1.

OCCURRENCE, VIRULENCE AND PATHOGENICITY OF *ESCHERICHIA* AND *SALMONELLA* FROM KALES IN NAIROBI AND ITS ENVIRONS

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2.
INTRODUCTIONAND LITERATURE REVIEW

- Vegetables play a role in nutrition as healthy diets and contribute to the economy of many countries.
- Consumers are encouraged to eat more of these products.
- In Kenya vegetables are consumed regularly by nearly every household in rural and urban areas.
- In Nairobi the vegetable sector has developed within the city and surrounding areas with kale (*sukuma wiki*) being the most grown vegetable.
- Kale are contaminated by microbial flora along food chain (farm to the table)

3.
Sources of contamination

- **Pre-harvest contamination sources:**
  - Feces
  - irrigation water
  - inadequately composted manure
  - wild and domestic animals
  - human handling

4.
Sources of contamination (cont.)

- **Post harvest contamination sources:**

- Feces
- human handling/cross-contamination
- harvesting equipments
- wash and rinse water
- transport vehicles and containers
- improper packaging
- wild and domestic animals
- Vermin and insects

5.
Urban agriculture in Nairobi

- Use of waste water and 'night soil' in peri-urban agriculture in Nairobi is extensive and unregulated (Kang'ethe *et al.*, 2007)
- 36% of the farmers in the city use sewage and waste water for irrigation subjecting the vegetables to contamination by human pathogens (Njenga *et al.*,2007)
- Farmers use untreated waste water to grow vegetables and have been reported to block the sewers to get the water
- Water samples from Nairobi rivers show fecal coliforms exceeds 1000FC/100ml recommended levels by WHO for unrestricted irrigation
- Potential health threats posed by use of polluted water has been raised but there is scanty information on specific pathogens (Hide, 2001)

6.
Possible vegetable contaminants

- Traditionally vegetables are considered low risk foods due to their low pH and natural barriers to pathogens.
- Currently increased disease outbreaks occur due to evolution of more pathogenic forms of bacteria that can now survive such conditions
- The pathogenic bacteria of major concern are: *Salmonella*, *Shigella*, *Escherichia* and *Klebsiella.* In other countries, *Salmonella* and *Escherichia (*serotype O157:H7) are commonly isolated**.**

7.
E.Coli and *Salmonella*

- Coliforms are a good indicator of fecal contamination and poor hygiene.
- *Escherichia coli* has been used as a non-pathogenic indicator of enteric pathogens, such as *Salmonella*.
- Some strains of *E.coli* are virulent:

1) Enterohemorragic (EHEC)
2) Enteroinvasive (EIEC)
3) Enteroaggregative (EaggEC),
4) Enteropathogenic (EPEC), and
5) Diffusely adherent (DAEC):

8.
E.Coli and *Salmonella* (cont).

- The worst of these is EHEC, O157:H7

Salmonella

- There are more than 2400 known serotypes, grouped in the two species *bongori* and *enterica*
- Have been associated with animal products but has been isolated from many types of vegetables grown in contaminated sites
- Recently *Salmonella* and *E. coli* O157:H7 have been isolated with higher frequency from fresh farm produce
- There is limited data available on level of vegetable contamination by these pathogens in Kenya.

9.
Salmonella and *Escherichia* in non-host environment

- Both *Salmonella* and *Escherichia* can survive well in the animal host.
- Outside the animal host they face limited nutrients, osmotic stress, large variation in temperature and pH
- Non-host environment has been shown to lower pathogenicity of these microorganisms
- However there is little documentation on this.
- There is, therefore, need to compare organisms isolated from kales and those from human origin with respect to their pathogenicity and hematological changes

10.
Pathogenicity of *Salmonella* and *Escherichia* in mice model

- Various animal models have been used to study the pathogenicity of bacterial isolates
- Mice have been used extensively to study pathogenicity of human enteric pathogens (Donald *et al*.,1984)

11.
Objectives

Overall objective

- To determine the occurrence,virulence and pathogenicity of *Escherichia* and *Salmonella* from kale in Nairobi and its environs

Specific objectives

- To determine the occurrence of *Escherichia* and *Salmonella* species on urban-farmed and market kales.
- To assess bacteriological quality of kale and water used for growing and washing/refreshing of market kales.
- Compare virulence and pathogenicity of recovered *Salmonella (S.*Typhimurium and *S.*Enteritidis*)* isolates with human clinical strains

12.
Hypothesis

- **Null hypothesis-Ho:** There are no *Escherichia* and *Salmonella* contaminants on kales and if present, they are not pathogenic to mice.
- **Alternate hypothesis-H_A:** There are *Escherichia* and *Salmonella* contaminants on kales and are pathogenic to mice.

