

Outbreak of *Escherichia coli* 0157:H7 related to animal contact at a petting zoo

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OBJECTIVE: To determine the cause of an outbreak of *Escherichia coli* 0157:H7 related to animal exposures so that further transmission could be prevented.

DESIGN: Description of laboratory investigations and a case control study.

SETTING: Agricultural pavilion at an annual fair in Ontario.

POPULATION: People with laboratory evidence of *E coli* 0157:H7 (seven people) and others with diarrhea (155 people) who called the health unit following a media release were interviewed. Animals that were accessed most frequently by the public in the agriculture pavilion were tested for *E coli* 0157:H7. In the case control study, a case was defined as someone with laboratory-confirmed *E coli* 0157:H7, or someone who developed severe or bloody diarrhea two to eight days after attending the agricultural pavilion at the fair (61 people). A convenience sample of people who attended the agricultural pavilion but did not develop diarrhea was selected as the control group (89 people).

INTERVENTIONS: Human and animal *E coli* 0157:H7 specimens were subtyped. Cases and controls were interviewed using a standardized questionnaire.

RESULTS: Subtyping of the seven human isolates of *E coli* 0157:H7 revealed five that were of an extremely uncommon phage type. Three samples from goats and one from sheep at the petting zoo in the agricultural pavilion were of this same phage type. The case control study also implicated goats (odds ratio [OR] 3.65; 95% CI 1.63 to 8.52) and sheep (OR 2.94; 95% CI 1.33 to 6.57) from the petting zoo.

CONCLUSIONS: Results of this investigation suggest strongly that the goats and sheep from the petting zoo were the source of this outbreak of *E coli* 0157:H7.

Key Words: *Animal exhibit; Escherichia coli* 0157:H7; Goats; Petting zoo; Sheep

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Flambée d'*Escherichia coli* 0157:H7 reliée à un contact animal dans un zoo pour enfants

OBJECTIF : Déterminer la cause d'une flambée d'*Escherichia coli* 0157:H7 reliée à l'exposition à des animaux afin de prévenir de nouvelles transmissions.

MÉTHODOLOGIE : Description des explorations en laboratoire et étude cas-témoin

LIEU : Pavillon agricole d'une foire annuelle en Ontario

POPULATION : Les personnes présentant des résultats en laboratoire d'*E coli* 0157:H7 (sept) et les individus atteints de diarrhée (155) qui ont téléphoné à l'unité de santé après un communiqué de presse ont passé une entrevue. Les animaux auxquels le public avait le plus accès au pavillon agricole ont subi des tests pour vérifier la présence d'*E coli* 0157:H7. Dans le cadre de l'étude cas-témoin, un cas était défini comme une personne présentant une confirmation en laboratoire d'*E coli* 0157:H7 ou qui avait souffert de diarrhée grave ou de diarrhée sanglante de deux à sept jours

après être allée au pavillon agricole de la foire (61 personnes). Un échantillon de commodité des personnes qui sont allées au pavillon agricole sans développer de diarrhée a été sélectionné pour servir de sujets témoins (89 personnes).

INTERVENTIONS : Les spécimens humains et animaux d'*E coli* 0157:H7 ont été sous-typés. Les cas et les témoins ont passé une entrevue au moyen d'un questionnaire normalisé.

RÉSULTATS : Le sous-typage des sept isolats humains d'*E coli* 0157:H7 a révélé que cinq d'entre eux présentaient une lysotypie très rare. Trois échantillons provenant de chèvres et un de moutons du zoo pour enfants du pavillon agricole présentaient la même lysotypie. L'étude cas-témoin mettait également en cause les chèvres (écart-type [ÉT] 3,65; IC 95 % 1,63 à 8,52) et les moutons (ÉT 2,94; IC 95 % 1,33 à 6,57) du zoo pour enfants.

CONCLUSIONS : Les résultats de cette recherche indiquent fortement que les chèvres et les moutons du zoo pour enfants étaient responsables de cette flambée d'*E coli* 0157:H7.

