean temp. 34.7˚C) and rainfall in association with stress of handling for force feeding or feeding of proportionately large size fish (at least in some percentage of deaths) or feeding too often could be responsible for these deaths. There is also some evidence that frequent filling of the stomach reduces the digestive efficiency of the system (Webb *et al*., 1991). Hatchlings and adult crocodiles need at least 36 hours of interval after a meal to empty their stomach; even though, the daily feeding is in practice everywhere (Huchzermeyer, 2003 p113). Crocodiles adapt best in an optimal temperature which is between 28 and 33˚C. Internal temperature >35˚C are lethal and several bodily functions cease to function below 25˚C in crocodilians; smaller crocodiles are more likely to suffer from heat stress than larger ones as their smaller mass heats up more quickly and this overheating is a very serious source of stress (Huchzermeyer, 2003). Heat stress simultaneously causes depression of appetite in the hatchlings.

Retention of the fish in the oesophagus that occurred in hatchlings from August to January coincided with force-feeding. The condition found in 52.00, 61.76, 42.00 and 40.00% of hatchlings in August, September, October and November respectively cannot be assumed normal. The oesophagus in reptiles does not act as crop as in fowls or a proventriculus in an ostrich (Huchzermeyer, 2003); all the swallowed materials are passed in to the stomach. The presence of small or relatively larger fishes that were fed forcefully to the hatchlings definitely signifies a stressful condition resulting in suffocation or compression of heart or lungs and death. This particular condition has never been reported earlier as a factor contributing to the death of gharial

hatchlings. The hatchlings in Nepal and India are on whole fish food, not like minced meat and pellets in farmed crocodiles in other countries. Most findings on farmed conditions have implicated heat and cold as the stressors to hatchlings, anorexia being manifested below 25 and above 35ºC in crocodile hatchlings (Lloyd, 2003). Change of natural abode and feeding habits has been blamed for digestive upset leading to toxemia in older crocodiles (Sinha *et al*., 1987), but the effect on gharial hatchlings in captivity is not known. Similarly, the putrefaction of food in the oesophagus or stomach may have detrimental effect on body systems. The putrefied food from the digestive tract is regurgitated by larger crocodiles (Fowler, 1978); however, it is not exactly known whether this phenomenon occurs in younger hatchlings. Similarly, toxicological composition of putrefied fish either in the stomach or oesophagus and its effect on mortality could not be studied. Gharial hatchlings in an undiagnosed disease outbreak manifesting distended stomach, sluggishness, shivering and death in Kukrail (India) neither produced any etiologically important organisms nor clostridial toxins could be detected in the stomach contents (Lal, 1982). Present study was conducted only in dead hatchlings, their anti-mortem signs without record, so could not establish a relation between signs and postmortem findings. The mortality in these months may be related to handling stress while forced feeding, piling up and suffocation and stress due to excessive temperature.

Piling up, a normal reaction to fear, may have contributed to death by suffocation and or by permitting infectious agents into the tissues through scratches in the skin (Huchzermeyer, 2003).

Cutaneous fungal lesions in gharial hatchlings reported in this study were similar to the description of others (Lal, 1982; Maskey, 1989) in terms of predilection sites but abscence of systemic involvement concluded that lesions observed were secondary to other diseases. Further more, infection due to a particular fungus as an outbreak could not be proved. The prevalence rate reported by Maskey (1989) was fairly high, but the lower prevalence (19.70%) found at present study may be either due to improvements in the structures of hatchling pen or due to teeth cleaning practice or both. The lesions on the snout and teeth are expected to occur more in young hatchlings (two to six weeks of age); the carcasses of this age were not included in the study population.

Drawn in muscles particularly of the neck and tail base eventually were the results of poor energy supply triggering metabolization of subcutaneous and muscle fat depots. This condition is regarded as an indicator of prolonged anorexia caused by septicaemic conditions. In this study, 19.70% of the carcasses were found to have drawn in neck and tail base. Huchzermeyer (2003) has stated that cold, overheating, overcrowding or handling and disturbances act as stress inducing factors in which normally present non invasive bacteria invade the blood circulation and various organs, seriously affecting growth performance and survival of crocodile hatchlings.

Yolk sac of the newly hatched crocodiles is resorbed probably with in three to four weeks under normal circumstances (Huchzermeyer, 2003). Hatchlings if not eating well after this, develop hypoglycemia; pronounced appetite depression results in emaciation but long time may elapse for the hatchlings to die because of the low metabolic requirements. Hypoproteinemia and ascites may be the consequences of appette depression in the hatchlings. The ascitic condition may have arisen either from hypoprotenemia or from a chronic septicaemic condition resulting into prolonged anorexia or from liver disorders. Out of ten septicaemic *C. porosus* hatchlings, two with excess serous fluid in body cavities were reported by Ladds and Sims (1990). Ascitic condition in hatchlings in this study was quite higher indicating the suffering of the hatchlings with septicaemia or exposed to primary or secondary (prolonged anorexia) nutritional deficiencies.

