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Research Article

Nitroxynil efficacy and some antimicrobials sensitivity against organisms isolated from Asiatic Elephant, Bangabandhu Safari Park, Dulahazara, Bangladesh

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ABSTRACT

Wild life conservation is immeasurably important for ecology and simultaneously periodical assessment of health status of these animals is necessary for public health safety. The aims of the present study were to estimate the nitroxynil efficacy and antimicrobial sensitivity against gastrointestinal parasite and microbiota, respectively, in Asiatic elephants. The study was carried out in a Safari Park of Bangladesh. Four fecal samples were collected and quantitative parasitological examinations were performed by McMaster technique before and after nitroxynil treatment. Furthermore, antimicrobial sensitivity tests were also performed on microbiota isolated from the feces through disk diffusion method. Eggs of *Strongyloides* sp were identified on fecal examination. The male had higher egg per gram (EPG) (18.20±2.80) than female (16.00±0.00) where as below 30 years of age group of elephants had higher EPG (18.50±3.53) than above 30 years of age group (16.80±1.97) before treatment. In both sex and age groups, eggs load were decreased up to 50% in feces after nitroxynil treatment. Moreover, gastrointestinal microbiota *(Salmonella sp, Shigella sp, Campylobacter sp* and *E. coli*) were also isolated from fecal samples. A total of 9 antimicrobials were tested and among them gentamicin (100%), ciprofloxacin (75%) ampicillin (25%) and cefadroxil (25%) were found strongly sensitive. It can be concluded that nitroxynil can reduce nearly half of the parasitic load whereas 100% sensitivity to only one antimicrobial (gentamicin) indicates emergence of resistance micro-organism even in semi captive conditions of wild elephants. Considering the semi-captive condition of wild elephants, further controlled drug trials are warranted.

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1. INTRODUCTION

Human population is increasing day by day around the globe. Due to over population and tremendous growth of agricultural production and industrializations, the habitats of the wild animals are changing and decreasing worldwide (Camill, 2010). Therefore, everywhere in the world the wild animals are facing threats to be endangered. Moreover, these are also

infected with different types of diseases due to change of habitats. Since, these animals are more important for ecology, thus conservation of endangered animals' species is very much important globally for ecological balance (The Convention on Biological Diversity 1993, article 2). For conservation of these animals, health status monitoring programs are also important. Elephant is the largest land dwelling animal, in the globe, which is found in wild, semi-captive and domesticated conditions. Almost 250-300 elephants (Sukumar, 2006) are found in Bangladesh of particular in the Chattogram Hill Tracts and in the north eastern part of Bangladesh. The government of Bangladesh has already taken initiative to conserve these endangered species by confinding in different safari parks. Importantly parasitic infestation and infectious diseases hindered the conservation of these animals. Internal parasites are of particular importance in the case of free ranging and semi-captive conditions. Helminthes and protozoa are responsible for most economic and production losses in livestock worldwide (Coop and Holmes, 1996; Waller 2006). Since, eradication of parasites is impractical; the aim of control measures in livestock is to limit parasitic populations to a certain level that are compatible with economic production (Brunsdon and Adam, 1975). These parasites can affect host survival and reproduction directly through pathological effects and indirectly by reducing host condition (Coop and Holmes, 1996). The general effects of gastrointestinal nematode infestation are partial or complete loss of appetite; interference with the production of digestive juices; damage to the lining of the alimentary tract so that materials such as proteins leak into the gut from the blood stream; diarrhea which leads to dehydration; and possibly interference with digestion and the absorption of digested nutrients (Fox, 2000). On the other hand, gastroenteritis is mostly caused by different agents including *E. coli, Salmonella, Shigella and Campylobacter* (McCambridge and McMeekin, 1981). Although a number of studies have been performed on the parasitic and microbial gastroenteritis of livestock and wild animals, but rarely any attempts was taken to study on the efficacy of anthelmintics and sensitivity of the antimicrobials that can effectively make clinical recovery in Asiatic Elephants *(Elephas maximus).* The purpose of the study was to assess the gastrointestinal parasites and response to anthelmintic treatment, to identify the gastrointestinal microbes *(E. coli, Salmonella sp, Shigella sp, Campylobacter sp)* and their antimicrobial sensitivity in semi-captive Asiatic elephant.

2. MATERIALS AND METHODS

Study Area

A cross sectional study was conducted on semi-captive elephants of Bangabandhu Safari Park, Dulahazra, Chakaria, Cox's Bazar for a period of 30 days, 25, December 2013 to 23, January 2014.

