ISSN: 2634 - 680X

Journal of Clinical Case Studies, Reviews & Reports

SCIENTIFIC Research and Community

Case Report

Open Access

A Case Report of a Typhoid Fever Outbreak with an Uncommon Vehicle and Source of Salmonella Enterica Serotype Typhi

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ABSTRACT

Two goat caretakers aged 19 and 25 years old were infected with *Salmonella enterica* serovar typhi (*S. typhi*); both had eaten raw carrots from a garden enriched with goat faeces in typhoid endemic region of Morogoro, Tanzania. *S. typhi* strains isolated from garden soils and carrots proved to be from goat faeces. This data provide evidence for the spread of typhoid fever through carrots contaminated by faeces from goats contained transient *S. typhi*.

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Received: November 30, 2020; **Accepted:** December 20, 2020; **Published:** January 19, 2021

Keywords: Typhoid Fever, Transient *S. typhi*, Goat faeces, Contaminated Carrots

Background

S. typhi is a bacterial pathogen which remains an important public health issue in developing countries especially that of African and Asian continents [1-5]. Worldwide, it is estimated that 94 million illnesses and 155,000 deaths per year are linked with *S. typhi* [6]. While these statistics are terrifying, little is known regarding the possibility of transient *S. typhi* in causing typhoid fever outbreak especially in the developing world where *Salmonella* infections are common and their treatments are becoming difficult due to emergence of drug resistant strains [4,7].

Efforts to avert typhoid fever in developing countries is limited to safe disposal of human faeces, efficient sewage treatment, drinking water treatment and frequent hand washing. Despite these measures, prevalence of typhoid fever is escalating in these countries [4,8,9]. This situation demands for an understanding of the sources of *S. typhi* and the way in which *S. typhi* from these sources get into human gastrointestinal tract. Previously reported outbreaks of typhoid fever were linked to human faeces and sewage [5,10]. However, studies reporting unusual sources and vehicles of *S. typhi* are limited despite heavy burden of typhoid fever in endemic typhoid environments [3,4,11,12]. Here, a discussion of a case of an occurrence of *S. typhi* in goat faeces and their link with a typhoid outbreak following consumption of raw carrots harvested from a garden enriched with goat faeces is presented.

Case report

In 2018, two goat caretakers from a small scale farm constituting 22 goats visited a public hospital. The first (19 years old) and second (25 years old) goat caretakers were responsible for the day to day management of goats and a vegetable garden located nearby goat farm. In the hospital, goat caretakers presented to a physician with worsening headache, strong fever, nausea and severe stomach pains. Based on these symptoms, fresh blood samples were taken from these patients and subjected to Widal test (Murex Biotech, Dartford, UK) after microscopic examination of malaria parasites tested negative. Briefly, fresh blood samples were immediately centrifuged to obtain serum. Two fold serial dilutions (1:20– 1:1280) of the serum sample were made and about 20µl of O antigen suspension was added to each tube containing the diluted sample. Afterwards, each tube was gently mixed and incubated for 4 hours at 50°C in order to check the agglutination. Tested samples had anti-TH antibodies of 1:8, signifying a recent infection of goat caretakers with *S. typhi*. In parallel, goat caretaker's stools were analyzed for *S. typhi* cells using standard methods in order to rule out false-positive emanating from Widal test. Briefly, 1g of stool sample was transferred to 10 ml of 0.067 M phosphate -buffered saline and sonicated before being allowed to settle. One milliliter from sonicated sample was inoculated onto xylose lysine deoxycholate (XLD) (Oxoid CM0469, Basingstoke, England) plates. Plates were incubated at 37°C for 24 hrs. After incubation, well isolated black colonies on XLD were separately streaked on the nutrient agar plates and incubated at 37 °C for 24 hrs to obtain pure isolates. These isolates were subjected to API 20E (bioMérieux, UK) in which 35 colonies out of 56 total colonies were identified as *S. typhi* (Table 1). Further confirmation was undertaken by extracting DNA from pure colonies of presumptive *S. typhi* isolated from nutrient agar using a QIAamp DNA Stool Kit (Qiagen countaboeuf, France) following the manufacturer's instructions and performs PCR reaction using a protocol and *S. typhi* primers [13]. Detected amplifications suggested that *S. typhi* was indeed responsible for the health deterioration of the goat caretakers. Upon subjecting the isolates of *S. typhi* to ampicillin, ciprofloxacin, ceftriaxone and chloramphenicol using Kirby-Bauer disks susceptibility testing method, *S. typhi* was susceptible to ciprofloxacin disk (5-µg) with screening zone size of 26 mm but resistant to the rest of the applied antibiotics. The patient was prescribed with oral ciprofloxacin by the treating physician, which made them to recover from typhoid fever. The fact that goat caretakers were responsible for the management of 22 goats presented in the considered farm with no history of clinical illnesses, analysis of *S. typhi* was determined in the faeces of each of the goat existed in the farm. Briefly, each goat

was subjected to a well cleaned cage for 6 to 8 hours to enable collection of representative fresh faeces. Collected faecal samples were kept in the sterile 50 ml wide mouthed bottles using a sterile spoon and transported to the laboratory while at cool condition (4OC). In the laboratory, goat faecal samples were processed in the similar procedure as for goat caretaker's stool samples. Of 22 goat faecal samples analyzed, two had a considerable number of presumptive *S. typhi* colonies ranging from 34 to 65 cfu/g and the remaining goats had undetectable *S. typhi* in their faeces (Table 1). DNA extracted from the pure culture of presumptive *S. typhi* colonies were subjected to PCR in order to test for the presence of invA, viaB, fliC-d, and prt genes of *S. typhi* using a procedure described by Kumar et al., [13]. Tested isolated were positive for invA, viaB, fliC-d, and prt genes except one which were false negative. To prevent spread of typhoid fever, all goats carrying *S. typhi* were euthanized and their remains disinfected using 24% formaldehyde solution diluted to 5% before interment.