Low rearing temperatures or infections has been found responsible for the retention of yolk sac in crocodilian hatchlings (Huchzermeyer, 2003). The environmental temperature in Chitwan during June-August (hatching period) is not less than 24ºC. Thus, this condition must have arisen due to infection of the yolk sac due to contamination of the egg and subsequent infection of the embryonic membranes before hatching. Other potential sources of yolk sac infection include unhealed navels allowing entry of environmental pathogens or patent vitelline canal allowing invasion by intestinal bacteria. Infection of yolk sac eventually causes the vitelline canal to close preventing flow, digestion and absorption of yolk resulting in its retention and malnutrition in hatchlings. The bacteria present in the yolk sac, may also precipitate septicaemia under certain conditions. Most retained yolk sac were cream colored, hard and bizarre in shape and of varying size similar to the fibriscess described by Huchzermeyer and Cooper (2000).

Gharial hatchlings having crossed snouts (the upper jaw crossing over the lower jaw and projecting to side) and some with short upper snouts with the exposition of nares have also been described in earlier reports (Maskey, 1989; Youngprapakorn *et al*., 1994). The defect is not much conspicuous during hatch but pronounced about a week after hatching; the deformity eventually leads to starvation and death. Hatchlings with snout defects in the study population survived about six to nine months because of hand feeding. In this study two hatchlings had snout deformity similar to the one described by Maskey (1989) and the other one had half shorter lower jaw than the upper one.

Death of gharial hatchlings due to prolapse of the vent reported as prolapsed rectum by Maskey (1989) were found in this study in a small percentage of hatchlings. The reason behind death due to this problem was bacterial invasion through the prolapsed organ, discomfort and related stress. Pseudomonas spp. from the kidney of one and the *Proteus mirabilis* from the liver of the other hatchling having prolapsed vent were isolated.

Hatchlings were thoroughly examined for enteritis but acute conditions were never found. Diarrhoea also could not be noticed, however, intestinal lumen with fibrinous deposits of flaky or mucoid consistency, dry, dirty or reddish contents were commonly found. The reporting of enteritis in a lower percentage was certainly due to our limited experiences with gharial pathology.

Competitive exclusion in crocodilian hatchlings prevents the entry of pathogenic organisms into the internal organs from the intestine (Huchzermeyer, 2003). However, stresses by reducing their resistance and immunity may lead to enteritis, where fibrin exudation into the lumen is copious. Such reptilian inflammatory process occludes the intestinal lumen, inhibits diarrhoeic signs and consequently produces anorexia, weight loss and emaciation. Among five types of enteritis described by Huchzermeyer (2003), only three types (exudative, nodular and haemorrhagic) were noticed in gharial hatchlings. However, the prevalence rate reported here could certainly increase if chronic cases would not have masked the acute form of enteritis. Exudative enteritis in hatchlings were not much manifested by distention of the abdomen or intestinal lumen where as nodular form of chronic enteritis were observed with nodular lesions seen externally as if the lesion were in the serosal layer. Gram-positive cocci were found in Gram's staining of the impression smear from the lesions but coccidial parasites were also found in histological sections.

Carcasses having reddish tinge on the skin of the ventral area of the belly and the chest, haemorragic costal musculature, engorgement of the splanchnic vessels, hyperemia of the intestinal serosa and pleural membranes were regarded as suffering from septicaemic conditions.

Many researchers are in the opinion that bacteria isolation from a crocodile should not be regarded as solely responsible for disease or death, because all the crocodile specific pathogens are opportunists, waiting for a weakened or stressed animal to produce the specific disease. Septicaemia is often produced by the fact that crocodilians are devoid of lymph nodes (Huchzermeyer, 2003). Crocodiles with septicaemic lesions due to intestinal bacteria have been reported suffering from severe stress (Huchzermeyer, 2000; Huchzermeyer and Cooper, 2000). This study also cannot exclude the probability of involvement of intestinal bacteria in causing septicemic death in gharial hatchlings due in period of stress.

Pseudomonas aeruginosa (Mishra *et al*., 1996; Mehrotra *et al*., 2000) and Clostridium spp. (Mishra *et al*., 1993) caused massive deaths of gharial hatchlings and *E. coli* (Sinha *et al*., 1988) was associated with septicemic conditions in mugger crocodiles even though such bacteria have been isolated from apparently healthy gharial hatchlings (Mishra *et al*., 1993); Gram negative rods including Salmonella spp., Proteus spp., *E. coli*, *Providencia rettgeri*, *Morganella morganii*, *Serratia marcescens* and *Aeromonas hydrophila* have been isolated from crocodilians and are implicated as responsible for diseases and death in many instances.

Citrobacter spp. has been isolated from captive Nile crocodile and from the faeces of a *Caiman crocodylus* (Roggendorf and Muller, 1976). *Citrobacter freundii* has been found associated with septicemia in American alligators (Novak and Seigel, 1986) and *C. koseri* has also caused meningitis in newborn human babies (Gross *et al*., 1973; Ross, 1979).