Management Practices

Elephants eat about 149~169kg (330-375 lb.) of vegetation daily. Ration for each elephant were- Green grass- 50kg; Banana tree- 200kg; Boiled rice- 04kg; Wheat bran- 04kg ; Jiggery- 250gm; Salt- 50gm. They usually drink about 80~150 liter water/day, depending on the temperature of the day. The elephants are usually kept in free range condition as semi-captive state. But during rainy season these are usually keep under a shed. No disease was recorded except non-descriptive diarrhea. Moreover, the authority gives Anthrax live vaccine and provides anthelmintic treatment in every four to six months. Commonly used anthelmintics were Ivermectin, Nitroxynil, Triclabendazole and Levamisole.

Data Collection

Demographic information on age and sex of individual elephants were gathered from the record keeping sheet kept by park authority.

Collection of Fecal Samples

A total of four elephants were brought under the current study. Fresh fecal samples of 200-300 gm were collected from four sites of boli of each elephant after defecation and kept in separate zipper bags and carried out within ice box from Safari Park to Biochemistry laboratory. Later parasitological and microbiological examinations were performed at Parasitology and Microbiology laboratory, respectively in Chattogram Veterinary and Animal Sciences University, Khulshi, Chattogram.

Preservation of Fecal Samples

Fecal samples were preserved in two different procedures. For parasitological examinations, each fecal sample was preserved with 2ml of 10% formalin and for microbiological test fecal sample was kept at 4°C in refrigerator for 2 days.

Examination of Fecal Samples for Gastro-intestinal Parasites

For examination of eggs, direct smear, sedimentation and floatation techniques were performed and eggs were quantified using McMaster techniques according to published protocols (Soulsby, 1982) and images were captured with computer interface (Progress Capture Version-2.8.8) with scale.

Drug Selection and Trial

Nitroxynil (Nitronex®, Renata Drugs Limited) 10mg/kg body weight was administered subcutaneously on day 1 after identification of *Strongyloides* eggs (Figure.1) and recollected fecal samples were examined after 14 days of treatment to know efficacy of nitroxynil against *Strongyloides sp* from the reduction of egg count.

Bacterial Culturing, Isolation and Identification

Rest of the samples, which were preserved in freezer, were transferred into Microbiology laboratory to identify the bacteria usually present in feces of the Asiatic elephants and antibiotic sensitivity by culture and sensitivity test. The fecal swab of the each sample was inoculated in test tubes containing Nutrient broth. Then these samples were incubated at 37ºC for 48 hours. Then the inoculums were streaked on the MacConkey agar followed by XLD agar (Xylose Lysine Deoxycholate) for isolation of *Shigella sp;* on RVS broth followed by XLD agar for isolation of *Salmonella sp;* in MacConkey agar and then EMB agar for isolation of *Escherichia coli;* Preston agar for isolation of *Campylobacter jejuni* and *Campylobacter coli*. All the samples were incubated again for 48 hours in 37ºC. Respective colonies for each bacteria were identified with the help of their colony characteristics on that particular medium.

Cultural and Sensitivity Testing

Determination of multidrug resistance pattern of bacterial susceptibility to different antimicrobial agents was measured *in vitro* by employing the modified Kirby-Bauer disk diffusion methods (Bauer *et al.*, 1966) by measuring zone sizes (in mm). Commercially available antimicrobials discs (Becton Dickinson, USA) were used for the test.

For sensitivity testing, the colonies from all the selective media were inoculated again in the blood agar media followed by incubation. By the standard method of inoculation, the top of a single and well-isolated colony was touched with a sterile loop and the growth was inoculated into 2 ml of Mueller- Hinton broth. The broth culture was then allowed to incubate at 37°C for 4 hours to obtain the young culture. The turbidity of actively growing broth cultures was then adjusted to a 0.5 McFarland standard and then a sterile cotton swab was dipped into the adjusted suspension within 15 minutes and excess broth was purged by pressing and rotating the swab firmly against the inside of the tube above the fluid level. The swab was then spread evenly over the entire surface of the plate of Mueller- Hinton agar to obtain uniform inoculums. The plates were then allowed to dry for 3 to 5 minutes. Antimicrobials impregnated discs were then applied to the surface of the inoculated plates with sterile forceps. Each disc was gently pressed down onto the agar to ensure complete contact with the agar surface. Even distribution of discs and minimum distance of 24 mm

Table 1: Antimicrobial, susceptibly intermediate and resistant categories according to the interpretation of the Becton Dickinson Microbiology Company

