

First isolation of *Escherichia coli* O157:H7 from faecal and milk specimens from Anatolian water buffaloes (*Bubalus bubalus*) in Turkey

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ABSTRACT

Three hundred rectal faecal samples and 213 raw milk samples obtained from the tanks and containers were examined using standard cultural methods. *Escherichia coli* O157:H7 was isolated from 11 (3.7 %) of 300 faecal samples and 3 (1.4 %) of 213 raw milk samples. It was determined that 8 (73 %) of *E. coli* O157:H7 strains isolated from faecal samples originated from water buffaloes younger than 2 years of age and 3 (27 %) from 2-year-old and older water buffaloes. This is the 1st isolation of *Escherichia coli* O157:H7 from faecal and milk samples of water buffaloes in Turkey.

Key words: *E. coli* O157:H7, faeces, isolation, milk, water buffalo.

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MATERIALS AND METHODS

Collection of specimens

The study was carried out on healthy Anatolian water buffaloes kept at the Ministry of Agriculture and Rural Affairs Kocatepe Agricultural Research Institute (KARI) and private breeding farms in Afyon region. KARI is a large state agricultural enterprise. Private breeding farms were located in 3 villages, Akçin, Küçük Çobanlar and Erenler. They are small farms belonging to villagers and have no connection with each other. The animals kept at the state-owned enterprise had a grain-based diet, while the diet of the animals kept at private breeding farms was hay-based. Water buffaloes were classified into 2 groups: 2 years and older ($n = 176$), and younger than 2 years of age ($n = 124$).

A total of 300 faecal samples from 158 healthy water buffaloes at KARI, and from 142 healthy water buffaloes on private farms were collected between July 2002 and July 2003. In order to prevent cross-contamination, samples were collected from the rectum with a change of gloves before the rectal examination and sampling of each animal. Faecal samples were transferred into single-use plastic containers and transported to the laboratory in a cool box on ice. During the same period a total of 213 raw milk samples were collected from 120 healthy water buffaloes milked with milking machines at KARI. A further 93 milk samples were collected from healthy water buffaloes that were milked by hand on private farms (Table 1). Samples were obtained from the tanks and containers after milking and not directly from the teats. Milk

INTRODUCTION

Escherichia coli was considered only to be an index of faecal contamination until the late 1950s, but this perspective changed with the discovery of pathogenic types that could cause deaths in both humans and animals^{8,21}. *E. coli* serotype O157:H7 was isolated for the first time from a female patient displaying symptoms of haemorrhagic diarrhoea in California in 1975²³. Epidemics during 1982 in Oregon and Michigan in the United States yielded similar strains^{2,15,24}. *E. coli* O157:H7 belongs to the enterohaemorrhagic *Escherichia coli* (EHEC) group. It was subsequently identified as a significant human pathogen that causes haemorrhagic colitis (HC), haemolytic uraemic syndrome (HUS), haemorrhagic or nonhaemorrhagic diarrhoea and thrombotic thrombocytopenic purpura (TTP), and may have lethal consequences. This serotype has been considered to be one of the most important pathogens of food-borne infections in the world in the recent years, posing a great challenge to public health^{19,24}. Ruminants, especially cattle, are accepted as the primary reservoirs for this serotype and consumption of foods originating from or contaminated by cattle are

connected to serious epidemic outbreaks^{7,30}.

Studies investigating the frequency of *E. coli* O157:H7 serotype in water buffaloes are limited. Anatolian water buffaloes are classified as a river subtype of the Mediterranean water buffalo group. According to the 2004 data of the Food and Agriculture Organization of the United Nations (FAO), populations of 9 800 000 cattle and 136 000 water buffaloes exist in Turkey¹³. Afyonkarahisar is located in the western region of Turkey and is becoming significant as a breeding area for water buffaloes owing to suitable conditions. Clotted cream, skimmed from the milk of water buffalo, is a sought-after Turkish product. Raw milk, not properly heated while skimming the cream, can pose a potential hazard for public health.

The aim of this study was to examine faecal and milk specimens collected from breeding stocks of Anatolian water buffaloes in Afyon region in Turkey for the presence of *E. coli* O157:H7.

Table 1: Number and sources of faecal and milk samples collected.

Sources of specimens collected	Isolation/ Faecal specimens (n)	Isolation/ Milk specimens (n)
Ministry of Agriculture and Rural Affairs Kocatepe Agricultural Research Institute	10/158	2/120
Akçin Village	1/74	1/51
Küçük Çobanlar Village	0/36	0/28
Erenler Village	0/32	0/14
Total	11/300	3/213

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samples were transferred to sterile glass bottles and transported to the laboratory in a cool box on ice. Samples (milk and faeces) were only collected once from each of the water buffaloes sampled.

Isolation of *E. coli* O157:H7 from faecal and milk samples

EHEC O157:H7 strain EDL 931²⁷, which produces Stx 1 and Stx 2, was used as a control in this study.