Aeromonas hydrophila is one of the most common bacteria associated with the aquatic environment and has caused skin lesions and septicaemia in a Nile crocodile (*C. niloticus*), yielding pure cultures from skin, internal organs and blood (Turutoglu *et al*., 2005). It was reported earlier as the sole agent but later reports showed Proteus spp., *Morganella morganii*, *Serratia marcescens* and *Klebsiella oxytoca* equally responsible for septicaemic lesions in crocodiles. It is emerging as a potential pathogen for the immunocompromized host (Chang *et al*., 1997). Various aeromonal infections, including septicaemia, have also been reported in apparently healthy individuals; the septicaemic course is often fulminant and fatal and may lack an obvious focus.

Morganella morganii is an opportunistic secondary invader originally thought responsible for summer diarrhoea in humans. Several reports implicate this organism for causing septicaemia and abscess in the brain and ovary in neonates. It had been found in a case of chorioamninonitis and meningitis in an immunocompromized patient. It has been an important cause of nosocomial infection, though it was regarded as a relatively unimportant human pathogen in the past. *Morganella morganii* has been found in cases of septic arthritis of African dwarf crocodiles (Heard *et al*., 1988) and was also isolated from juvenile *Crocodylus porosus* (Hibberd *et al*., 1996).

Providencia rettgeri infection have caused neurological disorders (swaying, swimming in circle and head tilting) and death in *Crocodylus porosus* (Ladds *et al*., 1996) and American alligator (Camus and Hawke, 2002) hatchlings in association with overcrowding or severe temperature stress respectively. The neurological disorders and death in gharial hatchlings described by Maskey (1989) seems to have caused by this species of bacteria. The frequency of infection and death of gharial hatchlings due to this organism was almost similar (2.5-2.7%) with the reports of Ladds *et al.* (1996) in *Crocodylus porosus* hatchlings. Providencia spp. has also been recovered from human urine, throat, faeces, blood and wound specimens (O'Hara *et al*., 2000).

Proteus mirabilis and *P*. *vulgaris* have been isolated from captive Nile crocodile and there are reports of septicemia in crocodiles due to these organisms. *Proteus mirabilis* has also been implicated in bacteremia, meingo-encephalitis, empyema and osteomylitis particularly in very young babies (O'Hara *et al*., 2000).

Klebsiella pneumoniae and *K. oxytoca* are opportunistic pathogens found in the environment and in mammalian mucosal surfaces. They can infect neonates having impaired respiratory host defenses and produce septicaemia.

Escherichia coli form a part of normal intestinal flora and it is the predominant bacteria responsible for urinary tract infections, neonatal meningitis, gastroenteritis and septicemia in man and animals (Chakraborty, 1995). Poor hygiene, intensive husbandry practices and younger age are common predisposing factors for *E. coli* infection in animals (Quinn *et al*., 1994). *E. coli* was found responsible for septicaemic death in gharial hatchlings (Mehrotra *et al*., 2000) and it was also recovered from severely stressed African dwarf crocodiles (Huchzermeyer and Agnagna, 1994). In this study *E. coli* comprised 12.16% among all the isolated bacteria from the internal organs of the hatchlings where as isolates from the culture of colon swabs reached 30%.

Staphylococcus is ubiquitous organism primarily found on mammalian skin and mucosal surfaces. *Staphylococcus aureus* is associated with most supporative lesions; however, it is the common cause of bacteremia, the infection reaching the blood through lungs, gastrointestinal tract, urinary tract and skin abrasions.

Mihsra *et al*. (1993) thought Clostridium spp. responsible for death of gharial hatchlings as they isolated it from the oedematous fluid of swollen limbs but such swelling in the carcassess were not observed in this study. Actinomyces spp. isolated from gharial hatchlings at present study are probably normal flora of oropharynx or gastrointestinal tract.

Several bacterial species from apparantly healthy gharials (Mishra *et al*., 1993) and wild caught African dwarf crocodiles (Huchzermeyer and Agnagna, 1994) have been isolated but the opportunistic bacteria can cause serious infections in reptiles under stress condition (Ebani and Fratini, 2005). Because of this fact, bacteria isolated from the gharial hatchlings at present study may be regarded as the evidences of the mortalities.

The number of skin scrapings of the fungal lesions that were cultured and identified in this study was very few but it revealed that all of them were involved only externally. Four species of fungi that were identified in this study were also reported to occur as oral flora of American alligator and intestinal flora of African dwarf crocodiles (Huchzermeyer, 2003) and two species of fungi isolated in this study are probably new records from gharial skin lesions. Extensive hatchling mortality due to fungal infections (over 50%) as described by Hibberd and Harrower (1993) in *C. porosus* was not recorded in this study. Similarly, systemic involvement in other species of crocodiles as reported by others (Fromtling *et al*., 1979; Frelier *et al*., 1985; Maslen *et al*., 1988; Hibberd and Harrower, 1993; Hibberd *et al*., 1996; Thomas *et al*., 2002) were not recorded both on gross and microscopic examination of the carcassess and tissue sections. Therefore, it can be concluded that all the isolated fungus were opportunistic pathogens and invasion was secondary to stress or debilitating diseases similar to the report of Migaki *et al*. (1984).