Since it was first identified in the early 1980s (1), verotoxigenic *Escherichia coli* 0157:H7 (*E coli* 0157:H7) has become recognized widely as an important cause of food-borne illness. *E coli* 0157:H7 infection is potentially life-threatening because it can lead to the hemolytic uremic syndrome (HUS). This occurs in 2% to 7% of people with bloody diarrhea that is caused by *E coli* 0157:H7 (2). Young children and the elderly are particularly susceptible to developing this complication. Most commonly, cattle are identified as a reservoir for verotoxin-producing *E coli* 0157 (3), although it has also been found in sheep, sea birds, goats, dogs and horses, as well as on flies (4-8). The first human outbreak of disease related to this bacteria was linked to undercooked hamburger (1). Since then, outbreaks have been associated with a variety of foods, including radish sprouts, lettuce, unpasteurized juices and apple cider, and raw milk from both cows and goats (9-15). Recently, *E coli* 0157:H7 outbreaks have been associated with deer jerky and salami (16,17). In addition, contaminated drinking water and swimming in contaminated water have been implicated (18,19). Several outbreaks have been related to farm visits, and there have been sporadic cases reported from direct and indirect animal contact (20-25). An outbreak of *E coli* 0157:H7 related to direct contact with goats and sheep from a travelling petting zoo is described in the present paper.

During a 10-day period from September 27 to October 6, 1999, the health unit in Middlesex-London, Ontario received four reports of people with *E coli* 0157:H7 infection, one of whom was hospitalized with HUS. This exceeded the health unit's average of three cases of *E coli* 0157:H7/month. All four cases reported having touched animals in the agricultural pavilion at the region's annual fair, held September 10 to 19, 1999. No other common links, including food or beverage, were identified among the four cases. The fair's water supply was from the municipal system and was not implicated as a source in this outbreak.

In September 1999, the fair attracted approximately 290,000 people from a major urban centre and the surround-

ing rural areas. There were more than 100 food vendors, a midway and numerous exhibition buildings, including an agricultural pavilion. This agricultural pavilion housed a travelling petting zoo, livestock from local farms, an animal maternity area and an area where cows could be hand-milked. Although fencing separated the livestock from the public, it was possible for passers by to touch the animals.

In the petting zoo area, feed for the animals could be purchased and was dispensed from a large barrel into ice cream cones. Visitors could walk among the goats and feed them. Also part of the petting zoo were rare and exotic animals such as a dwarf cow, a lemur and a kangaroo.

The purpose of the present investigation was to determine the cause of this outbreak, and to prevent further transmission and future outbreaks.

DATA AND METHODS

To determine if there were unrecognized cases in the community, the local health unit issued a media release. People who developed diarrhea after visiting the agricultural pavilion were requested to contact the health unit. Subsequent investigations included the subtyping of human isolates, the testing of potential animal sources and a case control study.

Stool specimens were submitted for bacteriological testing in Cary-Blair (Remel Inc, USA) transport medium. Presumptive *E coli* 0157 isolates obtained from the initial culture were confirmed serologically for 0157 by using standard slide agglutination, and were confirmed for H7 antigens by using tube agglutination techniques with in-house prepared antisera (26). Verotoxin production was detected using the ProSpecT Shiga-Toxin *E coli* Microplate Assay (Alexon-Trend, USA).

Subtyping was done using pulsed-field gel electrophoresis (PFGE) according to the Center for Disease Control and Prevention protocol (27), and was submitted to the National Laboratory for Enteric Pathogens, Health Canada for phage typing. Phage typing was performed by using standard techniques described previously (28). Briefly, *E coli*

O157:H7 strains were plated on nutrient agar and incubated for 18 h at 37°C. A single smooth colony was selected and inoculated in 4.5 mL of DIFCO Phage Broth (BD Diagnostic Systems, USA) (pH 6.8), and incubated at 37°C for 2.3 h in a shaking water bath (29). The bacterial cultures were inoculated by flooding the DIFCO Phage Agar Plate (BD Diagnostic Systems, USA) to form a smooth lawn. A panel of 16 phages at routine test dilution was spotted on the bacterial lawn, and the plates were allowed to dry. The dried plates were incubated at 37°C for 18 h before examination for lytic reactions.

The most publicly accessible dairy cows and calves at the pavilion were sampled by rectal swab five to six weeks after the end of the fair, when they had returned to their farms of origin. Goats, sheep and the dwarf cow from the travelling petting zoo were tested on their farm three and-a-half or more weeks after the end of the fair. Repeated samples from many of the goats and sheep were taken on four occasions over a six week period. In addition, composite fecal samples were obtained from the farm cages of several other petting zoo animals. Animal samples were transported, processed and subtyped by using the same methodology as the human samples described above.

