

RISK OF INFECTION FROM *E. coli* 0157:H7 THROUGH INFORMALLY MARKETED RAW MILK IN KENYA.

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Paper prepared for ORAL PRESENTATION at the 3rd All Africa Conference on Animal Agriculture. 6 – 9 November 2000.

Summary

E. coli 0157:H7 is a newly recognised bacterial zoonosis that originates from the gut of infected cattle. It causes potentially fatal haemorrhagic enteritis, haemolytic uraemic syndrome and kidney damage in humans. Epidemiological data on *E. coli* 0157:H7 infection and transmission in developing countries remain scarce but it is suspected that consumption of unpasteurised milk is an important vehicle for its transmission to humans, as milk can easily be contaminated with cattle faeces during milking. Given the high proportion of informal sales of unpasteurized milk in many tropical countries, *E. coli* 0157:H7 has been one of several zoonoses of concern.

Between January 1999 and January 2000, survey data and raw milk samples were collected seasonally from households consuming unpasteurised milk in rural and urban locations in central Kenya. Respondents were randomly selected within production system (extensive and intensive) and human population density (urban, peri-urban and rural) strata. Laboratory samples were assessed for bacteriological quality by total and coliform counts. Selective media were used sequentially to screen for faecal coliforms and *E. coli* 0157:H7. Suspect *E. coli* 0157:H7 colonies were also serotyped and tested for production of verocytotoxins.

E. coli was recovered from 91 out of 264 samples (34%) and *E. coli* 0157:H7 serotype identified in two samples (<1%). One of the two isolates produced verocytotoxins. As in many studies, the recovery rate of this serotype was low, but the finding is significant from a public health perspective. Our consumer studies have shown that over 95% of consumers of unpasteurised milk boil the milk before consumption and potential health risks from this zoonosis are therefore quite low. As informal milk markets without pasteurisation technology are likely to remain dominant for the foreseeable future, there is the need to further emphasise the importance of boiling raw milk before consumption, especially among pastoral communities where this practice is not common.

Key words: E. coli 0157:H7, unpasteurised milk marketing, Kenya

Introduction

Since the first reported foodborne illness associated with *Escherichia coli* 0157:H7 (*E. coli* 0157:H7) in 1982 in Michigan and Oregon, USA (Riley *et al.*, 1983), the organism has been isolated from a variety of foods and from cattle faeces in many countries (Jay, J.M., 1992; Abdul Tapif *et al.*, 1996; Youko Miyao *et al.*, 1998; Aloysio *et al.*, 1999). Accumulated research data have led to the recognition of this organism as an important foodborne pathogen and a zoonosis. Otherwise known as enterohaemorrhagic *E. coli* (EHEC), *E. coli* 0157:H7 causes haemorrhagic colitis (HC) leading to bloody diarrhoea and haemolytic uraemic syndrome (HUS) in humans due to the production of potent verocytotoxins; HUS is associated with serious kidney damage and renal failure (Jay, 1992; Besser *et al.*, 1993).

Human infection is associated with the consumption of a number of contaminated foods among them meat, especially undercooked ground beef, raw milk, yoghurt, salamis, cheese and unpasteurised apple cider (Riley *et al.*, 1983; Doyle and Shoen, 1984; Doyle, 1992; Tildenet *et al.*, 1996). Human beings and cattle carry the pathogen in their intestines and faeces are therefore a source of contamination of foods, water and the environment. The faeces and bacteria may contaminate udders and milking

equipment and get into the milk during milking and handling if adequate hygiene practices are not observed.

Most of what is known about *E. coli* 0157:H7 has emanated from developed countries. In Kenya, there is little information on the organism and the role milk and other foods play in its transmission. Milk is widely consumed in Kenya, mostly in its natural liquid form, or fermented, or in tea. Sales of raw (unpasteurised) milk captures over 85% of the marketed milk (Omore *et al.*, 1999). Since the liberalisation of milk marketing in the country in 1992 and subsequent increased sales of unpasteurised milk to urban consumers, concerns have been raised regarding transmission of foodborne diseases to consumers. As part of a larger consumer and milk market study, the microbiological quality of unpasteurised milk purchased by consumer households was assessed and coliform isolates screened for *E. coli* 0157:H7 to establish its occurrence and evaluate potential human health risks. Consumer practises that may reduce the risks of infection were also studied.