Gross examination of the carcassess did not reveal any lesions of mycobacteriosis (pale protruding nodules of uniform caseous consistency in the liver, lungs and kidney) which were earlier

described by Ariel *et al*. (1997a) in young fresh water crocodiles (*C*. *johnstoni*). Similarly, lesions of gout were not found in any of the hatchlings at present study but it was reported by Ariel *et al*. (1997b) in 10 month old *C*. *jonstoni* and *C*. *porosus* hatchlings and vitamin A deficiency was suspected as etiological factor. Cases of visceral gout in farmed *C*. *porosus* were also described by Ladds *et al*. (1995).

Coccidiosis, pentastomiasis, bacterial septicaemia and visceral gout were found as major cause of death in *C*. *novaeguineae* and *C*. *porosus* hatchlings ranging 44-136 cm in length and 140-4800g in weight in a study carried out by Ladds (1995) where as coccidiosis, bacterial septicaemia with gram-negative organisms, ascariasis and pentastomiasis were reported as major causes of death in *C*. *novaeguineae* and *C*. *porosus* having a mean length of 522 mm and weight of 284g in another study carried out by Ladds and Sims (1990). However, gharial hatchlings examined in this study (35-53 cm in length and 66-253g in weight) did not reveal any pentastomid and ascarid parasites in the lungs and gastrointestinal tract respectively. Likewise, gouty lesions both visceral and renal were completly abscent.

Two trematodes namely Exotidendrium spp. (Surahi fluke) and Proctocaecum sp. were found to cause significant lesions in gharial hatchlings. The former was found in the colorectum in huge numbers (100-300/animal) and the later in the duodenum. The Exotidendrium spp. probably causes certain degree of irritation to the mucosa; this was evidenced by certain amount of white or pinkish mucus in the lumen and fibrinoid whitish deposits sometimes with formation of tiny whitish balls. Most unique finding in gharial hatchlings was the “Sphincter Cap” formed arround the colorectal orifice. The trematode, in association with bacterial infections, probably have role on the formation of “Sphincter Cap” since the fluke were also found in the "Sphincter Cap" debris in heavy numbers. Mehra (1935) collected this fluke from rectum of gharial hatchlings in high numbers and described this trematode as *Exotidendrium gharialii* but this study assumed that more than one and different from the one described earlier by Mehra (1935) and Srivastava (1981) occurred. Prevalence rate of more than 25%, size and shape, capability to differ due to great extensions and contraction potential of the anterior part, armed cuticles and the structure and position of internal organs matches with the Exotidendrium spp. These flukes probably open an avenue for the penetration of Gram-negative rods into the tissues and blood stream causing septicaemia.

Considering Mehra’s description, the Surahi fluke just matched with the genus Exotidendrium. The parasite on our part was examined from dead and frozen gharial hatchlings where we did not see any contracted forms rather we found only fully extended forms. The description with regard to the ova as numerous, fairly thick shelled, yellow brown in colour and operculated with a fine filament at the non-operculated end perfectly matches with our observation. Mehra has not given any common name to this parasite; however, we named it as “Surahi fluke”. These flukes were also reported in the Nile crocodile (*C. niloticus*) from Madagascar and Southern Africa. Shrivastava (1981) has erected a separate family Exotidendriidae to accommodate the genus Exotidendrium based on certain morphological characters. However, both the authors did not describe any lesions in the host and this seems to be the first report on their pathogenicity to young gharial hatchlings of Nepal.

The colon in reptiles is believed to function as an organ for conservation of water by resorbing the later from the fecal masses (Wallach, 1971). Deposition of fibrin and mucus due to huge number of tiny and spiny trematodes in the lumen certainly reduces the absorptive capacity of the colon leading to dehydration and death of the gharial hatchlings.

Gharial hatchlings in Chitwan have high prevalence of Exotidendrium spp. and concurrent infections with Gram-negative bacteria may be responsible for high mortality. The relationship between heavy parasitism, bacterial infection and mortality may be similar to the systemic illness and death of Green sea turtles (*Chelonia mydas*) infected with higher number of spirorchid cardiovascular flukes, other internal parasites and simultaneous infections of Salmonella, *Escherichia coli* and other gram negative bacteria (Radial *et al*., 1998).

Fibrinecrotic colitis was observed repeatedly in gharial hatchlings mostly associated with the presence of hundreds of Surahi fluke (Exotidendrium spp.) and clear, pinkish or dirty mucus. Bacterial culture from these lesion produced members of Enterobacteriaceae. However, in two hatchlings, enlargement and shortening of the duodenum and the gross thickening of the mucosa at the opening of the bile duct into the duodenum was observed due to the effect of Proctocaecum sp. The pathogenicity of Paramphistomum-like sp. to gharial hatchlings was not established in this study.

