



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)
<u>Compulsory</u> REVISION comments	<u>All the manuscript</u> The review of writing by an expert in English language is highly recommended. Please, -write genes in italic: <i>eae</i>, <i>stx1</i>, <i>stx2</i> , etc. -do not confuse gene and its expression product (protein), e.g. “<i>eae</i>” and “intimin”. The gene was investigated in this study, the product was not. This flaw is observed along the manuscript. -when it is mentioned by the first time, the full name of microorganism is written followed by the initials “(STEC)”, afterwards continue using only the acronym “STEC”. - always write “<i>stx1</i>”, “ <i>stx2</i>” instead of StxI, StxII, if genes are referred. The toxins are called Stx1 and Stx2. <u>Abstract:</u> Please, -preferably “11% for STEC” should not be used since 9.4% was initially reported. -to improve the sentence: “..ushers a novel contribution to the epidemiology...”	We would sincerely want to thank the reviewer for taken the time to review our paper. We have point by point revised our work and it now makes more sense and interesting to read. An English speaker equally proof read our work. All genes have been written in italics We have made the corrections and pointed out that the genes (<i>stx1</i>, <i>stx2</i>, <i>eae</i>) were determined We have complied with this basic and simple rule We have also made the necessary corrections The correct value of 9.4% have been used



SDI Review Form 1.6

	<u>Introduction</u> This study constitutes an excellent opportunity to summarize previous reports of STEC from different origin in Trinidad and Tobago. Previous STEC isolates from humans, animals or environment sources would support the objectives of the present research. Please, -mention clearly which are the foods of animal origin that could cause STEC infections in Trinidad and Tobago's consumers. The authors can also include comments about the possible modes of STEC transmission from bovine stools to humans to highlight the value of positive results in the present work. Besides beef consumption, working or camping in rural areas and visiting farms are considered risk factors in other countries (Rivas M, Chinen I, Miliwebsky E, Masana M. 2014. Risk factors for Shiga toxin-producing <i>Escherichia coli</i>-associated human diseases. Microbiol Spectrum 2(5):EHEC-0002-2013. doi:10.1128/microbiolspec.EHEC-0002-2013. Page 1, line 28: what does "(EAC)" mean? The extended name should be written prior to the initials. Here, the authors could mention the most frequent STEC serotypes associated with human illness in their country and the world. Page 2, Line 32: "cow and bovine milk", bovines other than	We appreciate the suggestion and the statement now reads as follows '...therefore ushers a novel contribution to the epidemiology of STEC in the country and Caribbean region". We have done this and this section has extensively been revised We have done this. Thanks for the suggested reference which we have now used. Our write up now reads as follows "Besides beef consumption, working or camping in rural areas and visiting farms have been reported or considered risk factors for acquiring STEC infections [7, 14]. Shiga toxin-producing <i>Escherichia coli</i> (STEC) O157 have been shown to be an important cause of illness in the United States [15]. And in Mexico, surveillance studies have reported recovery of non-O157 STEC strains in a large proportion of ready-to-eat meals, suggesting that non-O157 could be a potential source of infection in humans [16]. The presence of STEC in water, cow and bovine milk sources have been established in rural north Trinidad by other researchers [17, 18, 19] and even in marine life or environment in the country [20], but no further studies have been carried out to characterize the virulent genes of <i>E. coli</i> isolated from farm animals" This statement has been deleted The most frequent STEC serotypes causing human illness has been indicated as follows
--	--	---



SDI Review Form 1.6

	cows? Could the authors mention them? Line 35: please, first the paragraph: "The documented presence of STEC in warrants the determination of their prevalence and characteristics" and second the sentence "The presence of STEC in <i>E. coli</i> isolated from farm animals". Line 34: Add references to support the sentence "...documented presence of STEC in milk, ground water and ready to eat items produced in Trinidad and Tobago". Line 40: "virulence genes" instead of "virulent genes" The following article about STEC recovery in Trinidad and Tobago could be cited in Introduction as a contribution to the knowledge of STEC epidemiology in the region: <u>Walker TJ¹, Bachoon DS, Otero E, Ramsubhag A. 2013. Detection of verotoxin producing Escherichia coli in marine environments of the Caribbean. <i>Mar Pollut Bull.</i> 2013 Nov 15;76(1-2):406-10. doi: 10.1016/j.marpolbul.2013.08.019. Epub 2013 Sep 12.</u> <u>Materials and Methods</u> Page 2, Line 45: "randomly selected <u>healthy</u> adult cattle" Line 47: juveniles? Line 49: "were processed for investigation of STEC" instead of "were processed for bacterial organisms"	<i>"Shiga toxin-producing Escherichia coli (STEC) O157 have been shown to be an important cause of illness in the United States [15]. And in Mexico, surveillance studies have reported recovery of non-O157 STEC strains in a large proportion of ready-to-eat meals, suggesting that non-O157 could be a potential source of infection in humans [16]."</i> We have made the corrections and it now reads as follows <i>"The presence of STEC in water, milk and bovine faeces sources have been established in rural north Trinidad by other researchers [17- 19]..."</i> This has been rephrased and reads as follows "As stated above, the documented presence of STEC in milk, ground water and ready to eat items produced in Trinidad and Tobago [17-19] in conjunction with the pathogenic capacity and the propensity to cause an outbreak, warrants the determination of their prevalence and genetic characteristics. Also, several prevalence estimates, ranging from 0.1% to 62% of STEC in cattle has been reported in several countries [21-23] and also in North and South American countries, namely the USA, Mexico, Argentina, Brazil, Colombia, Peru and Venezuela [4, 5, 15, 16, 24 - 29] neighbours to Trinidad and Tobago, but there is paucity of such estimates or information in the country. Thank you. This has been included [20]
--	--	---



