

## Human *Escherichia coli* O157:H7 infection associated with the consumption of unpasteurized goat's milk

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### SUMMARY

A cluster of four cases of haemolytic uraemic syndrome in children occurred in Northern Bohemia, Czech Republic, between 15 June and 7 July, 1995. All the cases had significantly elevated titres of anti-O157 lipopolysaccharide (LPS) antibodies as detected by the indirect haemagglutination assay. All but one of them had drunk unpasteurized goat's milk from the same farm within the week before the disease. Evidence of *E. coli* O157 infection was subsequently found in 5 of 15 regular drinkers of the farm's raw goat's milk; four of them were asymptomatic, 1 had mild diarrhoea at the end of June. Verocytotoxin 2-producing *E. coli* O157:H7 strains of phage type 2 and of identical pulsed-field gel electrophoresis patterns were isolated from 1 of 2 farm goats and from 1 of the asymptomatic goat's milk drinkers. The frequency of anti-O157 LPS antibodies found among regular drinkers of the farm's raw goat's milk (33%; 5 of 15) was significantly higher than that found in control population (0%; none of 45) ( $P = 0.0005$ ; Fisher's exact test). Our findings indicate that goats may be a reservoir of *E. coli* O157:H7 and a source of the infection for humans; raw goat's milk may serve as a vehicle of the pathogen transmission.

### INTRODUCTION

Verocytotoxin (VT)-producing *Escherichia coli* (VTEC) strains serotype O157:H7 are associated with the haemolytic uraemic syndrome (HUS), bloody and

non-bloody diarrhoea, or asymptomatic infection [1, 2]. Cattle are a major reservoir of *E. coli* O157:H7 and an important source of human infection [2–5], undercooked ground beef and unpasteurized milk being common vehicles of the pathogen transmission [2, 4]. Recently *E. coli* O157:H7 was isolated from other domestic animals including sheep [6], goats [7, 8], horses and geese [8], and the role of these animal

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species in epidemiology of the infection remains to be established. Here we report a cluster of human *E. coli* O157 infection associated with the consumption of raw goat's milk and provide evidence that goats may be a reservoir of *E. coli* O157:H7 and a source of the infection for humans.

## PATIENTS AND METHODS

### Cluster of HUS

Between 15 June and 7 July, 1995, four children residing in two neighbouring districts of Northern Bohemia were admitted to the local hospital for HUS preceded by diarrhoea. All of them were negative for *Salmonella*, *Shigella*, *Yersinia* and *Campylobacter* spp., and their stool and serum samples were sent to the Institute of Medical Microbiology, Charles University, Prague, for investigation of VTEC infection. Two serum samples that were received first showed significantly elevated titres of antibodies to *E. coli* O157 lipopolysaccharide (LPS). Preliminary investigation revealed that both the patients resided in the same village; one of them lived on a farm where two goats were kept, and raw goat's milk was consumed by the farm residents and distributed in the village. Based on these data an outbreak of *E. coli* O157 infection which might be linked to raw goat's milk was suspected, and a collaborative investigation was conducted by the district public health authorities, hospital, veterinary service, and the Institute of Medical Microbiology, Prague.

### Investigation of the cases and their contacts

#### *Microbiological*

To diagnose *E. coli* O157 infection, stools from the HUS patients and their household contacts were screened for the organism using the *E. coli* O157 Stool ELISA (LMD Laboratories, Carlsbad, USA) [9], tested for free faecal neutralizable VT (FVT) by the Vero cell assay [10], and cultured on sorbitol MacConkey agar (SMAC). Serum samples were tested for antibodies to the LPS O157, O26, O55, and O111 using the indirect haemagglutination assay [11, 12]; the titres  $\geq 640$  were considered significantly elevated.

#### *Epidemiological and environmental*

A case was defined as a patient with HUS [13] or with bloody diarrhoea, and with evidence of *E. coli* O157

infection, identified in the two districts in June and July 1995. Case finding was based on surveillance of HUS and bloody diarrhoea in the district hospital during the period; stool and/or serum samples from HUS patients and from those patients with bloody diarrhoea who were negative for *Salmonella*, *Shigella*, and *Campylobacter* spp. were investigated for *E. coli* O157 infection as described above. Because of limited diagnostic possibilities, no attempts were made to test for *E. coli* O157 patients admitted for non-bloody diarrhoea.