Cestodes have not been found in crocodilians though they are abundant in other reptiles; this is probably a reason for the lack of published reports of adult tapeworms in them. A very low gastric pH of crocodiles has been suggested for not having adult tape worms (Huchzermeyer, 2003). However, present study shows that intermediate stages of cestodes occurrs in gharial hatchlings. The plerocercoids when straightened after removing the encasing measured 5.33 x 0.73 mm with two pairs of sickle shaped rostral hooks. Proglottids ended in a round tip. However, cestode larvae detected in the internal body membranes in this study could not be blamed for hatchling deaths owing to their minute gross size which did not had effect such as pressure necrosis on organs immediate to them.The abscess like minute protrusions on the intestinal wall which were visible from outside as if they were located inside the serosa were similar to the cases reported as nodular enteritis by Huchzermeyer (2003).

Hemogregarine infection in gharial hatchlings were not studied, however, Ladds and Sims (1990) reported it to occur in 64% of crocodile hatchlings in Irian Jaya and was reported to occur in alligators, gharial and mugger crocodiles. The findings of this study support the findings of Ladds and Sims (1990) that massive infections of internal parasites in young and stressed hatchlings concurrently having other diseases certainly contribute to mortality.

Fatty degeneration in the liver was highly prevalent among liver sections. The lesions probably can be related to chronic anorexia. Disease and environmental stressors may have caused prolonged anorexia leading to fatty degeneration of the hepatocytes. Focal infiltration of liver, lungs and kidney tissues by mononuclear cells is associated with bacterial infections. Detection of massive number of coccidial parasites displacing the paranchymal tissues in liver and spleen by this study alerts that gharials suffer a great deal from coccidiosis. A high percentage of liver sections with golden brown pigments also indicate that hatchlings have suffered from intravascular hemorrhage probably caused by coccidial proliferation. The necrotic lesions in the wall of the colorectum were probably associated with Surahi fluke, coccidia and secondary bacterial invasions.

The present finding is similar to the findings of Ladds and Sims (1990) in *C. novaeguineae* and *C. porosus* with regards to coccidial infection in spleen but in gharial hatchlings coccidial infection also occurred in liver and intestinal mucosa and a few unsporulated oocysts were recovered in faecal examinations. Gouty lesions, blood fluke and pentastomes (Riley and Huchzermeyer, 1995; 2000) were conspicuous findings in their study; where as such lesions and parasites were absent in

the gharial hatchlings. The causes of death of gharial hatchlings in Chitwan were found multifactorial in contrary to multiple parasitisms in Irian Jaya in Indonesia.

Huchzermeyer (Personal communication, 2006) states that stress are the most important agent in diseases of captive reared crocodilians while most of the bacterial infections are only secondary to stress. It is therefore most important to determine the behavioural requirements of the hatchlings with regard to space, cover, heat and disturbances. Obviously nutrition also plays an important role, particularly the addition of good vitamin and mineral supplements in their diet are equally important.

6. SUMMARY AND CONCLUSION

The mortality rate of captive gharial hatchlings during the first nine months after hatching was found very high. This study investigated the causes responsible for these deaths and was conducted to evaluate the involvement of bacteria, fungus and parasitic agents in causing high mortality of gharial hatchlings with the aim that their viability in captivity could be increased.

One hundred and thirty seven captive gharial hatchlings (*Gavialis gangeticus*) that were 8-42 weeks old, hatched in June 15-28, 2006 at Gharial Breeding Center (GBC) of the Chitwan National Park comprised the study population. The study required a period of nine months- i.e. August 2006–April, 2007.

Retrospective mortality data analysis of four years revealed the mean hatchling death number for every month after hatching. The highest percentage of mortality in 2006 was found to have occurred in the six to ten week old hatchlings (17.56%). Monthly mean weight and length of the dead hatchlings were also obtained, which was 88.25g and 42.21 cm respectively in the August where as it was 111.27g and 47.55 cm respectively in the month of April.

The hatchlings subjected to general examination during necropsy revealed twelve gross pathological conditions in them. Of the studied population, retention of fish in the oesophagus (37.95%), septicaemic lesions (27%), drawn in neck and tail muscles (19.70%), fungal lesion on skin (18.97%), ascites (17.51%), retention of yolk sac (10.94%), enteritis (24%), anemic visceral organs (21%), cysts in the serous membranes (8.76%), nodular lesions in the intestinal wall (6.60%), snout deformities (2.18%) and prolapse of the vent (1.46%) were notable.

Bacteriological culture from four organs of 102 dead hatchlings revealed 15 genera of organisms and the most notable was Citrobacter spp. (32.43%), *E. coli* (12.16%) and Proteus spp. (12.16%). Infections among organs were found highest (25%) in the hatchlings that died in the month of September and lowest (3.38%) in December. The highest infections (42.57%) were found in the lungs and least infection (14.86%) in the heart blood. The highest infection (75%) was found in the 30-34 weeks old hatchlings where as the lowest (20.83%) in the 22-26 weeks old hatchlings. Infections with single species of bacteria were found in 70% of the hatchlings followed by two species (21.11%) and by three or more spp. (8.89%). Among the bacterial species, the *Citrobacter freundii* (33.33%) and *E. coli* (12.70%) were predominant among single bacterial infections where as Citrobacter and *Proteus vulgaris* infections (26.32%) dominated among the mixed infections. Combined infections of *E. coli* & Salmonella; Citrobacter & Salmonella; Citrobacter & *Staphylococcus aureus*; and *Klebsiella oxytoca* & *Staph. aureus* were equal in magnitude (10.53%). It was also noted that 11.76% of the dead hatchlings were negative in organ cultures.