Materials and Methods

Between January 1999 and January 2000, 212 and 222 raw (unpasteurized) milk consuming households were surveyed in the dry and wet season, respectively. Respondents were randomly selected within production system (extensive and intensive) and human population density (urban, peri-urban and rural) strata. The geographical units selected from each site also covered a variation from low to high income classes. Nakuru district represented extensive production systems and low population density (also medium market access). Nairobi represented intensive production systems and high population density (also high market access). Attempts were made during the second (wet) season to interview and sample the same respondent as in the first (dry) season. Where this was not possible, substitution was made within the same locality. *E. coli* 0157:H7 was isolated after screening for coliforms by plating and counting of colony forming units (c.f.u). Milk samples were collected in sterile 50ml plastic tubes in the mornings and transported to the laboratory in ice-cooled boxes. Analysis commenced within six hours of sample collection. This report covers results from 264 samples that were processed and that had plates with coliform colony forming units.

Sample preparation and culture for bacteriological quality assessment.

For each sample, tenfold serial dilutions (10^{-1} to 10^{-7}) were prepared in sterile phosphate buffered water diluent (0.0425g of potassium dihydrogen phosphate per litre (final concentration) of distilled water), pH 7.2. Dilutions to culture for total counts and coliform counts were based on the expected microbial load in the samples.

Total plate count and coliform count

One millilitre of 10^{-4} to 10^{-7} dilutions of milk was pipetted into 90mm diameter disposable petri dish and mixed well with 20 ml of sterile standard plate count (SPC) agar (APHA; Oxoid). The SPC agar was prepared by dissolving 23.5g of powder in one litre of distilled water, sterilised by autoclaving at 121°C for 15 minutes and cooled to 45° – 47° in a waterbath. The sample cultured for total counts was also cultured for coliform counts. Sample dilutions from 10^{-1} to 10^{-4} were cultured; 10^0 and 10^{-5} dilutions were sometimes included. One millilitre of each dilution was pipetted into 90 mm diameter disposable petri dish and mixed with about 20 ml of violet red bile (VRB) agar (Oxoid). After cooling and solidification of the medium, all the plates were covered with a thin layer of the same VRB agar medium. The medium was prepared according to the recommendations of the manufacturer by suspending 52g of powder in one litre of distilled water, bringing it to boil to dissolve completely and cooling to 45° – 47°C in a waterbath.

After cooling and solidification of the poured media, SPC and VRB agar plates were incubated inverted, at 32°C for 48 hours for total counts and at 37°C for 24 hours for coliform counts. SPC agar plates with countable colonies between 25 and 250 c.f.u./plate and VRB agar plates with countable colonies between 15 to 150 c.f.u./plate were chosen for counting with the aid of colony counter (Gerber).

Screening for E. coli 0157:H7

After counting the number of coliforms for a sample, the coliform c.f.u were examined for the presence of *E. coli*. In order to increase chances of detecting *E. coli* and strain 0157:H7 in particular, up to six colonies per plate were purified on MacKonkey agar (Oxoid) and differentiated for *E. coli* by plating

on eosine methylene blue agar (Oxoid) and testing suspect colonies for indole, methyl red, vogues proskauer and citrate (IMViC) reactions. Identified *E. coli* isolates were further cultured by streaking onto selective indicator Biosynth culture medium (BCM™ 0157:H7(+)) (Biosynth Biochemica, Biosynth International Inc., USA) and incubated at 35°C for 24h for identification of blue black colonies of *E. coli* 0157:H7. The BCM™ 0157:H7(+) medium was prepared according to the instructions of the manufacturer. Briefly, 80g of the powder was dissolved completely in 1 litre of distilled water containing 5ml N, N-dimethylformamide (Sigma). After cooling to 50°C in a water bath, 5ml of 0.2% (w/v) sodium novobiocin (Sigma) and 0.2ml of 0.1% (w/v) potassium tellurite (Sigma), both filter sterilised, were added to the medium, mixed and the medium poured into petri dishes. It was then allowed to solidify and dry at room temperature.