Standardized questionnaire and interview with the parents were used to obtain data on the cases' food history within the week before the onset of their disease including consumption of raw goat's milk, their exposure to animals, contact with each other, and a day-care centre attendance. Cases' household contacts were questioned about recent diarrhoeal episodes, and about goat's milk consumption. Drinking water from the households was tested for *E. coli* O157 using filtration (membrane filters, pore size 0.22  $\mu$ ) and plating the filters on SMAC.

### Animal investigation

Investigation of two goats kept on the farm which distributed raw goat's milk was conducted on 13 July, 1995. No cattle were kept on the farm. Faecal and serum samples from the goats were tested for *E. coli* O157, FVT and anti-O157 LPS antibodies as described above. Milk samples were filtered through membrane filters and these were cultured on SMAC. In order to determine a shedding pattern of *E. coli* O157:H7, the goats' faeces were monitored for the organism using the *E. coli* O157 ELISA and culture in 1- to 7-day intervals. The goats' state of health was reported by the farmer to find out whether the *E. coli* O157 shedding was associated with symptoms of disease.

### Investigation of goat's milk drinkers and comparison with controls

The farmer was interviewed to identify people who consumed the farm's raw goat's milk. Fifteen regular drinkers of the milk were found including the farm residents (except the farm HUS patient) and their relatives and neighbours in the village. These persons were questioned about diarrhoeal episodes within the month before the investigation, and their stools and sera were tested for *E. coli* O157 infection as described

above. The frequency of anti-O157 LPS antibodies found in the 15 regular goat's milk drinkers was compared with that found in 45 age-matched controls (three for each goat's milk drinker); these were patients hospitalized for respiratory diseases who have never drunk goat's milk; the results were analysed by the two-tailed Fisher's exact test.

Although the farmer also sold goat's milk at local markets, he was not able to identify any of his clients.

### Characterization of *E. coli* O157:H7 isolates

*E. coli* O157:H7 isolates of human and animal origin were characterized by phage type [14] and VT phenotype [10], and for macrorestriction fragment length polymorphism by pulsed-field gel electrophoresis (PFGE) using restriction enzyme *Xba* I [15].

## RESULTS

### Investigation of the cases and their contacts

#### *Microbiological*

As shown in Table 1, all four HUS patients admitted between 15 June and 7 July had serological evidence of *E. coli* O157 infection based on significantly elevated titres of anti-O157 LPS antibodies in their initial sera. Detection of free VT2 in stools of case No. 3 indicated infection with a VT2-producing *E. coli* O157 strain.

One of 12 cases' household contacts, the mother of case No. 1, had evidence of *E. coli* O157 infection based on the anti-O157 LPS antibody titre of 640 in her serum taken 2 weeks after HUS diagnosis in her son (data not shown).

#### *Epidemiological and environmental*

No more HUS cases and no cases of *E. coli* O157-associated bloody diarrhoea were identified.

As demonstrated in Table 2, 3 of the 4 HUS cases had drunk unpasteurized goat's milk from the goat farm within the week before the onset of their prodromal diarrhoea. All of them, including the farm case (No. 3) consumed the farm's raw goat's milk only once. The milk for cases No. 1 and 2 had been bought by their mothers from the farmer at markets held on 9 and 10 June in the village and in the city where the cases lived. No milk consumed by the cases remained for investigation.

Consumption of beef products, or exposure to cattle or to other animals were not found in any of the cases. None of the cases was in contact with each other during the 1-month period before the onset of disease, and none of them attended a day-care centre. Drinking water in the cases' households was from the municipal water-supply and did not show any coliform contamination.

All 12 cases' household contacts were asymptomatic for 1 month before and 1 month after the onset of disease in the cases. All five contacts of the farm case but none of the contacts of the other three cases consumed the farm's raw goat's milk.