13.
Justification for the study

- Rapid population growth in Nairobi city has led to high demand for vegetables such as kales. Farmers in trying to meet this demand have resorted to production of these vegetables under informal irrigation
- This practice, coupled with poor hygiene practices amongst those concerned along the distribution chain, pose a great risk of pathogenic infections to farmers and consumers alike.
- There is scanty information on contamination level of vegetables by pathogens such as *Salmonella* and *Escherichia* as well as their pathogenic characterization.
- This information is important to health policy makers responsible for protecting the public. It will help them in formulating respective control strategies

14.
MATERIALS AND METHODS

Sites

Purposive sampling will be carried out in the following sites:

- **Farms-**Athi River, Ngong and Wangige due to increased vegetable production using waste water
- **Markets-**Kawangware, Githurai, Kangemi,Uchumi (Sarit and Buruburu) and Zucchini grocery. This markets were chosen for comparison of high and low class markets
- Human-clinical *S.*Typhimurium/Enteritidis will be obtained from KEMRI and will include respective case history

15.
Sample size

- Sample size calculation will be done as described by Pfeiffer (2002).
- In total 150 vegetable samples and 150 water samples will be collected from the identified farms and markets in Nairobi and its environs

16.
Sampling method

- Pre-survey will be done and the list of farmers and traders obtained. Samples will be picked from every other third farmer and trader.
- 500g of leaves will be randomly picked from each of the farms and display units at the markets; put in sterile paper bags
- Water samples (500ml) will be collected aseptically into sterile sample bottles
- The samples will be put into cool boxes and transported immediately to laboratory for bacterial analysis.

17.
Isolation and characterization of isolates

- Isolation and characterization of pathogens will be done according to Bacteriological Analytical Manual (FDA, 2007)

18.
VIRULENCE TESTING

- This will be done through determination of LD_{50}/ID_{50}
- LD_{50}/ID_{50} determination of the isolates and reference strain will be done according to Reed and Muench (1938)

19.
Pathogenicity testing

- Mouse model will be used to establish the pathogenicity of the most virulent *S*.Typhimurium/Enteritidis isolate by determining:
  - PM lesions
  - Liver/ body weight ratio
  - Hematological parameters
- The dose will be 2x LD50 of the respective isolates
- 0.2ml will be inoculated intraperitoneally

20.
Pathogenicity testing (cont.)

- Sampling intervals-0, 1, 3, 7, 14, 21, and 28 days post inoculation
- Two mice sacrificed each sampling time
  - Blood collected
  - Body weight
  - Liver weight
  - PM lesions
  - Liver- bacterial isolation

21.
Hematological parameters to be determined

- Packed cell volume
- Haemoglobulin content
- Total leukocyte count
- Differential cell count
- Erythrocyte sedimentation rate

22.
Statistical analysis

Data collected will be entered in microsoft excel. Statistical analysis will be done using t-tests and Analysis of Variance (ANOVA).

23.
Workplan.

Activity	Feb 2010	Mar 2010	Apr 2010	May 2010	Jun 2010	Ju 2010	Aug 2010	Sep 2010
Proposal writing and presentation								
Procurement of materials, reagents and Logistics								
Sample collection and analysis								
Data analysis								
Thesis writing								

24.
BUDGET

ITEM	TOTAL COST (KSh)
Laboratory reagents and materials	70 000
Equipment	50 000
Per diem	30 000
Field work	40 000
Proposal and thesis writing	10 000
5% contingencies	10 000
Total	210 000

END

THANK YOU!