Name of anti-microbial	Strongly Sensitive (mm)	Moderately Sensitive (mm)	Resistant (mm)
Gentamicin 10µg	≥18	13-14	≤12
Doxycycline 30µg	≥16	13-15	≤ 12
Cotrimoxazole 25µg	≥ 18	13-14	≤ 12
Penicillin 10IU	≥ 18	13-14	≤ 12
Chloramphenicol 30µg	≥ 18	13-14	≤ 12
Ampicillin 10µg	≥ 18	13-14	≤ 11
Cefadroxil 30µg	≥ 18	13-14	≤ 16
Tetracycline 30µg	≥ 18	13-14	≤ 14
Ciprofloxacin 5µg	≥ 18	13-14	≤ 15

from center to center were ensured. Ten discs (five antibiotics discs and one blank disc as control) were placed in each petri dish. Within 15 minutes of the application of the discs, the plates were inverted and incubated at 37°C. After 16 to 18 hours of incubation, the plates were examined, and the diameters of the zones of complete inhibition to the nearest were measured as millimeter. The zone diameter for individual antimicrobial agents was then translated into susceptible, intermediate and resistant categories according to the interpretation (Table 1) of the Becton Dickinson Microbiology Company, USA (Saifuddin *et al.,* 2016).

Statistical Analysis

The raw data were sorted, entered into the computer software using Excel 2000 and exported to Statistical Package for the Social Sciences SPSS 16.0. The data were analyzed for descriptive statistics (mean, SD and percentage). The proportion of positive cases was calculated and the response to treatment was evaluated by the presence or absence of egg in faeces.

3. RESULTS AND DISCUSSIONS

Parasitological Tests Results

Table 2: Number of eggs of *Strongyloides sp* in feces of elephants before and after nitroxynil treatment according to the demographic variables

Variables	Category	Eggs per gram (EPG)		
		Before	After	% reduction
		Mean ± SD	Mean ± SD	
Sex	Male (n=3)	18.20 ± 2.80	8.93 ± 1.60	50.93
	Female (n=1)	16.00 ± 0	7.00 ± 0	56.25
Age	<30 year (n=2)	18.50 ± 3.53	8.80 ± 2.54	52.43
	>30 year (n=2)	16.80 ± 1.97	8.10 ± 0.98	51.78

Table 2 shows the average number of eggs/gm of feces according to sex and age before and after nitroxynil treatment. The mean score for the EPG in male elephant decreased after 2 weeks of nitroxynil treatment from 18.20±2.80 to 8.93±1.60 and the mean score for the EPG in female elephant declined from 16.00±0 to 7.00±0. And the mean score for the EPG in less than 30 years of aged elephant found decreased after 2 weeks treatment of nitroxynil from 18.50 ± 3.53 to 8.80±2.54 while in above 30 years of aged elephant found reduced from 16.80±1.97 to 8.10±0.98.

Microbiological Tests Results

Table 3: Sensitivity of gastrointestinal microbiota to different antimicrobials

Types of Bacteria	Strongly sensitive	Moderately sensitive	Resistance
E. coli	Gentamicin Ciprofloxacin	Ampicillin Cefadroxil Cotrimoxazole Chloramphenicol Tetracycline	Penicillin Doxycycline
Salmonella sp	Gentamicin Ciprofloxacin	Ampicillin Cefadroxil Cotrimoxazole Tetracycline	Penicillin Doxycycline Chloramphenicol
Shigella sp	Gentamicin Ciprofloxacin	Ampicillin Cefadroxil Cotrimoxazole Tetracycline	Penicillin Doxycycline Chloramphenicol

Campylobacter sp	Gentamicin Ampicillin Cefadroxil	Ciprofloxacin Chloramphenicol Tetracycline	Penicillin Doxycycline Cotrimoxazole

Among the 9 antimicrobials, both penicillin and doxycycline found resistance to *E. coli;* penicillin, doxycycline and chloramphenicol to both *Salmonella sp* and *Shigella sp;* doxycycline, penicillin and cotrimoxazole to *Campylobacter sp;* both gentamicin and ciprofloxacin showed strongly sensitive against *E. coli, Salmonella sp,* and *Shigella sp.* while *Campylobacter sp* found sensitive to gentamicin, ampicillin and cefadroxil (Table 2).

Table 3: Proportion of different antimicrobials sensitivity pattern against inolated gastrointestinal microbials

Antimicrobials	**Strongly sensitive %**	**Moderately sensitive %**	**Resistant %**
Gentamicin (10µg)	100	0	0
Doxycycline (30µg)	0	0	100
Cotrimoxazole (25µg)	0	75	25
Penicillin (10 IU)	0	0	100
Chloramphenicol (30µg)	0	50	50
Ampicillin (10µg)	25	75	0
Cefadroxil (30µg)	25	75	0
Tetracycline (30µg)	0	100	0
Ciprofloxacin (5µg)	75	25	0

The Table-3 shows that, among 9 antimicrobials, gentamicin found strongly sensitive to all isolated fecal bacteria *(E. coli, Salmonella sp, Shigella sp and Campylobacter sp)*. The proportion of strongly sensitive antimicrobial was gentamicin (100%), ciprofloxacin (75%), ampicillin (25%) and cefadroxil (25%). The moderately sensitive antimicrobials were tetracycline (100%), cotrimoxazole, ampicillin, cefadroxil (all are 75%), chloramphenicol (50%) and ciprofloxacin (25%). Finally Doxycycline (100%), cotrimoxazole (25%), penicillin (100%), chloramphenicol (25%) were found resistant.