### Animal investigation

On 13 July 1995, faeces from one of the farm goats (goat A) gave a strongly positive result in the *E. coli* O157 ELISA ( $OD_{450} = 2.650$ ), yielded a heavy growth of VT2-producing *E. coli* O157:H7 strain, phage type 2, and contained free VT2 (1:8). Faeces from the other goat (goat B) were *E. coli* O157 negative. None of the goats showed significantly elevated titre of anti-O157 LPS antibodies. Milk samples from both of them were negative for *E. coli* O157 but heavily contaminated with goat faecal flora (*E. coli*, *Enterobacter cloacae*, *Streptococcus faecalis*).

As shown by monitoring of the goats over time, goat A was shedding *E. coli* O157:H7 for 11 days since the first detection, with rapid clearing of the organism from faeces during this period; its faeces were then negative for *E. coli* O157 till 28 July when goat A was slaughtered. Goat B was *E. coli* O157 negative from 13 July to 9 August. Heavy faecal shedding of the organism detected by the ELISA appeared on 16 August ( $OD_{450} = 1.90$ ) and continued till 18 August ( $OD_{450} = 3.00$ ) when the goat was slaughtered; *E. coli* O157 was not isolated from these faecal specimens which were examined the day after collection. Signs of disease including apathy, anorexia and weight loss were observed in both the goats throughout the period of *E. coli* O157 shedding, and in goat A also for 4–5 weeks beforehand.

### Investigation of goat's milk drinkers and comparison with controls

Evidence of a recent *E. coli* O157 infection was found in 5 of 15 regular drinkers of the farm's raw goat's

Table 1. Evidence of *E. coli* O157 infection in HUS cases

Case no. (age in months, sex)	Diarrhoea/ blood	<i>E. coli</i> O157 Stool ELISA (OD <sub>450</sub> )*	FVT titre	Culture for <i>E. coli</i> O157	Serum no.	Week of serum collection†	Antibody titre to the LPS	
							O157	Non-O157‡
1 (16, M)	+ / +	-ve	< 1:2	-ve	I	1	10240	40-80
					II	3	5120	80-160
					III	25	80	40-160
2 (37, M)	+ / -	NA§	NA	NA	I	10	1280	40-80
					II	41	80	40-80
3 (15, F)	+ / +	-ve	1:8 (VT2)¶	-ve	I	1	5120	80-160
					II	6	640	80-160
					III	8	320	40-80
					IV	16	160	40-160
					V	51	20	40-80
4 (10, F)	+ / +	-ve	< 1:2	-ve	I	1	20480	80-160
					II	3	5120	80-160
					III	8	640	40-80
					IV	38	80	40-80

\* Optical density values at 450 nm (OD<sub>450</sub>) ≥ 0.2 were considered positive as recommended by the manufacturer: -ve, negative.

† Weeks from onset of prodromal diarrhoea to serum collection.

‡ Range of the antibody titres to the lipopolysaccharides (LPS) O26, O55 and O111.

§ NA, stool not available.

|| Acute serum not available.

¶ Free faecal VT (FVT) was neutralized with antiserum to VT2.

Table 2. Consumption of the farm's raw goat's milk by HUS cases

Case no.	Place of residence*	Date of raw goat's milk consumption (1995)	Volume consumed (ml)	Date of onset of prodromal diarrhoea (1995)
1	Village	9 June	150	13 June
2	City	10 June	150	16 June
3	Village (the goat farm)	24 June	200	29 June
4	City	Consumption not found	0	4 July

\* The same village and the same city located at a distance of 10 km.

milk (Table 3). Surprisingly, none of the farm residents had evidence of the infection (Table 3) despite their extensive exposure to goat A and its milk. Four of the five individuals with evidence of *E. coli* O157 infection were asymptomatic within the month before the investigation; one 4-year-old boy experienced a 3-day episode of mild watery diarrhoea in the last week of June.