In order to isolate *E. coli* O157:H7 from water buffalo faeces and milk, the stages of pre-enrichment and inoculation onto selective differential solid medium were performed. The method recommended by Wells *et al.*²⁹ was modified in pre-enrichment procedures for faecal specimens. Novobiocin (20 mg/) (Oxoid SR0181) was added to modified Tryptone Soy Broth (mTSB, Oxoid CM989) to use as a medium of pre-enrichment. One gram of faeces was transferred into tubes containing 9 m mTSB and homogenised by vortexing for 5 minutes at 120 rpm. The vortexed mixture was incubated at 37 °C for 6 hours. The same pre-enrichment procedure was performed for the milk samples. Twenty-five m of milk were added to glass flasks containing 225 m mTSB. The glass flasks were placed on the shaker and incubated for 24 hours at 37 °C¹¹. Cefixime-Tellurite (Oxoid SR0172) added to sorbitol MacConkey agar (SMAC, Oxoid CM981) containing 5-bromo-4-chloro-3-indolyl- β -D-glucuronide (BCIG) was used as the selective differential solid medium; 0.1 m was taken from pre-enrichment liquid medium and sub-inoculated onto CT-SMAC agar. The solid medium was incubated at 37 °C under aerobic conditions for 24 hours. Lack of sorbitol fermentation and β -glucuronidase enzyme activity was noted in colourless colonies growing on CT-SMAC agar, and these were regarded as suspect³².

Identification of *E. coli* O157:H7

Gram staining, oxidase, indole, methyl red (MR), Voges-Proskauer (VP), citrate, urease, hydrogen sulphide (H₂S), lysine decarboxylase, glucose, sucrose, lactose, salicin, adonitol, inositol, cellobiose, arabinose, trehalose, mannitol, rhamnose, xylose, raffinose and dulcitol tests were performed to identify suspected colonies^{18,25}. The *E. coli* O157 Latex Test (Oxoid, DR620M) and immobilization test¹² by H7 antisera (Difco, 221591) were performed on isolates. Additionally, the enterohaemolysin test on 5 % defibrinated sheep blood agar, washed in buffered peptone water, was performed on all isolates confirmed as *E. coli* O157:H7⁴.

Statistical analysis

Chi-square tests were used to determine the relationship between the presence of *E. coli* O157:H7 in faecal and milk samples.

RESULTS

E. coli O157:H7 was identified from 11 (3.7 %) of 300 rectal faecal sample and 3 (1.4 %) of 213 milk samples. Ten (90 %) of the strains isolated from the faecal samples originated from water buffaloes at KARI and 1 (10 %) from a water buffalo from a private breeding farm in Akçin Village. Two (67 %) of the strains isolated from raw milk originated from milk obtained from water buffaloes at KARI and 1 (33 %) from milk obtained from a water buffalo from a private breeding farm in Akçin village (Table 1). There was no statistically significant difference ($P < 0.05$) between the presence of *E. coli* O157:H7 in faeces and milk samples. Additionally, 8 (74 %) of *E. coli* O157:H7 strains isolated from faecal samples were recovered from water buffaloes younger than 2 years and 3 (27 %) from 2-year-old and older water buffaloes. Three (100 %) of *E. coli* O157:H7 strains isolated from milk samples originated from water buffaloes older than 2 years.

Oxidase, H₂S, citrate, VP, urease, salicin, adonitol, inositol, cellobiose reactions of all isolated strains were determined to be negative; motility, indole, MR, lysine decarboxylase tests and glucose, sucrose, lactose, arabinose, trehalose, mannitol, rhamnose, xylose, raffinose and dulcitol fermentations were positive. Also, all strains produced enterohaemolysin (Table 2).

DISCUSSION

Ruminants, especially cattle and dairy cows, are the primary reservoirs for *E. coli* O157:H7. Low and high levels of prevalence have been reported in various isolation studies concerning these animals. The prevalence of *E. coli* O157:H7 has been reported to vary between 2 % and 45 % in the United States²⁹, 4 % and 15.7 % in England⁷ and the rate has been stated to be between 1 % and 13 % in various European countries⁶. *E. coli* O157:H7 has been detected in live cattle and cattle carcasses in Turkey and the significance with respect to public health has been emphasised³¹. However, studies concerning the isolation of this pathogen from water buffaloes are limited.

Mohammad *et al.*²⁰, the first researchers to have shown the presence of VTECs in water buffalo calves with diarrhoea in Sri Lanka, reported that *E. coli* O157 serotype was not found among the isolated verotoxigenic *E. coli* (VTECs). Another

Table 2: Biochemical characteristics of *Escherichia coli* O157:H7 strains isolated from faecal and milk samples.

Tests	Results
Gram stain	Gram-negative
Oxidase	–
Motility	+
Indole	+
MR	+
VP	–
Citrate	–
Urease	–
TSI agar	Glucose
	Sucrose
	Lactose
	H ₂ S
Lysine decarboxylase	+
Salicin	–
Adonitol	–
Inositol	–
Cellobiose	–
Arabinose	+
Trehalose	+
Mannitol	+
Rhamnose	+
Xylose	+
Raffinose	+
Dulcitol	+
Enterohaemolysin	+

study analysed rectal faecal specimens collected from 289 breeding water buffaloes in Italy and 42 (14.5 %) isolates of verotoxin-producing *E. coli* O157 were made. It was stated that water buffaloes could be reservoirs, like other ruminants, for VTECs¹⁴.

