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A case report on acute bacterial enterocolitis in an Asian Elephant (*Elephas maximus*)

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Abstract
The present investigation was based on the sudden death of an Asian elephant (*Elephas maximus*) in Bonai Divison of Sundargarh forest range of Odisha, India. Bacterial enteritis in elephants is rarely reported according to the published cases and limited knowledge is available regarding etiology and pathogenesis of bacterial microflora in the large herbivores. It has been reported that the anatomy and physiology of the elephant gastrointestinal tract have many similarities to that of horse. So like enterocolitis in horses the microbial etiology in elephant was detected in this case. Routine microbial analysis of the samples collected from liver, lung, intestine and impacted food material revealed the presence of *Salmonella* species, *Escherichia coli*, Clostridial species and other enterobacterial organisms like *Klebsiella* sp. and *Pseudomonas* sp. Antimicrobial sensitivity pattern of the six isolates of *E. coli*, six isolates of *Salmonellae* and 4 isolates of *Clostridium* sp. were analysed and it was found that Enrofloxacin was highly sensitive followed by Gentamicin. While screening for biofilm formation all the 6 isolates of each *Salmonella* spp., *E. coli* and *Clostridium species* it was revealed that both *E. coli* and *Salmonella* spp. produced white color colonies on Congo Red Agar while clostridial species were found also negative with white colour colonies when isolated in anaerobic condition after cultivation with Robertson's cooked meat medium and screened with Congo Red Agar. Further analysis on microbiological and molecular characterization is required.

Keywords: Wild Asiatic elephant, *Elephas maximus*, microbial analysis, Antibiotic susceptibility test, Biofilm formation

Introduction
Elephant is the largest herbivore and is a simple stomach animal like horse. Also its anatomical and physiological structure and function of gastrointestinal tract is like that of a monogastric animal and have voluminous small and large intestine for fermentative digestion (Bojesen *et al.*, 2006) [3]. The microbial digestion of cellulose mostly takes place in the huge sacculated caeco-colon of elephant. The intestinal bacterial microflora plays a significant role in maintaining the health condition of an elephant. The microflora of intestine plays a vital role in the defence mechanism against the pathogenic bacterial invasion (Iacob *et al.*, 2019) [7] (Kanauchi *et al.*, 2005) [8] (Ubeda *et al.*, 2017) [10]. So in the present study an attempt has been carried out to isolate and characterize the microflora from the visceral organs of 30 year old female Asian elephant and its antibiotic sensitivity pattern and biofilm forming capacity has been carried out.

Materials and Methods
A 30 year old female elephant viscera consisting of stomach, liver, lung, small intestine with intestinal content, spleen and impacted food materials (200 – 300 gm) were supplied by Additional Veterinary Assistant Surgeon, Lahunipara, Sundargarh dist. of Odisha, India to dept. of Veterinary Microbiology Laboratory, College of Veterinary Science and Animal Husbandry, OUAT, Bhubaneswar to ascertain the microbial etiology of death. The death of the animal occurred on 28.10.2019 at 6.15 AM as mentioned in the postmortem report. As per the local people the elephant had trumpeted loudly inside the forest and had grazed the radish cultivated field.

Gross pathological examination
Postmortem examination was carried out by the Veterinary Assistant Surgeon, Bonai,

Sundargarh, Odisha, India and within 24 hr respective tissue samples, with ice, were transported to Veterinary Microbiology laboratory.

Bacteriological identification and characterization

Samples from stomach, liver, spleen, lung, intestine and impacted food material were collected aseptically (Fig. - 1.1, 1.2, 1.3 and 1.4) and routine microbial isolation procedure was carried out (Cruickshank *et al.,* 1980) [5]. The samples were processed with BHI Agar, BHI Agar + 0.3%Bentonite clay, Blood Agar, MacConkey Lactose Agar, Congo Red Agar, Robertson's cooked meat medium and Eosin Methylene Blue Agar (HiMedia, Mumbai). Enterobacteria and Gram positive bacteria were identified by colony morphology, staining and biochemical testing. For the bacterial isolates, antimicrobial sensitivity test was also carried using agar gel disc diffusion technique i.e., according to CLSI protocols (Bauer *et al.,* 1966) [1] (Wayne *et al.,* 2009) [11]. All the antibiotic discs used were procured from the HiMedia, Mumbai. The evaluation of biofilm forming capacity of both aerobic and anaerobic microbes by Congo Red Agar method was carried out (Freeman *et al.,* 1989) [6].