One of the asymptomatic goat's milk drinkers, a 6-year-old girl, harboured VT2-producing *E. coli* O157:H7 strain of phage type 2 whose PFGE pattern (Fig. 1, lane 4) was identical with that of the *E. coli* O157:H7 isolate from goat A (Fig. 1, lane 2); this

indicated that these isolates belonged to the same strain. Although slight variations in PFGE patterns were observed in both the isolates later, being associated with morphological dissociation of their colonies on Endo agar following subcultures, PFGE patterns of the resulting colonial morphological variants of both the isolates (light pink colonies; Fig. 1, lanes 3 and 5) were closely related to PFGE patterns of the respective original isolates (dark pink colonies; Fig. 1, lanes 2 and 4). In contrast, PFGE patterns of the cluster *E. coli* O157:H7 isolates and of their colonial morphological variants differed substantially from those

Table 3. Evidence of *E. coli* O157 infection in 15 members of 3 families who regularly consumed the farm's raw goat's milk

Family	No. of members positive for/No. of tested				
	<i>E. coli</i> O157 Stool ELISA	FVT (VT2)*	<i>E. coli</i> O157 strain	Anti-O157 LPS antibodies†	Positive in total
Farm residents‡	0/5	0/5	0/5	0/5	0/5
Relatives	0/4	1/4	0/4	3/4	3/4
Neighbours	0/6	1/6	1/6	2/6	2/6
Total	0/15	2/15	1/15	5/15	5/15

\* Free faecal VT (FVT) was neutralized with antiserum to VT2.

† Significantly elevated titres of anti-O157 antibodies in serum samples taken between 13 and 17 July; none of the five seropositive individuals had significantly elevated titre 6 weeks later.

‡ The farm HUS case not included.

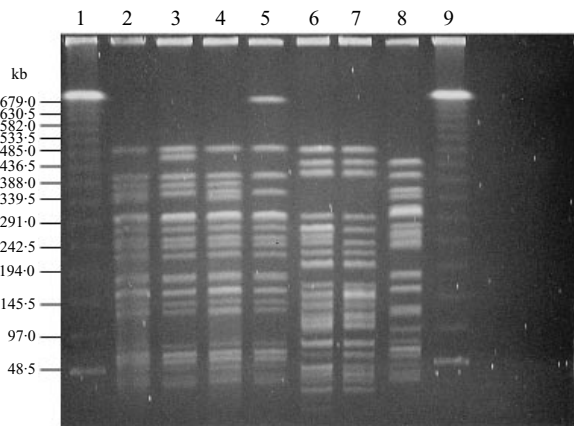


Fig. 1. Pulsed-field gel electrophoresis (PFGE) patterns of *E. coli* O157:H7 isolates generated by restriction enzyme *Xba*I. Lanes 1 and 9, molecular size markers (lambda ladder). Lane 2, original isolate from goat A (dark pink colonies); pattern A1. Lane 3, colonial morphological variant of the isolate from goat A (light pink colonies); pattern A2. Lane 4, original isolate from the goat's milk drinker (dark pink colonies); pattern A1. Lane 5, colonial morphological variant of the isolate from the goat's milk drinker (light pink colonies); pattern A3. Lanes 6–8, *E. coli* O157:H7/H- isolates not linked to the cluster; patterns B1, B2, and C, respectively.

of three previously isolated *E. coli* O157:H7/H- strains (Fig. 1, lanes 6–8) that were not linked to the cluster (two of them had atypical phage type, one other belonged to phage type 1).

The frequency of anti-O157 LPS antibodies found in the 15 regular raw goat's milk drinkers (33%; 5 of 15; Table 3) was significantly higher than that found in 45 controls that were not exposed to goat's milk (0%; none of 45; not shown) ( $P = 0.0005$ ).