To understand weather the carrots uprooted from the garden enriched with goat faeces and eaten by the goat caretakers was the vehicle of *S. typhi* responsible for the typhoid outbreak, three samples each with 100g of carrot roots from the same garden were separately placed in to 500 ml of 0.067 phosphate-buffered saline and by scrubbing the carrot with the aid of sterile toothbrush, bacterial cells attached on the surface of carrot roots were made available in the saline solution. Of the 500 ml used to scrub bacterial cells from the carrot roots, 100 ml was filtered through a polycarbonate filter with 0.2 µm poresize and the DNA was extracted from the filter using the protocol described by Djurhuus et al., [14]. Obtained DNA was tested for the presence of *S. typhi* genes using the PCR procedure and primers similar to that used for human stools and goat faces in this study. Amplification was detected in all of the three samples tested (Table 1), leading to the disinfection of harvested carrots using 0.6% chlorine solution to prevent further outbreak of typhoid fever.

Table 1: Detection of *S. typhi* in patients and environmental samples

Samples	Age	No. of samples	No. of positive samples on PCR	Number of positive isolates
Goat caretaker 1	(19 years old)			
Blood		1	1	
Faeces		1	1	35
Goat caretaker 2	(25 years old)			
Blood		1	1	
Faeces		1	1	46
Environmental samples				
Goat Faeces		22	2	34 – 65
Carrots from garden		3	3	5 – 8
Soils from carrot garden		3	3	10-16
Soils without goat manure		3	0	Not determined

To confirm the source of *S. typhi* in the investigated area, samples of soils (10 g each) from garden enriched with goat manure (n=3) and that without goat manure (n=3) were taken and separately analysed for *S. typhi* using the procedure similar to that described for carrots samples. In this analysis, *S. typhi* was detected in soil samples enriched with goat faeces and never in the soils without goat faeces (Table 1).

Discussion

Despite many efforts undertaken to improve sanitation and hygiene in the public and domestic domains, the prevalence of typhoid fever is continuously increasing while the sources and vehicles of *S. typhi* remain unattended. This case report appears to be the first evidence that *S. typhi* can be transmitted to humans through raw carrots contaminated with transient *S. typhi* in the faeces of goats [4,15]. Nineteen and twenty five -year-old goat caretakers were infected with Salmonella enterica serotype typhi after eating raw carrots harvested from a garden enriched with goat's faeces. This serotype typhi strain was simultaneously detected on the surface of carrots and in the soil collected from the carrot garden but never from the soil free from goat faeces during this episode, suggesting that carrots were a vehicle of the infection. In connection to this, the serotype typhi strain was detected in the faeces of health goats present in the investigated small scale farm in Tanzania at a concentration between 34 and 65 cfu/g. This concentration was substantially higher than that detected in the surface of the carrots (5-8 cfu/g) and garden soil (10-16 cfu/g) where carrots were harvested. This observation does not only suggests that goat faeces were the source of *S. typhi* but also indicates that *S. typhi* cannot survive for long in the environmental substances such as soil and carrots.

The typical *S. typhi* genes identified from the patients, carrots, soils of the carrot garden and goat faeces strongly support the assumption that *S. typhi* can be transmitted to humans through environmental substances such as soils and carrots contaminated by goat faeces containing transient *S. typhi*. These results are in line with the previous observation that reported passage of a significant number of bacterial pathogens from animals to human through different types of vehicles [16].

The fact that *S. typhi* is host restricted to humans, the occurrence of this pathogens in the faeces of 2 out of the 22 investigated goats in this study is not only an indication that *S. typhi* was a transient bacterium in the gut of the investigated goats but also suggested that individual goats differ in resisting transient *S. typhi* [17]. This observation is in line with the previous studies that detected *S. typhi* in fish gut as well as in faeces of poultry [11,12]. Further, the study showed that transient *S. typhi* in guts of animals is capable of initiating an outbreak of typhoid fever. However, further studies are needed to investigate the transient behavior of this pathogen in various animals interacting with human such as livestocks and domesticated animals so that an appropriate managerial plan can be developed and utilized to prevent public health from contracting typhoid fever.

Conclusion

For the first time *S. typhi* were detected in faeces of health goats in the small scale farm of developing country. Faeces of these goats applied in the carrot garden as manure led to the contamination of carrot with *S. typhi*. Raw carrots frequently eaten by goat caretakers were a vehicle for the outbreaks of typhoid fever in the goat farm. This case report is the remainder that previously unrecognized sources and vehicles of *S. typhi* are sufficient to induce typhoid outbreaks.

Acknowledgments

I thank the owner of the farm for supporting this study, laboratory technicians of the Sokoine University of Agriculture (SUA), Department of Biosciences for their assistance in sampling and analyzing the faecal samples and SUA for giving me time and laboratory facilities for performing this work.

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