Fig.- 1.1

Fig.- 1.2

Fig.- 1.3

Fig.- 1.4

Fig. 1: Gross samples from Impacted food material (1.1), Liver (1.2), Small Intestine (1.3) and Lungs (1.4) obtained from the Asian Elephant

Fig. 2

Fig 2: Showing the gram staining of spleen samples after enrichment

Table 1: Antibiotic susceptibility profile of *E. coli* and *Salmonella* sp. isolates

Sl. No.	Antibiotics	Disc potency (µg)	*E. coli* isolates (n = 6)		*Salmonella* sp. Isolates (n = 6)	
			Average zone of inhibition (mm)	Interpretation	Average zone of inhibition (mm)	Interpretation
1	Pipercillin + Tazobactam	100/10	-	Resistant	10	Resistant
2	Enrofloxacin	10	31	Sensitive	34	Sensitive
3	Streptomycin	25	19	Sensitive	21	Sensitive
4	Tetracycline	30	17	Sensitive	20	Sensitive
5	Cefoperazone	75	14	Resistant	21	Sensitive
6	Vancomycin	30	-	Resistant	-	Resistant
7	Gentamicin	10	23	Sensitive	22	Sensitive

Table 2: Antibiotic susceptibility profile of *Clostridium* sp. Isolates

Sl. No.	Antibiotics	Disc potency (µg or IU)	Average zone of inhibition (mm) of *Clostridium* sp. isolates (n = 4)	Interpretation
1	Enrofloxacin	10	29	Sensitive
2	Gentamicin	10	19	Sensitive
3	Streptomycin	25	-	Resistant
4	Amoxycillin	10	24	Sensitive
5	Kanamycin	30	-	Resistant
6	Penicillin-G	10	-	Resistant
7	Oxytetracycline	30	15	Resistant