Serogrouping of isolates

Blue black colonies on BCM™ 0157:H7(+) medium were cultured onto non-selective tryptose soy agar (Oxoid) and serogrouped using latex slide agglutination test (oxoid) to confirm that they were *E. coli* 0157:H7 and hence potential producers of verocytotoxin (VT). Latex beads coated with specific rabbit antibody reacts with the 0157 somatic antigen causing agglutination.

Test for production of verocytotoxins

The organisms were cultured onto brain heart infusion agar (Oxoid) at 37°C for 24h and toxins extracted from the growth using polymyxins B sulphate (sigma) solution. The polymyxin B extracts were tested for VT1 and VT2 in V-bottom microtitre plates using reverse passive latex agglutination (RPLA) test kit (Oxoid).

Results and Discussion

Total counts and coliform counts

The bacteriological quality of the milk (total viable counts and coliform counts) was interpreted according to the Kenya Bureau of Standards (KEBS) guideline specifications for whole unpasteurized milk (1976). According to the standard, milk containing a total bacterial count of up to 1 million per millilitre is classified as very good; 1 million to 2 million as good; 2 million as bad and >5 million as very bad. Similarly, milk containing coliform counts up to 1000 per millilitre is classified as very good; 1000 to 50,000 as good; 50,000 to 500,000 as bad and >500,000 as very bad. Milk classified as bad is not acceptable within the regulations for marketing.

Eighty six percent of the milk samples in Nairobi and 88% in Nakuru urban had total counts of >2 million/ml with no significant difference between the two towns (Table 1). A repeat sampling in Nairobi showed the same high proportion (85%) of unacceptable bad quality milk. In Nakuru rural a fairly high proportion of milk, but relatively less than that from Nairobi and Nakuru, had total counts >2 million/ml. The results of the coliform counts showed a picture similar to total counts in both urban and rural areas. In Nairobi, 46% of the milk had coliform counts >50,000/ml and in Nairobi urban 45%. Repeat sampling in Nairobi showed an increase in count from 46% to 71%. These high counts show that milk bought by households for consumption in the two urban centres is of poor bacteriological quality. By contrast, only 12% of the milk from Nakuru rural had coliform counts >50,000/ml showing that most of the milk was of good quality.

Table 1: Milk samples from consumer households containing unacceptably high total and coliform bacterial counts

District/area of Study	Samples with unacceptable high counts			
	Total Counts >2,000,000 c.f.u. ^a /ml		Coliform counts >50000 c.f.u./ml	
	n	%	n	%
Nairobi urban (dry season)	49	86	46	46
Nairobi urban (wet season)	53	85	52	71
Nakuru urban (dry season)	58	88	58	45
Nakuru rural (dry season)	104	41	104	12

^ac.f.u. = colony forming units

The high number of bacteria in raw milk is a reflection of poor production and handling hygiene during milking, transportation to the market, storage at selling points and even at home. Initial loads at the production stage may be high. Unsanitary handling during transportation from source to sale points may add to the contamination. Coupled with these, long holding times in warm tropical weather by vendors and even by households before pasteurisation or boiling encourages rapid microbial multiplication. In the areas studied and particularly in the urban centres, milk goes through a number of handling stages without adequate control of hygiene or cooling and this favours contamination and multiplication of bacteria in the milk before the household buys it. Many households in the urban centres, and especially those with low incomes, buy small quantities of raw milk from traders or from nearby milk shops, milk bars, kiosks, and street vendors (stationary or mobile on bicycles or motorised vehicles). Most households lack cooling facilities and use plastic containers, which are difficult to clean. The distances travelled and/or the time spent on the way from producer to consumer is sometimes long. All these factors contribute to the poor bacteriological quality of the milk.