A case control study was undertaken to determine the factors associated with this outbreak. People who contacted the health unit following the media release, and who had developed diarrhea within one to 10 days of attending the agricultural pavilion at the fair, were asked to complete a structured telephone questionnaire. In addition, those people who were identified with *E coli* O157:H7 by laboratory testing were interviewed. Standardized interviews were conducted by health unit staff members for some participants from October 15 to 18, 1999. Other participants were interviewed by staff members of a local survey unit from October 19 to 29, 1999.

Controls were defined as people who had attended the agricultural pavilion at the fair, but did not develop diarrhea in September 1999. A convenience sample of controls was obtained from a variety of sources, including several local businesses, people who were identified by cases and people who had contacted the health unit with questions

about the outbreak. The survey unit administered the same structured telephone questionnaire to the controls from October 18 to October 29, 1999. Up to four attempts were made to contact both cases and controls.

For inclusion in the case control study, a more specific case definition was used. A case was defined as someone with laboratory-confirmed *E coli* O157:H7, or someone who developed severe or bloody diarrhea two to eight days after attending the agricultural pavilion at the fair. Severe diarrhea was defined as five or more loose bowel movements/day, with a duration of at least 24 h.

Data were entered into SPSS Version 8.0 (SPSS Inc, USA) for analyses. Logistic regression was used to quantify and evaluate (two-sided tests, P<0.05) unadjusted associations between case status and potential risk factors. Fisher's exact 95% CI were constructed for each odds ratio using PEPI Version 3.00 (30).

RESULTS

There were seven primary cases with laboratory evidence of *E coli* O157:H7 infection related to contact with the agricultural pavilion, four of whom were identified before the media release and three after. In six of the seven cases, laboratory confirmation was obtained from the primary case, while in one case who was not tested, a symptomatic family member was found to have laboratory confirmed *E coli* O157:H7. Phage typing revealed that five of the seven isolates were phage type (PT) 27, and two were PT 14. Four of the five PT 27 isolates were PFGE A, and one was PFGE A1. PFGE A and PFGE A1 are related closely, indicating that all PT 27 isolates were from a common source. The five PT 27 isolates were from people who had attended the fair on September 18 or 19. Both PT 14 isolates were PFGE A1 and were obtained from cases who attended the fair on September 15. Table 1 summarizes these results and additional demographic information for the primary cases.

Of the 18 dairy cattle from local farms that were tested, none were positive for *E coli* O157:H7. However, three samples from goats and one sheep sample taken from animals of the travelling petting zoo were found to have *E coli* O157:H7. Subtyping revealed that *E coli* O157:H7 isolates

TABLE 1
Phage typing and pulsed-field gel electrophoresis (PFGE) results, laboratory confirmed cases of *Escherichia coli* O157:H7, Middlesex-London, Ontario, 1999

Case number	Age (years)	Sex	Date at fair	Incubation (days)	Phage type	PFGE
1	5	Male	September 18	4	27	A
2	9	Male	September 18	3	27	A1
3	35	Female	September 15	4	14	A1
4	14	Female	September 18,19	7 or 6	27	A
5*	5	Male	September 19	6	27	A
6	3	Female	September 18	24	27	A
7	1	Female	September 15	7	14	A1

*Subtyping associated with the patient in Case 5 is from his father's stool sample. His father acquired the infection through spread within the family. The father's isolate likely reflects what would have been found in Case 5 if the patient had been tested

TABLE 2
Case symptoms in case control study of patients with *Escherichia coli* 0157:H7, Middlesex-London, Ontario, 1999

Symptoms	Frequency	Percentage
Five or more loose bowel movements/day	61	100.0
Abdominal cramps	53	86.9
Fever	24	39.3
Vomiting	19	31.1
Bloody diarrhea	15	24.6
Nausea or vomiting	8	13.1