Hatchlings infected by "Surahi fluke" (Exotidendrium spp.) and having fibrinous deposits in the mucosa of the colorectum either with or without distinct “Sphincter Cap” (40 hatchlings) were subjected to microbiological studies. It resulted in the isolation of eight genera of Gram-negative rods and two genera of Gram-positive organisms. In this study, Citrobacter spp. (30%) dominated the spectrum followed by *E. coli* (20%) and *Proteus vulgaris* (12.5%).

Lesions of fungal infections in the skin were recorded in 18.9% of the hatchlings. Of these, skin scrapings from 23.07% hatchlings were subjected to mycological studies. Six different species of fungus were isolated indicating that no particular species of fungus is involved. All of these were saprophytic one and infection was the result of a consequence of immuno-suppression in the hatchlings.

Parasitological studies revealed that several trematode species (Paramphistomum- like species, Pseudoneodiplostomum spp., Proctocaecum sp. and Exotidendrium spp.) infected the gharial hatchlings. The fecal examination of 137 hatchlings revealed the occurrence of Paramphistomum-like ova in the stomach contents of 29.92% hatchlings; however, the ova of other recorded species were absent in the faecal matter. A characteristic trematode “Surahi fluke” (Exotidendrium spp.) was recorded in 40.95% of the hatchlings dead over the period of nine months. Their number in the colorectum were very high (ranging 100-300/animal) and were associated with colitis and a characteristic black, white, greenish or yellowish green granulomatous scab formed around the colo-rectal orifice opening into the cloaca. A typical finding was that hatchlings that died November onwards had this fluke in all of them (100% prevalence). The typical trematode has been given a Nepali name “Surahi fluke” and the distinct lesion observed was named a "Sphincter Cap”. It is concluded that this parasite irritates the mucosal layer and incites the production of mucoid mass as well as opens an avenue for the *Citrobacter freundii*, *E. coli* and other members of Enterobacteriaecae normally present in the intestine to enter the blood stream and cause septicemia and death of the hatchlings.

Concurrent with the detection of trematode eggs in faecal examination, coccidial oocysts were observed in 21.90% of the hatchlings.

A fileroid parasite (Micropleura spp.) was found in only one hatchling. A single type of nematode egg was found in 12.40% of the dead hatchlings, but the adult could not be found and the species could not be identified on the basis of the eggs.

Histopathological examination of the nodular lesion of the intestine revealled an abscess in the submucosal layer indicated by heterophilic infiltration, loss of cells, accumulation of tissue debris and coccidial parasites. Coccidial species were found in 58.22% and 68% of the liver and spleen and colon sections respectively. It indicated the occurrence of this protozoon in gharial tissues. Oedema and thickening of alveolar septa were found in the lung sections. A considerable number of liver sections were presented with fatty degeneration and hemosiderosis.

Present findings were unique to indicate that gharial hatchlings suffer a number of health problems caused by bacteria, fungi, coccidia and trematodes. There were number of hatchlings in which the involvement of these agents were not found, thus a substantial number of hatchlings were assumed to have died from extreme environmental, managemental and immunosuppressive factors. Extreme heat, cold or other stress factors are regarded as primary factors triggering the proliferation of bacteria, fungus and parasites resulting into the death of the hatchlings.

Other microbial agents involved as pathogens in several other crocodilian species particularly Mycobacteria, Mycoplasma and Chlamydae could not be examined at present study which deserves a substantial attention in future research.

7. RECOMMENDATIONS

The near extinction of wild gharial population from habitat rivers of India and Nepal was solely prevented by captive rearing and release programs implemented since 1970s. Hadn't these ideas developed and implemented in time, the species would have been lost forever by now. Therefore, the marvelous idea and effort of the scientists, which initiated the captive rearing and release programs for saving the species from extinction, was highly appreciable. Furthermore, the following recommendations are added to the list of previous researchers as a crucial tool to increase the viability of captive hatchlings and to foster release population:

1. Feeding of gharials with locally collected fishes from Rapti river probably cannot be avoided in GBC facilities; but fishes can be frozen for 72 hours to kill the parasitic larvae before feeding (Foggin, 1992). Reliable sources of fish and fish fries for hatchlings must be established rather than depending on fish catching in the river and streams.
2. The microbial quality of the water supplied to the hatchling pool should be assessed and use of underground water particularly for hatchlings is recommended. The hatchling pool must maintain a high standard of sanitation since gharial hatchlings have been found affected by microbial load in it. Environmental, physical and other stress factors should always be minimized.
3. CNP should be able to issue permits to researchers to use some of the normal, sick and dead hatchlings for recording normal physiological parameters, though gharial is an endangered and protected species. Periodic examination and treatment attempts may be beneficial.
4. Further research on gharial health and husbandry is recommended.