In Nakuru rural, milk was relatively of better bacteriological quality (Table 1). Although cooling facilities were not readily available, time spent from producer to consumer was generally shorter than in the urban centres especially in Nakuru. Some of the respondent households were also milk producers themselves, consuming some and selling the remainder to neighbours.

E. coli 0157:H7

A total of 264 milk samples that were cultured for coliform counts yielded 845 coliform colonies that were screened for *E. coli* and subsequently *E. coli* O157:H7. Three *E. coli* isolates from three different samples, one from Nairobi and two from Nakuru urban, produced blue black colonies on BCMTM 0157:H7 medium and were regarded highly suspect for strain 0157:H7 (Table 2). Two of these three isolates reacted positively with 0157:H7 specific antibodies. Thus two isolates (one from Nairobi and one from Nakuru urban) out of 264 milk samples were serologically confirmed to be *E. coli* 0157:H7. This translates to a recovery rate of 0.8%. The organism is a rare strain among a huge population of *E. coli* organisms (Jay, 1992) which therefore requires examination of a large number of isolates in order to detect it. The finding is, however, significant considering the importance of the pathogen in causing haemorrhagic colitis with bloody diarrhoea and haemolytic ureamic syndrome which often leads to kidney failure (Jay, 1992; Besser *et al.*, 1993). One of the isolates from Nakuru urban produced verocytotoxin one (VT1). The finding is also significant considering the low infective dose of 700 organisms or less (Turtle and Gomez, 1999) and, in this case, the large number of people who buy unpasteurised milk for consumption. They, if consuming unboiled or unpasteurized contaminated milk, stand a high risk of getting infected. Fortunately, over 95% of the households boil milk before consumption, which destroys this and other pathogens. Consumers should therefore protect the milk from recontamination after the heat treatment.

Table 2: Numbers of unpasteurised consumer milk samples screened and isolation and identification of *E. coli* 0157:H7

Milk sample and test details	Number			
	Nairobi urban	Nakuru urban	Nakuru rural	Total
Examined for coliforms	102	58	104	264
Positive for <i>E. coli</i>	37	21	33	91
Suspect <i>E. coli</i> 0157:H7 on BCM TM medium	1	2	0	3
Serologically confirmed <i>E. coli</i> 0157:H 7	1	1	0	2
Verocytotoxin1 producing <i>E. coli</i> 0157:H7	0	1	0	1

It is clear from the total bacteria and coliform counts that milk sampled from consumer households, particularly in the urban centres, had heavy loads of coliforms. Consequently, faecal *E. coli* is expected to be in high numbers, which increases the chances of some milk being infected with strain 0157:H7. Since the milk were from consumers, it is difficult at this point to indicate the main sources and entry points of 0157:H7 into the milk. However, contamination with cattle or human waste and contaminated water (Jay, 1992; Cobbold and Desmarchelier, 2000) at the different stages of handling (farm level, market level and consumer level) are the broad possible sources. In many areas, there have been difficulties with obtaining water, especially in the dry seasons. At the farm level, besides cows faeces, *E. coli* mastitis could contribute to the presence of 0157:H7 in milk. Unhygienic handling and

infected handlers may also contaminate marketed milk. Since the results under discussion are only a part of an ongoing study, a clearer picture of the occurrence of *E. coli* O157:H7 in the milk will emerge after completion of the market and farm level studies.

In Kenya, diarrhoea is one of the commonest diseases of children caused by a variety of pathogens including *E. coli* (Sang *et al.*, 1992). Cases of kidney failure in humans are fairly common. The role of *E. coli* O157:H7 in the causation of enteritis and renal failure in Kenya is yet to be established and needs to be given attention. One of the O157:H7 isolates from the milk produced verocytotoxin and these toxins are associated with kidney damage and kidney failure. As far as we know, this is the first time *E. coli* O157:H7 has been isolated from milk in Kenya. Its origin could have been from cows or from human beings.

Acknowledgements

This research was funded by the Smallholder Dairy (R&D) Project (SDP) of the Kenya Ministry of Agriculture and Rural Development, KARI and ILRI. SDP is funded by the UK Department for International Development (DFID). The views expressed are those of the authors and not necessarily those of DFID or SDP.

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