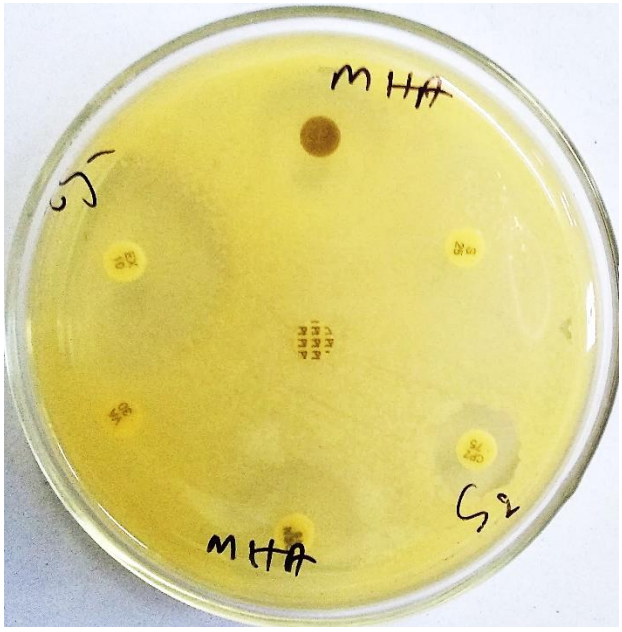

Fig. 3

Fig 3: Antibiotic sensitivity pattern of *E. coli* and *Salmonella* sp. Isolates

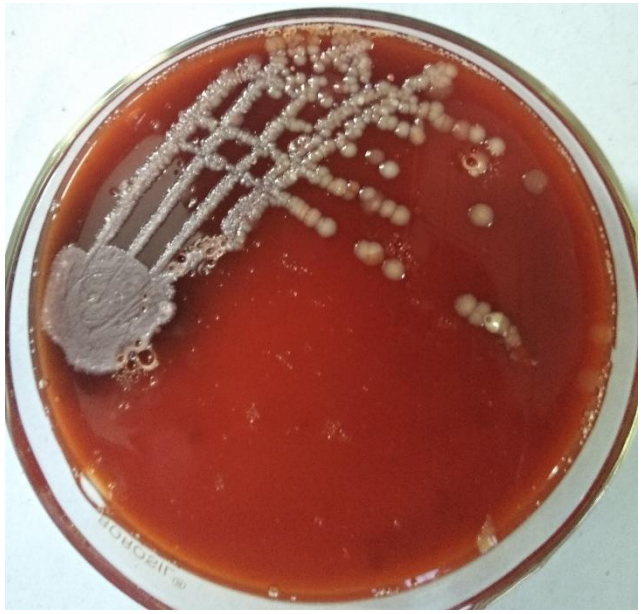

Fig. 4

Fig 4: Revealing no biofilm forming enteric bacteria on Congo red agar

Results and Discussion

According to postmortem examination, it was revealed that the animal was severely bloated. There were no external injuries and any discharges like blood, pus, mucus etc. Petechial hemorrhages were there on the pleural surface of both the lungs. Huge quantity of ingesta had lodged inside the stomach and was unusually dry, hard and lack of moisture. It could not move further in the alimentary canal.

Bacteriological analysis revealed the presence of Gram positive rods with Gram negative coccobacillary organisms. A total of six isolates of *E. coli*, six isolates of *Salmonella spp.* and four isolates of Clostridial species which were indistinguishable (*Clostridium perfringens* and *Clostridium difficle*) and other enterobacterial organisms like *Klebsiella* sp., *Pseudomonas* sp. were also found in the intestinal content. Fig.2 shows the presence of gram positive rods and gram negative coccobacillary organisms.

Isolation of Clostridial spp. along with *Salmonella* spp. and *E. coli* revealed in the present case, correlated with the postmortem findings, suggesting the main factor for death of the elephant. Earlier *Clostridium* spp. *(Cl. septicum*, *Cl. perfringens* and *Cl. difficle)* were isolated from liver and intestine causing enterocolitis as reported by several researchers (Biberstein *et al.*, 1990) [2] (Bojesen *et al.*, 2006) [3] (Songer *et al.*, 1996) [9] which corroborates with the present finding.

As there was no earlier history of the present elephant but on enquiry from the nearby villagers it was noted that the elephant had consumed huge amount of radish. Radish, a member of the plant *Brassica* known to cause potential livestock poisoning (Cheeke *et al.*, 1985) [4] from SMCO or glucosinolate toxin and it causes excessive gas. It has also been reported that large amount of radish eating can lower the blood sugar (Hypoglycemia) and increases the bile flow. This

may be the cause during postmortem examination that there was huge bloat observed in the dead elephant.

Enrofloxacin was found to be potent antimicrobial followed by Gentamicin for all the isolates of *Clostridium* spp., *E. coli* and *Salmonellae* spp. The *E. coli* and *Salmonellae* spp. isolates were sensitive for Tetracycline whereas Pipercillin+Tazobactam and Vancomycin were found to be highly resistant. Cefoperazone was found resistant in *E. coli* but sensitive for *Salmonella* spp. For *Clostridium* spp. Amoxycillin was also found sensitive whereas Streptomycin, Kanamycin, Penicillin-G and oxytetracycline were resistant (Table: 1 & 2). All the identified bacterial isolates were found negative for the biofilm formation as no bacterial isolate showed binding with Congo red and gave white color colonies (Fig.-4). Multidrug resistant *E. coli* and *Salmonellae* spp. along with *Clostridium* spp. may cause septicemic infection and acute colitis which may be the cause of death of the elephant.

Conclusion

The present findings emphasize the need of specific antibiotic therapy for treatment of enterocolitis in elephants. In microbiological examination gram stained smear from small and large intestine showed gram positive rod shaped bacilli so also culture of mucosal scrapings from small intestine, liver, and lungs revealed presence of Clostridial spp. in large number along with other organisms. Isolation of multidrug resistant microbes which was found to be highly sensitive to Enrofloxacin followed by Gentamicin. The present findings report the use of both Enrofloxacin and Gentamicin for treatment of acute enterocolitis in Asian Elephants.

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