



SDI Review Form 1.6

Journal Name:	American Journal of Experimental Agriculture
Manuscript Number:	Ms_AJEA_28207
Title of the Manuscript:	Shiga toxin Producing Escherichia coli (STEC) in Food Producing Animals from Trinidad and Tobago
Type of the Article	Original Research Article

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

(<http://www.sciencedomain.org/page.php?id=sdi-general-editorial-policy#Peer-Review-Guideline>)



SDI Review Form 1.6

PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments	The material and methods, and results and discussion need revision. It is not clear what is done. • How was the fecal samples collected? Was it fecal swabs or fecal material? • Why was fecal samples from young animals (juveniles) excluded? It is well known that younger animals shed more in their feces. • How were the samples analysed? Were the fecal samples enriched prior to isolation of E. coli? On which plates where the samples plated and how many colonies were tested from each sample? • Why was not a method for STEC (ISO 13136) used? • I do not understand how the colonies were tested as stx-PCR is listed in more than one section. In 2.1 it is stated that E. coli isolates were tested for eae, stx1 and stx2 by PCR. In 2.2 it is stated that all isolates testing positive for eae, were further tested for stx1 and stx2. What is the difference between 2.1 and 2.2? Since you are looking for STEC would it not be more logical to test all the isolates for stx1 and 2 first and then further test for the presence of eae? Unless you have a stx-gene present, you do not have an STEC whether or not it is of an STEC associated serogroup with an eae gene.	We sincerely would like to thank the reviewer for really making out time to read through our paper. The constructive comments has helped us to be focused in reporting what we did. 1. As we have now explained,..” Approximately 10 – 20g faecal materials were collected from healthy and dairy cattle that were observed defecating in their pens during the time of visit to the farms. The faecal pat was stirred and collected using a sterile wooden spatula or tongue depressor and gloves. Samples were placed into a labeled dry sterile containers or whirlPak bags”. 2. Why was faecal samples from young animals (juveniles) excluded? We have now explained why we excluded juveniles. We now have written and explained as follows “Juveniles were excluded from sampling and analysis because most of the farm owners were uncertain about the age of their juveniles. So to preserve uniformity and present a more accurate picture of the prevalence in Trinidad and Tobago, they were excluded. Also as reported in literature, rate of shedding is variable. Depending on age, shedding in very little amount occur pre weaning or under 2 months and shedding at a much higher volume occur post weaning after 2 – 6 months [35, 36]” 3. The laboratory analysis has been explained as follows “In the lab, minimal pre-



SDI Review Form 1.6

	• In the results and discussion, last paragraph, testing for verocytotoxicity was mentioned. I do not understand if the authors have done this in the presented study or if they are referring to a paper. Please make this clear and if it is carried out here, put it in the M&M. • The results and discussion should be revised. Please put the results in a table and enumerate the tables. • Figure 2 should be deleted as it does not give any new information and the gel is of a rather poor quality. • What does it mean that the E. coli isolates were sulphur negative? • In the same paragraph it is referred to figure 1 for results of stx1 and 2 genes, but figure 1 is a map. Please correct. • In the results and discussion, it is often referred to stx toxins, f.ex: "three farms were positive for both toxins". I assume that this means toxin genes? Please revise the section with respect to this. • The sentence ".... implying that new STEC serotypes might be present in Trinidad". First it should be serogroups, and also there has been described numerous serogroups with stx-genes, but one cannot test them all, so to imply "new STEC serogroups" might be too strong. Also, if the authors had used another method the results might have been different. By testing random E. coli isolates, it is expected that many will not be found and therefore the method and the number of isolates tested are very important. It would	enrichment was done which involved using a buffered peptone water at a ratio of 1:10 to 8 to 12g of the fecal materials and homogenized for about half an hour. This was then plated onto sorbitol-MacConkey (SMAC) agar plates and incubated for 18 – 20 hours at 37°C. Suspected pure colonies for E. coli were sub cultured on MacConkey and blood agar plates. Colonies were confirmed as E. coli if they were lactose-fermenting colonies on MacConkey agar plates, and biochemical tests including, non-hydrogen sulphide production, positive indole and motility (SIM) test, positive beta-glucuronidase and methyl red tests, and negative result for citrate tests according conventional microbiology procedures." 4. Our lab uses the ISO 16654 :2001 protocol 5. The duplication in the methods of PCR analysis have been deleted. This section now reads as follows "2.3 Detection of the virulent markers – stx₁, stx₂, eae, tir, by colony Polymerase Chain Reaction <i>Escherichia coli</i> isolates were screened for several virulent markers including the eae gene (coding for the intimin adhesion), Shiga toxin 1 (stx₁) and Shiga toxin 2 (stx₂) genes using colony Polymerase Chain Reaction (PCR) method as described in literature [30, 31] with minimal modifications. Briefly, the reaction mixture comprised the following: 12.5 µl GoTaq® Green Master Mix (2X), 2 µl of each primer (forward and reverse), tiny portion of colony as a source of template DNA, made to 25 µl by water nuclease-free (Promega, Madison, USA). The GoTaq® master mix contains two dyes (blue and yellow) that allow
--	---	---



