SCREENING OF ELEPHANTS PARTICIPATING IN THE ESALA PERAHERA FOR ZOOONOTIC AND MULTIDRUG RESISTANT BACTERIA

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SUMMARY: During religious, cultural and other human entertainment activities domesticated elephants come into close contact with humans creating a potential disease transmission threat between elephants and humans. The main objective of the study was to screen the elephants participated in the Esala Perahera 2015 for the zoonotic bacterial pathogens namely *Salmonella*, *Campylobacter* and *Mycobacterium tuberculosis* complex. Further, the antimicrobial susceptibility of the fecal *E. coli* and zoonotic pathogens isolated were determined to identify multidrug resistant organisms. The isolation rates for *E. coli* and *Salmonella* were 100% and 8%, respectively while *Campylobacter* was not isolated from any of the fecal samples collected. Three of the four *Salmonella* isolates were resistant to ampicillin and tetracycline and all four isolates were susceptible to nalidixic acid, streptomycin, gentamicin, ciprofloxacin, sulfamethoxazole and trimethoprim combination, cefotaxime, ceftazidime, ceftriaxone, imipenem and amikacin. A number of *E. coli* isolates were resistant to ampicillin (8%), sulfamethoxazole and trimethoprim combination (8%), tetracycline (8%), ceftriaxone (8%), amikacin (6%), nalidixic acid (4%), imipenem (4%), gentamicin (2%), streptomycin (2%), ceftazidime (2%) and ciprofloxacin (2%). However, all tested *E. coli* isolates were susceptible to cefotaxime. Further, 8% of the *E. coli* isolates showed resistance to three or more antimicrobial groups used and can be classified as multidrug resistant. None of the elephants yielded a positive result for the fecal PCR assay indicating that the animals did not excrete pathogenic mycobacteria in their feces.

INTRODUCTION

The captive elephants have been a part of Sri Lankan culture for hundreds of years and have played an important role in warfare, economy, and religious activities. Currently Sri Lanka has about 150 domesticated elephants and many of them are owned by temples, zoos, elephant orphanages, non-profit organizations and private owners. These captive elephants take part in elephant shows, safaris, hauling logs in difficult terrains, religious and cultural activities and other human entertainment work. During these activities, elephants come into close contact with humans creating a great potential to transmit diseases from elephants to humans (zoonotic diseases) and *vice versa*. The Esala Perahera of the Sri Dalada Maligawa in Kandy, a spectacular and probably the oldest procession in Asia, is held annually with the data (WHO, 2015). Many bacterial strains, resistant to a range of antimicrobials including cotrimoxazole, ampicillin, tetracycline, methicillin and vancomycin have been isolated from elephants (Sthitmatee *et al.*, 2013; Barman *et al.*, 2013). At present antimicrobial resistance has become a global public health issue due to the widespread

participation large number of elephants. During this procession thousands of people, including tourists, come into a very close contact with the elephants. The objective of this study was to screen elephants that took part in Esala Perahera in the year of 2015 for a range of zoonotic pathogens including *Salmonella*, *Campylobacter* and *Mycobacterium tuberculosis* complex and to determine the occurrence of multidrug resistance among fecal *E. coli* and isolated pathogens.

Scientific literature provides ample evidences for the high prevalence of *Escherichia coli*, and *Salmonella* infections in captive Asian elephants residing in a many countries (Chooi *et al.*, 1988, Mikota, 2006). However, there is not much information available on the status of *Campylobacter* infections in elephants. The aforementioned organisms top the list as human gastrointestinal pathogens according to the recent and inappropriate use of antibiotics by both human and animal health sectors. Such pathogenic microorganisms and/or the antimicrobial resistance acquired by the organisms residing in elephants can easily be transmitted to mahouts and the general public. Multidrug resistant Gram negative bacteria pose the biggest threat to the global health as

plasmids of Gram negative bacteria has the capacity to acquire resistance faster than the Gram positive organisms. This acquired resistance gets disseminated horizontally across the related and non-related groups of bacterial populations via plasmids resulting in multiple multidrug resistant groups (Bennett, 2008).

Tuberculosis (TB) has been frequently reported in Asian elephants (Mikota *et al.*, 2001). TB in elephants is commonly caused by *M. tuberculosis* and *M. bovis* (Lyashchenko *et al.*, 2006) which are zoonotic pathogens. All human pathogenic mycobacteria are classified into a group called *Mycobacterium tuberculosis* complex. Elephants in captivity can get TB from humans and other mammals (Michalak *et al.*, 1998). Further, elephant to elephant transmission of the disease may be very likely due to certain behavioral habits such as placing their trunks inside the mouths of other elephants. The elephant TB poses a great risk to elephant handlers, and to the general public especially to those who are immunocompromised and not immunized against tuberculosis. As in humans, TB in elephants can be a chronic, debilitating disease which manifests as weakness, weight loss, exercise intolerance, diminished appetite, chronic nasal discharge and coughing. Elephants may not show signs until the disease is quite advanced. Even long before appearing of the clinical signs, infected elephants can spread the disease (Greenwald *et al.*, 2009).