In this study 300 faecal samples collected from clinically healthy water buffaloes were examined for the presence of *E. coli* O157:H7. Of the specimens examined 11 (3.7 %) were positive for *E. coli* O157:H7. This is the first report of *E. coli* O157:H7 from faeces of water buffaloes in Turkey. The 3.7 % isolation rate in this study was lower than the rate of isolation (14.5 %) obtained by Galiero *et al.*¹⁴ in Italy. The difference between the methods of isolation and geographical variations may be the cause of the discrepancy.

Beutin *et al.*⁵ reported that there was a close association between enterohaemolysin and VT production in strains of *E. coli*. They emphasised that determining enterohaemolysin was a useful tool for the rapid detection of VT-positive strains. In the present study, enterohaemolysin was shown to be produced by all strains.

Besser *et al.*³ have shown that *E. coli* O157:H7 seems to colonise cattle only transiently and long-term carriers have not been found. The shedding of *E. coli* O157:H7 in herds of cattle is also intermittent and, during the majority of sampling visits, the organism cannot be detected¹⁶. In this study it was impossible to categorise

the *E. coli* O157:H7-positive animals as temporary or permanent carriers because they were only sampled once.

Several factors can influence the faecal shedding and prevalence of *E. coli* O157:H7 in cattle. Examples are age, diet, breed, season, immune response and competing microbial flora. Young animals appear to carry *E. coli* O157:H7 more frequently than adults^{16,33}. In another study⁹ it was found that after experimental infection of calves and adult cattle with *E. coli* O157:H7, calves remained positive for longer periods. Wells *et al.*²⁹, in a study where they classified the animals into age-groups as calves, heifers and adult cows, reported that *E. coli* O157:H7 was isolated from the faeces of 17 (2.8 %) of 604 calves and heifers and only from 1 (0.15 %) of 602 adult cows. In another study¹⁴ 289 faecal samples from water buffaloes between the ages of 6 and 18 months were examined. Isolations were generally made from the younger animals. In this study, 8 (73 %) of the isolated strains were from water buffaloes younger than 2 years old and 3 (27 %) from 2-year-old and older water buffaloes. The longer duration of faecal shedding in young animals may be attributed to higher prevalence. It is reported that in the fully-developed rumen of adult cattle the combination of high concentrations of volatile fatty acids and a low pH inhibit the growth of *E. coli* O157:H7²⁶. In the present study, the age-related differences in rumen function may be another factor that influences the higher prevalence in young water buffaloes.

Diez-Gonzalez *et al.*¹⁰ reported that cattle fed grain diets have large numbers of acid-resistant *E. coli* organisms in their faeces, while cattle fed hay diets do not. In this study, 10 (90 %) of the strains isolated from the faecal specimens originated from the grain-fed water buffaloes at KARI and only 1 (10 %) from a water buffalo from a private breeding farm on a hay-based diet. This difference may explain the higher isolation rates in the state-owned buffaloes.

The relationship between the presence of *E. coli* O157:H7 in the milk of cows and prevalence of disease in humans due to consumption of raw or pasteurised milk is of great significance to the dairy industry²⁸. In various studies on the prevalence of *E. coli* O157:H7 from raw milk the isolation rate was reported to be quite low and varied between 0 % and 10 %²². It has been suggested that the microorganisms are not actually excreted in the milk but probably result from faecal contamination of milk during the milking process¹⁷. Abdul-Raouf *et al.*¹ reported that *E. coli* O157:H7 was detected in 3 of 50 samples of unpasteurised cows' milk. In another

study concerned with isolation of *E. coli* O157:H7 from 23 raw milk samples, the agent was detected in only one²⁹. In this study, *E. coli* O157:H7 was detected in 3 (1.4 %) of 213 raw milk samples obtained from bulk milk tanks and milk containers. This result is consistent with the results of other studies. As the isolates from milk and faeces originated from the same places, faecal contamination of milk is a possibility. The higher prevalence (67 %) detected in the milk of buffaloes from KARI in comparison with private farms is probably related to the number of *E. coli* O157:H7-positive animals in the state-owned enterprise.

In conclusion, *E. coli* O157:H7 was cultured from the faeces and milk of water buffalo for the first time in Turkey. This demonstrated that water buffaloes, like other ruminants, are reservoirs for the *E. coli* O157:H7. Faecal contamination due to poor hygiene is a risk factor for the presence of pathogenic organisms in milk. Although no cases of human disease connected to *E. coli* O157:H7 have been reported in Turkey, people need to be strongly dissuaded from consuming raw milk. There is also a need for awareness that either state- or privately-owned or private animals can be a source of *E. coli* O157:H7 infections in humans.

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