## DISCUSSION

While goats have been reported to excrete non-O157 VTEC with high frequency [16], isolation of *E. coli* O157:H7 has been rare [7, 8]. *E. coli* O157:H7 was for the first time isolated from goats in a 1994 outbreak of *E. coli* O157:H7 infection in the United Kingdom which was associated with a farm visitor centre and was likely to be caused by direct contact of visitors with the farm animals [7]. Similarly as the *E. coli* O157:H7 strain isolated from goat A in our study, the UK *E. coli* O157:H7 outbreak strain produced VT2 and belonged to phage type 2. The role of goats as a source of human infection could not be determined unequivocally in the UK study since *E. coli* O157:H7 strains of undistinguishable restriction fragment length polymorphism patterns were isolated from human cases, and from both goats and cattle in the farm visitor centre [7]. In comparison, epidemiological, microbiological and serological findings in our study clearly demonstrate that goat A was a source of human *E. coli* O157 infection including 3 of 4 HUS cases and 5 of 15 regular goat's milk drinkers. Although *E. coli* O157:H7 was not isolated from milk of goat A, epidemiological data implicating raw goat's milk as a vehicle of infection are supported by the finding of contamination of the milk with goat faecal flora; it suggests that *E. coli* O157:H7 could be present but was not detected because of low sensitivity of direct culture [17]. No more cases occurred after 15 July when the consumption of the farm's goat's milk was stopped.

Two individuals in our study, who had evidence of *E. coli* O157 infection although they did not drink the farm's goat's milk could have acquired infection by

person-to-person transmission [2]. While the mother of case No. 1 was likely to be infected from her son, HUS case No. 4 was not found to be in contact with any of the individuals who had evidence of *E. coli* O157 infection; however, such contacts could not be excluded as the case was exposed to multiple person-to-person contacts, spending a lot of time at markets where her parents worked.

One interesting observation in the study was lack of symptoms and lack of evidence of *E. coli* O157 infection in the farm residents (Table 3), all of whom were extensively exposed to the source of the infection. This observation is consistent with the findings of Karmali and colleagues [18] in a family outbreak of VTEC infection caused by VT1-producing O111:H-strain. In this outbreak, lack of symptoms and of bacteriological evidence of the infection in the farm members of the family who were exposed to the infection closely correlated with the presence of pre-existing anti-VT1 antibodies (neutralizing, IgG) in their sera suggesting a protective role of anti-VT1 antibodies [18]. High frequency of VT1-neutralizing antibodies among dairy farm residents (42%) that is consistent with repeated, cumulative exposure of this population to VTEC, has been reported by Karmali's group in a recent study [19]. Drawing parallels between Karmali's and our observations we could assume that the farm residents in our study might possess, as a consequence of previous repeated exposure to VTEC through regular longstanding consumption of raw goat's milk, anti-VT2 antibodies that protected them from infection with the VT2-producing *E. coli* O157:H7 cluster strain. Investigation is in progress to study anti-VT2 immune response in the individuals involved in the cluster and to gain insight into the protective role of anti-VT2 antibody.

*E. coli* O157 shedding appeared to be transient in the goats in our study, as has been found in cattle [5] and sheep [6]. Our finding is consistent with the observation by Shukla and colleagues [7] that only 1 of 7 goats that were shedding *E. coli* O157:H7 during initial investigation in the farm visitor centre was shedding also on follow-up investigation 6 weeks later. In contrast, association of *E. coli* O157:H7 shedding with symptoms of disease as observed in our goats has not been reported previously in goats [7] or in cattle and sheep [5, 6].

Our observations that goats may be a reservoir of *E. coli* O157:H7 and a source of human infection, and the infection may be transmitted via raw goat's milk are of a considerable public health importance in our

country. Consumption of unpasteurized goat's milk, cheese and yoghurt markedly increased in the Czech Republic in the last few years, although the actual number of the consumers is not known. Most importantly, raw goat's milk is widely used as a nutritional substitute for cow's milk particularly in young children. Raw goat's milk and dairy products are sold on farms and at public markets, and there is no mandatory control of foodstuffs for *E. coli* O157 at present. Preliminary measures were taken to reduce the risk of human *E. coli* O157 infection from goats, and to prevent a recurrence of the outbreak. These included: (i) a newspaper report on the outbreak including warning against consumption of unpasteurized goat's milk and dairy products especially by young children; (ii) regulatory measures to limit distribution of unpasteurized goat's milk and dairy products at public markets. Moreover, the establishment of surveillance systems for HUS and for *E. coli* O157:H7 infection in the Czech Republic which is in progress is needed to better define spread of the organism, populations at risk of the infection and of severe disease, and effective preventive measures.

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