MATERIALS AND METHODS

Freshly voided fecal samples were collected from 50 elephants participated in Esala Perahera in August 2015. The fecal samples were immediately transported to the laboratory at 4°C and a fraction of the samples were immediately utilized for bacteriological culture. The rest of the samples were stored at -20°C to be used for molecular diagnostic work.

Salmonella and *Campylobacter* were isolated following the standard analytical methods elaborated under ISO 6579 and ISO 10272E standards, respectively (ISO, 2002 and ISO 2006). *E. coli* were isolated by direct plating of feces on McConkey agar. Colony morphology and conventional biochemical tests were used to identify the organisms. The antimicrobial sensitivity tests

Therefore, the study did not find a risk of transmitting tuberculosis from elephants to humans during the Esala Perahera held in 2015. However, elephants acquiring infection from humans is always a possibility during such close human-elephant interactions.

A total of 50 *E. coli* isolates were tested for susceptibility to different antimicrobials described in the method section and the percentages of the isolates resistant to respective antibacterials are

were performed on all isolates of *E. coli* and *Salmonella* using the Kirby-Bauer disk diffusion method according to the standard operating protocols described by the Clinical Laboratory Standard Institute (Watts and CLSI, 2008). The isolates were tested for the susceptibility to ampicillin, nalidixic acid, streptomycin, gentamicin, tetracycline, ciprofloxacin, sulfamethoxazole and trimethoprim combination, cefotaxime, ceftazidime, ceftriaxone, imipenem and amikacin. The antimicrobial disk strengths used are given in Table 1.

A PCR assay was conducted to detect *Mycobacterium tuberculosis* complex specific DNA in fecal samples. The total fecal DNA was extracted using Qiagen Mini Stool Kit following manufacturer's protocol (Qiagen Sciences, Maryland, USA). The PCR assay targeted a 123bp fragment of IS 6110 insertion element specific to *M. tuberculosis* complex. The PCR assay was conducted according to a previously published procedure (Ozkara *et al.*, 1998). Figure 2 shows a gel electropherogram of the fecal PCR assay.

RESULTS AND DISCUSSION

Isolation rates for *E. coli* and *Salmonella* were 100% and 8% (only 4 isolates), respectively while *Campylobacter* was not isolated from any of the samples. One larger study conducted in Thailand has also reported very low prevalence of *Salmonella* in elephants (Thitaram *et al.*, 2004). Neither the elephants participated in the study had acute gastroenteritis nor yielded *Campylobacter* upon culture.

None of the samples became positive for the fecal PCR assay indicating that the elephants were not shedding organisms belonging to *Mycobacterium tuberculosis* complex in their feces. Previously our group has validated the fecal PCR assay using human subjects and shown that it is a sensitive (~80%) and a specific (100%) assay to detect zoonotic mycobacteria (Kulasooriya *et al.*, 2016a and Kulasooriya *et al.*, 2016b). Further, this same method has been applied to confirm the diagnosis of tuberculosis in elephants previously (Kumara *et al.*, 2015).

given in Table 1. Three of the four *Salmonella* isolates were resistant to ampicillin and tetracycline and all four isolates were susceptible to the rest of the antimicrobials used. Eight percent of the *E. coli* isolates exhibited resistance to three or more antimicrobial groups used in the study (Figure 1). Therefore, these isolates can be concluded as multidrug resistant (Magiorakos *et al.*, 2012).

Coliforms, particularly *E. coli* is a useful indicator organism for screening antimicrobial resistance. This study revealed that a number of *E. coli* isolates tested were resistant to many important antimicrobial drugs including ceftriaxone. Ceftriaxone is a third generation cephalosporin and listed as a high priority critically important antimicrobial drug by the World Health Organization. Third generation cephalosporin is one of the few available therapies for serious *Salmonella* and *E. coli* infections, particularly in

children. Moreover, ceftriaxone is one of the several antibiotics with a high probability of non-human sources resulting in transmission of resistant bacteria to humans (Winokur *et al.*, 2000). Such important antibacterial drugs may become ineffective in treating human diseases in the near future if not used sparingly and carefully on animals. Therefore, it is very important to closely monitor the antimicrobial resistance in bacteria isolated from animals.

Table 1. The percentage of the *E. coli* isolates resistant to different antibacterials tested.

Antimicrobial agent	Disk Strength	% Resistance
Ampicillin	10µg	8%
Tetracycline	30µg	8%
Sulfamethoxazole+trimethoprim	25µg	8%
Ceftriaxone	30µg	8%
Amikacin	30µg	6%
Nalidixic acid	30µg	4%
Imipenem	10µg	4%
Gentamicin	10µg	2%
Streptomycin	10µg	2%
Ceftazidime	30µg	2%
Ciprofloxacin	5µg	2%
Cefotaxime	30µg	0%

Figure 1. *E. coli* isolates showing multiple drug resistance

Figure 2. A 1.2% agarose gel electropherogram showing outcome of the fecal PCR assay
L: DNA ladder, S: fecal sample, P: positive control, N: negative control, IS: IS 6110 insertion element specific to *M. tuberculosis* complex

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