SDI Review Form 1.6

	since the presence of other microorganisms was not studied. Lines 50-51: "...using molecular methods for intimin, Stx I or Stx II."? The experimental design in this study aims to demonstrate the presence of the genes, not their expression products. Line 55: delete "amplification" Line 57: "the following compounds:..." Line 58: "in a final 25 µl volume". Add trademark, city and country of chemical compounds. A Table including all the primer pairs used in the amplification of <i>eae</i>, <i>stx1</i> and <i>stx2</i> by PCR is suggested. It could include the following items: gene to amplify, primer forward 5'→3' sequence, primer reverse 5'→3' sequence, amplicon size in bp. Idem for primer pairs used in serotyping PCR and to determine genes <i>escN</i>, <i>espA</i> and <i>tir</i>. Number Table: "Table 1." Lines 69-70: "All isolates positive for the <i>eae</i> gene were further subjected to colony PCR to establish the presence of the Stx-I and Stx-II genes as previously described". This sentence is not consistent with results showed in "Table 1. Distribution of bovine faecal samples and recovered <i>E. coli</i> genes from 177 animal farms in Trinidad and Tobago". For instance, in the row "Kernaham 2 Manzanilla 11 5 4 4 1", there is one STEC strain <i>eae</i>+ but also there are four strains	This has been clarified. <i>"Both the farms and the regional corporation areas were selected from a pool of the list to avoid bias"</i>. Exclusion of juveniles has been explained in lines 153 – 158 of the revised paper This has been corrected This has been corrected Done Done Done Done The table has been created Thanks for the suggestion
--	--	--



SDI Review Form 1.6

	<i>stx1</i> and other four <i>stx2</i> . For a better comprehension, it is suggested to mantain Table 1 with information limited to regional prevalence, and open a new Table containing the 15 STEC strains, the farm or region where each one was isolated, and results corresponding to virulence gene. So, the virulence genetic profile of each strain will be easily visualized. Line 74: "LEE" instead of "LLE", "...to confirm absence or <u>presence</u> of LEE?" Paragraph 2.2 duplicates information included in 2.1? Please, rewrite. Line 92, Statistical analysis: The confidence index (CI) and significance level of <i>P</i> should be reported. This "<i>P</i>" is different to the "<i>P</i>" written in the heading of Table 1. To avoid confusion, "<i>P</i>" in Table 1 should be changed by other letter. <u>Results and Discussion</u> Page 3, Lines 106-108: the sentence "The isolate that tested positive for <i>eae</i> was further screened by PCR amplification and was positive for <i>stx1</i> but negative for the <i>stx2</i> gene as depicted on Figure 1" will be consistent when virulence genetic results of each STEC strain be showed in a new Table as proposed above. Line 108: Figure 1 does not correspond. Line 109: "an overall frequency rate of 9.4 or 11%?"	This has been revised This has been corrected The duplicate has been amended This has been done and included on Table 2. There the STEC acronym and p denoting positive samples and p values have been used. This has been amended This has been corrected The correct value is 9.4. Significant p values, OR and RR have been calculated and included
--	--	---



SDI Review Form 1.6

	The value 9.4% is preferred; please, include the confidence index (CI) and significance level of <i>P</i>. Line 111: "Samples of three farms were positive for both Shiga toxin genes." Lines 109-119: Are there any reports of STEC recovery from foods or clinical cases in these areas? Line 128: "this gave an overall prevalence of 10.9% (.../...)." Please, write the absolute values as seen in other cases. It would be appropriate to emphasize that "Stx 2 is 100-1000-fold more potent than Stx1 (Gyles 2006)". Line 129: compare the present results with reports from other Caribbean regions (Venezuela, Colombia, others), if any. Is the Trinidad y Tobago's cattle imported from surrounding countries where STEC in bovines has been demonstrated? Lines 132-133: "implying that other STEC serotypes..." The word "new" seems to imply unknown serotypes to date. Page 4, Line 138: "stx positive intimin negative" could be written "stx⁺ eae⁻", when genes are mentioned. Line 151: the sentence "The presence of non-pathogenic bacteria can also influence the production of the Shiga toxin" should be supported by references. Lines 158-159: "In our study, testing for verocytotoxicity	in the text. This has been corrected and now reads as follows "Samples from three farms produced STEC that were positive for both toxin genes <i>stx</i>₁ and <i>stx</i>₂" Not to the best of our knowledge This has been done We have included this statement and reference. Thanks We have done so with data from Argentina, Brazil, Venezuela etc, not too distant neighbors to Trinidad and Tobago Not really Done Done Done Deleted
--	--	--



SDI Review Form 1.6

	was done with Vero cell monolayers on cultures positively identified as O157", add "(data no shown)". If possible, add Figure with results of PCR for <i>stx1/stx2</i>.	Not possible as we did not have good quality picture
<u>Minor</u> REVISION comments	<u>References:</u> Please, review details of cite transcription, fit the journal style and when possible, include references published after 2010.	We have done so
<u>Optional/General</u> comments	- a recently published article in AJEA should be used as a format and style guide for the present manuscript.	We have done so too