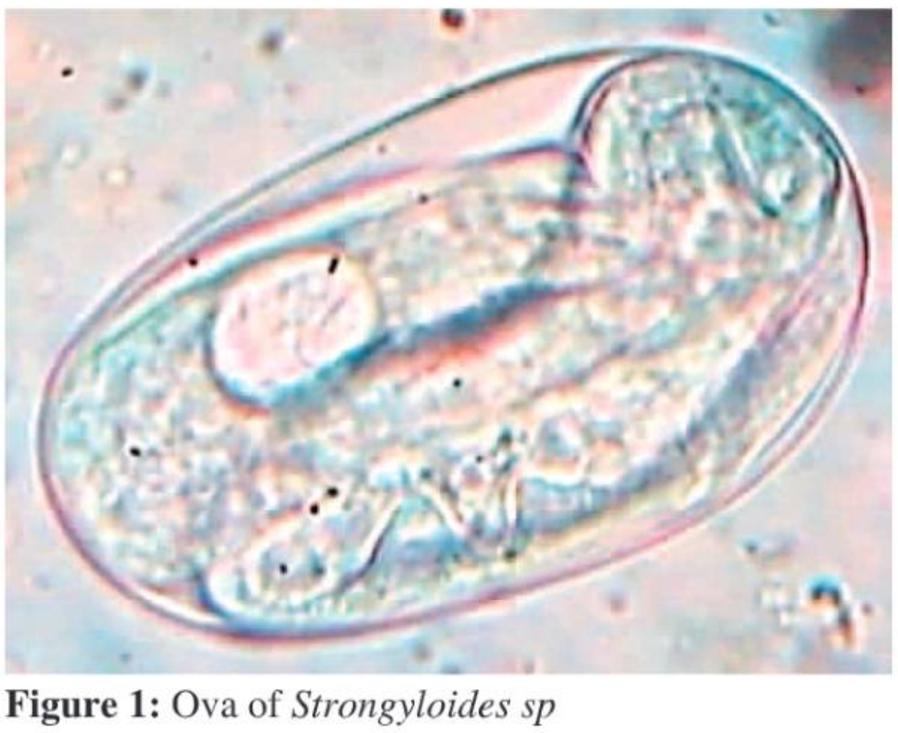

Figure 1: Ova of *Strongyloides sp*

In this study we investigated the gastrointestinal parasites especially *Strongyloides sp* and the efficacy of nitroxynil treatment against them, concomitantly elucidate the gut micro-biota of *Enterobacteriaceae* and *Campylobacteraceae* and their sensitivity to some selected antimicrobials. The present study showed significant reduction of *Strongyloides sp* eggs load in faces of Asiatic elephants in semi captive condition after nitroxynil treatment. More reduction of EPG was evidenced in female as compared to male counterpart. The similar trend of reduction of eggs count was observed in both age groups. Gentamicin showed 100% sensitivity to *E. coli, Salmonella sp Shigella sp and Campylobacter sp.* The number of *Strongyloides sp* found higher in male as compare to female. That might be due to male animals move in wide range of area for vegetation (Barnes, *et al.,* 1999). The number of eggs was reduced after Nitroxynil treatment, however, were not eliminated completely. That could be due to nature of narrow spectrum of nitroxynil and/or less sensitive to the *Strongyloides sp* in Asiatic elephants.

Gastrointestinal bacteria were also cultured from feces in selective media, from which we isolated four different types of bacteria. Similar types of gastroin-

testinal bacteria were also reported by Craig (2009). Antimicrobial sensitivity test revealed that, gentamicin showed 100% sensitivity to all isolated bacteria *(E. coli, Salmonella sp, Shigella sp, Campylobacter sp),* which is coincided with the previous study (Craig, 2009). These findings revealed the multi-drug resistance even in wild captive elephants and pose threats to human and animal health. This study indicates mammals may play role as host reservoirs and potential vectors for the spread of antimicrobial resistant bacteria, which leading to emergence of multi-drug resistance superbug.

4. CONCLUSION

In the study demonstrated nearly fifty percent reduction of *Strongyloides sp* eggs/gram feces with nitroxynil after 14 days of treatment and only gentamicin showed 100% sensitivity to *E. coli, Salmonella sp, Shigella sp, Campylobacter sp.* The strength of the present study that we identified the gastrointestinal parasites especially *Strongyloides sp* and some antimicrobial sensitivity to pathogenic bacteria isolated from captive wild elephants respectively. However, there are some limitations, which include small number of sample size, short duration of the study and of particular no molecular study were performed.

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