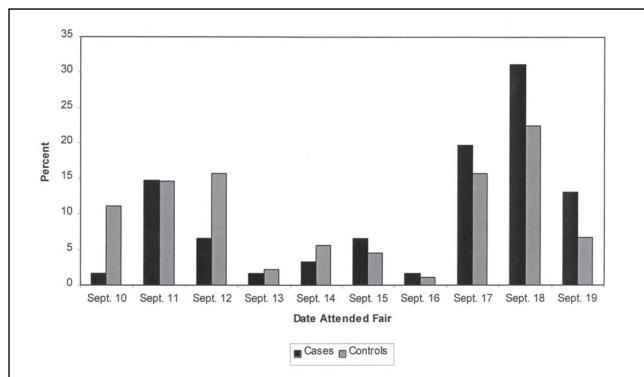


Figure 1 Percentage of cases and controls attending the fair on each day, September (Sept) 1999. Controls were more likely than cases to attend the fair during the first weekend (September 10 to 12), while cases were more likely than controls to attend during the second weekend (September 17 to 19) ($P < 0.05$)

from these animals were PT 27 and PFGE A. The sample from the dwarf cow and composite samples from the other petting zoo animals were negative.

Of the 443 people who called the health unit after the media release, 155 developed diarrhea within one to 10 days of attending the agriculture pavilion. Attempts were made to recontact those 155 people, as well as the initial four laboratory-confirmed cases, to administer the study questionnaire. One hundred and thirty-one (82%) of the 159 people completed the structured telephone questionnaire. Twenty-two (14%) people could not be reached, and six (4%) refused to complete the questionnaire. For the case control study, 61 people met the more specific case definition. Eighty-nine controls were obtained from the community.

The cases ranged in age from 10 months to 77 years. Controls ranged in age from eight months to 59 years. Of the cases, 67% were 15 years of age or younger compared with 74% of the controls. This difference was not significant statistically. Approximately half of the cases and controls were female. Among the cases, diarrhea lasted an average of nine days (range two to 28 days) and was accompanied by abdominal cramping in 87% of cases. Case symptoms are summarized in Table 2. Nearly half of the cases (47%) saw a physician for their symptoms. Twenty-four of

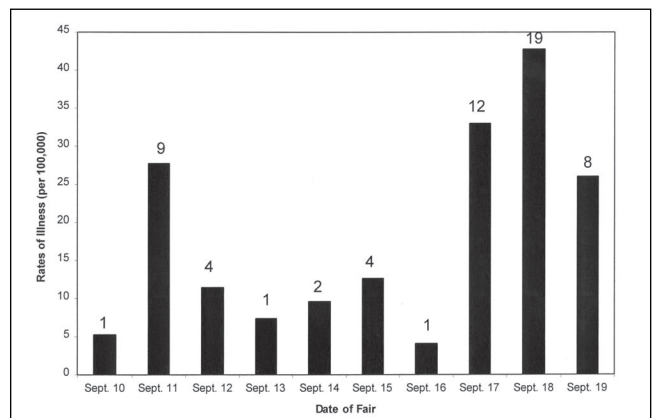


Figure 2 Rates of illness by date of attending the fair, September (Sept) 1999. The numbers above the bars indicate the number of cases who visited the agriculture pavilion each day

the 29 (83%) people who saw a physician reported that they were given stool sample bottles, but only 20 actually submitted stool samples to a laboratory. Nine of these samples were submitted after October 8, 1999, when fecal shedding may have already stopped.

Controls were more likely than cases to attend the fair during the first weekend, while cases were more likely than controls to attend the fair during the second weekend ($P < 0.05$) (Figure 1). However, a bimodal distribution of illness rates is noted with peaks on September 11 and the weekend of September 17-19 (Figure 2).

Table 3 shows the odds ratios (ORs) associated with exposures at the agricultural pavilion at the fair. Cases were 8.55 (95% CI 1.92 to 77.65) times more likely than controls to have visited the petting zoo and 3.65 (95% CI 1.63 to 8.52) times more likely to have touched goats at the petting zoo. As well, cases were 2.88 (95% CI 1.35 to 6.26) times more likely than controls to have walked among the goats in the petting zoo and 2.94 (95% CI 1.33 to 6.57) times more likely to have touched the sheep in the petting zoo. All of these associations were significant statistically ($P < 0.005$). Cases were significantly ($P < 0.05$) less likely than controls to have visited the maternity ward, the pigs and piglets, birds, and rabbits. Cases were no more likely than controls to have touched either beef (OR 0.71; 95% CI 0.35 to 1.44) or dairy cattle (OR 1.18; 95% CI 0.50 to 2.92).