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APPENDICES

Appendix I

Postmortem examination report sheet for gharial hatchlings 2006-2007

Case No. Date of death:

Weight: (gm.) Date of examination:

Length: (cm.) Photographic record no.

External Examination

Body condition	poor	perfect	moderate	
Drawn in muscles of neck and tail	yes	no		
External fungal lesions	yes	no		
Skeletal deformities	yes	no		
External parasites	yes	no		
Pinkish abdominal area	yes	no		
Prolapse of the vent	yes	no		
Eye lesions	yes	no		
Fight injuries	yes	no		
Joint lesions	yes	no		

Internal Examination

Lungs and trachea	Congestion	yes	no	
	Nodules	yes	no	
	Parasites	yes	no	
Liver	Abscess	yes	no	
	Hemorrhage	pin point	massive	
Gallbladder	Color: specify			
	Status	full	empty	partially
Kidney	Color	dark red	whitish	pink
	Congestion	yes	no	
	Abscess	yes	No	
	Parasites	yes	no	
Heart	Color	red	dark red	
Pericardial fluid	Excess fluid	yes	No	
	Hemorrhagic	yes	No	
Spleen	Color	black	pink	
Oesophagus	Mucus	yes	no	
	Intact fish	yes	no	
	Regurgitated mass	yes	no	
Stomach	Sand	yes	no	
	Ulcers	yes	no	
	Parasites	yes	no	
	Food and mucus	yes	no	
	Reddish look externally	yes	no	
Intestine	Reddish look	yes	no	
	Contents	full	empty	
	Color of contents	bloody	black	
	Fibrin deposits on mucosa	yes	no	
	Parasites	yes	no	
	Abscess in the wall	yes	no	

	"Sphincter Cap"			yes	no	
	Yolk sac			absorbed	retained	
Ventral abdominal muscles	Haemorrhagic			yes	no	
Pleural and peritoneal membranes	Cysts			yes	no	
Body cavities	Excess fluid			yes	no	
	Engorgement of vessels			yes	no	
	Hemorrhage			yes	no	
	Parasites			yes	no	
Cloaca	Distention			yes	no	
	Samples					
Tissues for histopathology	lung	liver	heart	kidney	spleen	intestine
Samples for microbiology	lung	liver	heart	kidney	spleen	intestine
Samples for parasitology	Stomach contents			+ve	-ve	ova
	Intestinal contents			+ve	-ve	ova
	Type			trem.	nem.	other
	Drawings					
Skin scrapings				yes	no	
Skin scrapings for culture				yes	no	
Adult parasites	Collected			not collected		
	Identified?			yes	no	
	Genus			species		

Describe remarkable lesion in detail:

Tentative diagnosis: ----------
Comments----------
Laboratory findings: ----------
Confirmatory diagnosis: ----------

Signature
Date:

Appendix II

General pathological conditions observed in dead gharial hatchlings

Month / Pathological. conditions	Aug (46)	Sept (34)	Oct (7)	Nov (5)	Dec (6)	Jan (14)	Feb (4)	Mar (15)	Apr (6)	Total Cases
Fungal lesion on skin	6	8	2	3	1	6	0	0	0	26
Drawn in muscles	1	4	2	3	1	5	0	7	4	27
Retention of fish in oesophagus	24	21	3	2	0	2	0	0	0	52
Retention of yolk sac	11	1	0	0	0	0	1	2	0	15
Ascites	6	7	2	0	3	4	1	0	1	24
Cysts in body membranes	0	0	2	1	0	3	2	2	2	12
Abscess in intestinal wall	0	1	0	1	0	2	0	3	2	9
Vent prolapse	0	0	0	0	0	1	0	1	0	2
Snout deformity	0	0	0	0	0	1	1	1	0	3
Septicaemic lesions	11	6	2	2	3	4	2	5	2	37
Enteritis	5	8	4	2	1	4	0	4	1	33
Anaemic internal organs	5	2	1	2	2	11	2	7	1	29

Figure in parentheses indicates number of hatchlings examined

Appendix III

Parasites: Eggs, adults and related lesions in dead gharial hatchlings

Months Parasites and lesions▼	Aug (46)	Sept (34)	Oct (7)	Nov (5)	Dec (6)	Jan (14)	Feb (4)	Mar (15)	Apr (6)	Total Cases
Helminth eggs and coccidial oocysts										
Trematode egg	1	5	2	2	3	7	4	13	4	41
Nematode egg	4	2	0	1	2	2	1	4	1	17
Coccidial oocysts	5	11	2	0	2	2	2	5	1	30
Adult nematodes										
Micropleura spp.♠	0	1	0	0	0	0	0	0	0	1
Adult trematodes										
Pseudoneodiplostom. spp.♣	0	0	0	0	0	0	0	1	0	1
Paramphistomum like (UI)	0	1	0	0	0	1	0	1	0	3
Proctocaecum spp.♦	0	0	0	0	1	0	0	5	2	8
Surahi (Exotidendrium spp.)*										
Immature forms	20	11	0	0	0	0	0	0	0	31
Mature forms	0	0	0	2	5	14	4	15	6	46
Larval cestodes	0	0	2	1	0	3	2	2	2	12
Lesions										
Distinct "Sphincter Cap"	0	0	0	2	5	8	2	6	2	25