SDI Review Form 1.6

	have been interesting to have had the results the other way round, by starting to screen an enrichment broth and then going for isolation. I think that the work is interesting and gives information from a part of the world from where such information is scarce. However, there are issues with the materials and methods that needs clarifying.	<i>monitoring of progress during electrophoresis. Reactions with the master mix have sufficient density for direct loading onto agarose gels and did not require loading dyes. The PCR cycling program was performed using a Techne Flexigene Thermal-cycler (American Laboratory Trading East Lyme, CT). Primers used in this study are displayed in Table 1 and were all purchased from Sigma (St Louis, MO, USA). Samples were subjected to 25 PCR cycles, each consisting of an initial denaturation of 5 min at 94°C, then 25 cycles of 1 min of denaturation at 94°C; 1 min of annealing at 52°C and 1 min of elongation at 72°C and a final extension time of 10 min at 72°C. Amplicons (5 µl) were resolved on 1% agarose gel in Tris-Acetate-EDTA (TAE) buffer alongside a 100bp ladder (New England Biolabs, Ipswich, MA) and viewed on a UV trans-illuminator after staining with Gelred™ dye (Biotium Inc., Hayward, CA)...."</i>
--	---	--



SDI Review Form 1.6

Minor REVISION comments	Please put the primer sequences used either in a Table or only as a reference, not as it is now in the text. Please have a look at the structure of the introduction. In the beginning, you are discussing diarrheal diseases, and suddenly the text jumps to <i>E. coli</i> as a part of the microbiota. Please insert a shift or paragraph or something.	6. In the results and discussion, last paragraph, testing for verocytotoxicity was mentioned. <i>This has been deleted, as it was mistakenly written</i> 7. The results and discussion should be revised. Please put the results in a table and enumerate the tables. <i>The results and discussion section have completely been revised. Results have also been placed in a tabular format.</i> 8. Figure 2 should be deleted as it does not give any new information and the gel is of a rather poor quality. <i>We do agree that the figure is of poor quality. We have deleted it</i> 9. What does it mean that the <i>E. coli</i> isolates were sulphur negative? This has been corrected as above. "... non-hydrogen sulphide production, positive indole and motility (SIM) test..." We were trying to indicate that based on biochemical tests, <i>E. coli</i> strains were confirmed based on their inability to produce Hydrogen sulphide (H₂S) 10. In the same paragraph it is referred to figure 1 for results of stx1 and 2 genes, but figure 1 is a map. This has been corrected 11. In the results and discussion, it is often referred to stx toxins, f.ex: "three farms were positive for both toxins". I assume that this means toxin genes? <i>We have made the corrections in all places e.g. Three farms were positive for both toxin genes stx₁ and stx₂.</i> 12. The sentence ".... implying that new STEC serotypes might be present in Trinidad". First it should be serogroups, and also there has been described numerous serogroups with stx-genes, but
---------------------------------------	---	--



SDI Review Form 1.6

		one cannot test them all, so to imply “new STEC serogroups” might be too strong. We agree and have rephrased the statement. This now reads as follows “None of the <i>E. coli</i> strains harboring Shiga toxin genes was positive for the serotype analyzed using multiplex PCR for the seven commonly known STEC serogroups (O157, O145, O121, O111, O103, O45 and O26) implying that other STEC serotypes might be present in Trinidad and Tobago. The seven O groups tested for are commonly associated with human infection [8] but not exhaustive, therefore a greater number of faecal samples and broader spectrum of testing could have yielded positive results. Indeed, work by others have reported up to twenty-eight different serogroups associated with STEC [37]. In this present study it was noted that only one isolate was positive for intimin and the rest were negative. Recent studies have shown a trend of stx+ eae- occurrence with a high incidence of exhA presence [37]’.
Optional/General comments		