The ORs associated with nonanimal exposures were also determined. Cases were significantly more likely than controls to have spent more than 1 h in the agricultural pavilion (42.6% versus 19.1%). Although not significant statistically, cases were more likely than controls to have eaten while in the agricultural pavilion, and less likely to have washed their hands. Illness was not associated with any foods eaten at the fair (Table 4).

DISCUSSION

The present investigation identified seven people with laboratory evidence of *E coli* 0157:H7 associated with animal contact at the agricultural pavilion at a regional fair.

TABLE 3
Odds ratios associated with exposures at the agricultural pavilion at the fair, Middlesex-London, Ontario, 1999

Risk factor	Cases n=61 (%)	Controls n=89 (%)	Odds ratio	95% CI	P
Visited any dairy cattle	49 (80.3)	69 (77.5)	1.18	0.50–2.92	0.84
Visited sheep pen (not in petting zoo)	35 (57.4)	70 (78.7)	0.37	0.17–0.80	<0.007*
Visited any beef cattle	27 (44.3)	47 (52.8)	0.71	0.35–1.44	0.32
Visited goat pen (not in petting zoo)	40 (65.6)	54 (60.7)	1.24	0.60–2.59	0.61
Visited petting zoo	59 (96.7)	69 (77.5)	8.55	1.92–77.65	<0.001*
Touched goats in petting zoo	49 (80.3)	47 (52.8)	3.65	1.63–8.52	<0.001*
Walked among the goats	45 (73.8)	44 (49.4)	2.88	1.35–6.26	<0.004*
Bought feed at petting zoo	35 (57.4)	41 (46.1)	1.58	0.78–3.21	0.19
Fed the goats in petting zoo	34 (55.7)	41 (46.1)	1.47	0.73–2.99	0.32
Touched sheep in petting zoo	25 (41.0)	19 (21.4)	2.94	1.33–6.57	<0.005*
Visited maternity area	36 (59.0)	75 (84.3)	0.27	0.12–0.62	<0.001*
Visited pigs and/or piglets	45 (73.8)	78 (87.6)	0.40	0.15–1.01	0.04*
Visited birds	23 (37.7)	51 (57.3)	0.45	0.22–0.92	0.02*
Visited rabbits	24 (39.3)	53 (59.6)	0.44	0.21–0.90	0.02*
Touched horses	24 (39.3)	30 (33.7)	1.28	0.61–2.64	0.49
Rode pony	17 (27.9)	25 (28.1)	0.99	0.45–2.17	1.00
Ate while in pavilion	13 (21.3)	11 (12.4)	1.92	0.73–5.13	0.18
Did not wash hands after touching animals	38 (62.3)	57 (64.0)	0.93	0.45–1.93	0.86
Did not wash hands before eating	37 (60.7)	41 (46.1)	1.81	0.89–3.70	0.10
Spent more than one hour in pavilion	26 (42.6)	17 (19.1)	3.15	1.42–7.02	<0.003*

*Statistically significant result, $P < 0.05$

TABLE 4
Odds ratios associated with foods at the fair, Middlesex-London, Ontario, 1999

Food	Number of cases (%)	Number of controls (%)	Odds ratio	95% CI	P
Corn dogs	12 (19.7)	12 (13.5)	1.57	0.59–4.16	0.37
Pizza	11 (18.0)	22 (24.7)	0.67	0.27–1.60	0.42
Candy floss	11 (18.0)	17 (19.1)	0.93	0.36–2.32	1.00
Souvlaki	3 (4.9)	8 (9.0)	0.52	0.09–2.31	0.53
Hamburger	1 (1.6)	4 (4.5)	0.35	0.01–3.72	0.65
Fries	21 (34.4)	31 (34.8)	0.98	0.47–2.05	1.00
Ice cream	4 (6.6)	11 (12.4)	0.50	0.11–1.80	0.28
Elephant ears	5 (8.2)	9 (10.1)	0.79	0.20–2.81	0.78
Beer nuts	1 (1.6)	3 (3.4)	0.48	0.01–6.14	0.65
Hot dogs	12 (19.7)	10 (11.2)	1.96	0.71–5.46	0.16
Bacon on a bun	4 (6.6)	8 (9.0)	0.71	0.15–2.81	0.76

Subtyping revealed that five of the seven primary cases were the extremely uncommon *E. coli* O157:H7 PT 27, while two were the common *E. coli* O157:H7 PT 14. The rare PT 27 was confirmed in three samples from goats and one sheep sample from animals of the travelling petting zoo. Results from the case control study suggest strongly that the goats and sheep from the petting zoo were the source of this outbreak.