Figure in parentheses indicate number of hatchlings examined. *Only 94 hatchlings were examined ♣Digenea: *Proterodiplostomatidae* ♦Digenea:*Cryptogonimidae* *Digenea: *Exotidendriidae* ♠Nematoda: Micropleurinae UI= unidentified

Appendix IV

Frequency of bacteria isolated from 102 gharial hatchlings

Bacterial isolates	Month									Total
	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	
Actinomyces spp.	2	0	0	0	0	0	0	2	0	4
Aeromonas spp.	0	0	0	0	0	2	0	0	0	2
Bacillus subtilis	0	0	1	0	0	0	0	0	1	2
Citrobacter freundii	4	12	1	4	1	11	8	6	1	48
Clostridium spp.	0	0	0	0	1	0	1	0	0	2
E. coli	5	7	1	0	1	1	1	1	1	18
Klebsiella oxytoca	1	4	2	0	1	1	0	2	0	11
Klebsiella pneumoniae	0	0	1	0	0	0	0	0	0	1
Morganella morganii	0	1	0	0	0	2	2	1	0	6
Proteus mirabilis	2	2	0	0	0	0	0	3	0	7
Proteus vulgaris	3	4	1	1	0	0	0	4	1	14
Providencia rettgeri	2	0	0	0	0	1	0	0	1	4
Pseudomonas spp.	0	0	0	0	0	1	0	0	0	1
Salmonella paratyphi A	0	1	1	0	0	0	0	0	0	2
Salmonella paratyphi B	0	0	0	1	0	0	0	0	0	1
Salmonella typhimureum	0	0	0	1	0	0	0	0	0	1

Salmonella spp.	0	0	0	0	0	0	0	0	1	1
Shigella dysenteriae	0	0	1	0	0	0	0	0	0	1
Staphylococcus albus	2	2	1	1	0	0	0	1	0	7
Staphylococcus aureus	3	3	0	1	1	1	0	1	0	10
Streptococcus β-hemolytic	0	0	0	0	0	1	0	0	0	1
Streptococcus viridans	1	1	1	0	0	0	0	1	0	4
Total	25	37	11	9	5	21	12	22	6	148
No growth	4	8	0	4	4	4	0	20	4	48
Grand total	29	45	11	13	9	25	12	42	10	196

Appindix V

Colon and "Sphincter Cap" isolates of 40 gharial hatchlings

Organisms	Months						Total
	Nov (2)	Dec (5)	Jan (12)	Feb (4)	Mar (15)	Apr (2)	
Aeromonas spp.	0	0	1	2	0	0	3
Bacillus subtilis	0	0	0	0	1	0	1
Citrobacter freundii	1	3	3	2	2	1	12
E. coli	0	0	1	0	6	1	8
Klebsiella oxytoca	1	0	0	0	1	0	2
Morganella morganii	0	0	2	0	0	0	2
Proteus mirabilis	0	0	0	0	2	0	2
Proteus vulgaris	0	1	3	0	1	0	5
Providencia rettgeri	0	0	0	0	1	0	1
Pseudomonas spp.	0	0	1	0	0	0	1
Streptococcus viridans	0	1	1	0	1	0	3

Figure in parentheses indicates number of samples taken for culture. Only hatchlings with specific lesion were cultured.

Appendix VI

Hatchling death numbers (July-April) of four consecutive years

Month	Years			
	2003	2004	2005	2006
July	4	10	11	11
August	31	12	58	46
September	38	12	29	34
October	51	9	13	20
November	29	2	20	5
December	7	11	7	6
January	1	6	14	14
February	5	22	18	4
March	5	12	56	15
April	3	32	30	6
Total dead (no.)	174	128	256	161
Obtained (no.)	179	298	333	262
Survived (no.)	5	170	77	101

BIOGRAPHICAL SKETCH

The author was born in Sabhung Bhagawatipur VDC Ward No. 2, Gairabari, Tanahun as the third son of Mrs. Nanda Kumari and late Mr. Bama Dev Gairhe on October 11, 1960. He grew in a rural environment getting primary education in Shree Sharada Primary School, Jana Jyoti Lower Secondary School and Meen Vocational High School at home district. He passed Certificate level (Animal science) in 1978-79 from IAAS and served as a school teacher for a couple of years.

He is a graduate in Veterinary Science from Bangladesh Agricultural University; was appointed Veterinary Officer in 1988 and is still working in the same capacity at Chitwan National Park under the Department of National Parks and Wildlife, Ministry of Forests and Soil Conservation, Nepal.

He is devoted to wildlife conservation in Nepal and working hard for the protection of health of captive and free ranging wild animals. He is always entertaining sharing of field and academic knowledge among the students and professionals.

He is married with Sabita and is blessed with a son.