Phage type 27 is an extremely rare subtype of *E. coli* O157:H7 in Canada. Each year, there is an average of 1000 to 1200 isolates of *E. coli* O157:H7 submitted to the Canadian Laboratory Centre for Disease Control for phage typing. Since 1991, PT 27 had previously been identified in only six people and one animal in Canada (R Ahmed, personal communication, October 1999). The rarity of PT 27

indicates strongly that these five cases acquired their infection from a common source. Identification of this same rare phage type in the petting zoo animals indicates strongly that these animals were the source of this outbreak.

Phage type 14 is the most common phage type of *E coli* 0157:H7 found in Ontario (31). The two cases with PT 14 may have contracted their infection from a different source at the fair or from some other exposure unrelated to the fair. If these two cases acquired their infection from animal contact at the fair, this implies a second unrecognized source of infection among the animals. The second animal source may have been missed because not all animals in the pavilion were tested. As well, it is known that animals shed intermittently and are more likely to shed while under stress (32). Testing of the animals was done several weeks after the fair, when they may no longer have been shedding.

The clustering of cases on the two weekends of the fair may indicate that manure disposal and environmental cleaning may have been more difficult on these days, when the volume of visitors was high. Detailed histories from two of the primary cases suggest that either the rails or the environment surrounding the petting zoo were possible sources of *E coli* 0157:H7 in this outbreak. Case 4 (Table 1) only leaned against the rails at the petting zoo, while Case 6 only walked by the petting zoo. Neither case actually touched the animals. The rails have been implicated as a source of infection in an outbreak of salmonella from a reptile exhibit at a zoo in Colorado (33). The long incubation period for Case 6 suggests that she may have ingested the organism several weeks after the fair through contact with fecal matter remaining on her shoes or stroller. It is known that *E coli* 0157:H7 can survive for several months in soil samples (34). The fact that the cases were less likely than controls to visit the maternity area, pigs and piglets, birds, and rabbits indicates that people either chose to go the petting zoo area or to these other animals that were distant from the petting zoo in the agriculture pavilion.

There are several possible sources of bias in this investigation. Any case control study is subject to differential recall between the cases and controls. Cases tended to be interviewed earlier than controls, which may have resulted in increased recall for the cases. Also, the cases in this study

were interviewed at least twice, while the controls were interviewed only once.

A second source of bias may have resulted from the misclassification of the cases because laboratory evidence of *E coli* 0157:H7 was obtained only in seven cases. The limited laboratory confirmation occurred because only 20 stool samples were submitted, and almost half of those submissions were obtained several weeks after the onset of symptoms, when shedding may have ceased.

Another possible source of bias in this investigation was some erroneous media reporting. On October 14, 1999, the headline "Petting zoo ruled out in *E coli* outbreak" appeared on the third page of a local newspaper (35). This headline appeared before the controls were recruited for the study. Thus, it is possible that potential controls who visited the agricultural pavilion, but who went only to the petting zoo, may not have volunteered for the study because they understood from the media reporting that the petting zoo had been ruled out. This bias could have pointed erroneously to the petting zoo as the cause of the outbreak. However, it is unlikely that the conclusion is erroneous because animal and human laboratory testing results indicate strongly that the petting zoo was the source of this outbreak.

Previous investigations have implicated animals on farms (20,21) as the source of *E coli* 0157:H7 infection. This is the first time that contact with a petting zoo has been implicated in an *E coli* 0157:H7 outbreak. Because of the popularity of petting zoos, petting zoo animals with *E coli* 0157:H7 have the potential to make large numbers of people ill. This highlights the need for operating standards in settings where the public has exposure to animals. Standards should outline the need for adequate hand-washing facilities, appropriate disposal of manure and proper cleaning of the environment, including rails and floors.

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