

## Community-wide outbreak of *Escherichia coli* O157:H7 associated with consumption of frozen beef burgers

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### SUMMARY

On 24–25 October 2005 a cluster of five haemolytic uraemic syndrome (HUS) cases was reported in southwest France. An investigation was undertaken to identify the outbreak source and implement control measures. Cases were defined as individuals with HUS or diarrhoea with isolation of *Escherichia coli* O157:H7 in stools or a positive antibody response to *E. coli* O157 lipopolysaccharide, resident in southwest France with symptom onset after 19 September 2005. Sixty-nine identified patients had symptom onset between 5 October and 3 November 2005, including 17 cases of HUS. One brand of frozen beef burgers produced on 22 August 2005 was consumed by all patients in the week before symptom onset. *E. coli* O157:H7 strains from patients, patients' burgers and the manufacturing plant were genetically related. This is the largest community-wide outbreak of *E. coli* O157:H7 in France to date and the first associated with consumption of contaminated frozen beef burgers.

**Key words:** *Escherichia coli* O157, France, haemolytic uraemic syndrome, meat products, shiga-toxigenic *Escherichia coli*.

### INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC), particularly *E. coli* O157:H7, are an important cause of foodborne disease in industrialized countries [1, 2]. Since their identification in the 1980s knowledge of the role of these pathogens in human disease has expanded rapidly. Clinical manifestations of STEC infection

range from mild diarrhoea to severe and specific complications such as the haemolytic uraemic syndrome (HUS), which occurs primarily in young children [2, 3].

STEC infections are not notifiable in France and most primary diagnostic laboratories do not routinely examine stool samples for STEC. Surveillance of these infections is thus based on surveillance of STEC-related HUS. A national surveillance system was set up in France in 1996 in order to monitor trends in the incidence of HUS in children aged <15 years and to detect outbreaks of STEC-related HUS. This system

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is based on a voluntary network of 34 paediatric nephrology departments across the country reporting cases of HUS to the French national public health surveillance institute (Institut de Veille Sanitaire) in Paris. The system identifies on average 70–100 HUS cases per year and *E. coli* O157:H7 has been identified as the primary cause of HUS in French children [4, 5].

On 24 and 25 October 2005, two paediatric nephrology departments in two administrative districts in southwest France, Gironde and Pyrénées-Atlantiques, reported five cases of HUS to the national public health surveillance institute. The five patients had symptom onset between 9 and 21 October and were resident in two neighboring administrative districts. An outbreak investigation was begun on 25 October to identify the causal agent and the vehicle of transmission, and to implement control measures.

## METHODS

### Case definition

A confirmed case was defined as a person with HUS or diarrhoea with isolation of *E. coli* O157:H7 in stools or a positive antibody response to *E. coli* O157 lipopolysaccharide, with symptom onset after 19 September 2005 living in southwest France. An individual with bloody diarrhoea and an epidemiological link to a confirmed case (sharing same household, attendance at a common crèche/school/place of work, sharing a meal or any other common activity involving direct contact between the individuals) was defined as a probable case.

### Case finding

Active case finding was carried out using hospitals, laboratories, paediatricians and general practitioners in the affected administrative districts and surrounding districts in southwest France. Physicians were asked to systematically request stool samples from patients with bloody diarrhoea be screened for STEC and laboratories were requested to transfer all positive samples to the national reference laboratory for *E. coli* for further identification and molecular typing. Patients, or their parents, were interviewed by telephone or in person with a standard questionnaire covering food consumption, presence of other patients in the entourage (familial, educational, professional, social), travel, social and recreational activities and contact with farms or animals in the 7 days before symptom

onset. Initial interviews with the parents of HUS-infected children failed to identify a probable vehicle of transmission. On 28 October three sets of parents with a HUS-infected child hospitalized in the same hospital were brought together to discuss the food histories of their children. As a result of this meeting, a common risk exposure was identified and patients, or their parents, were re-interviewed.

### Microbiological investigation

Stool specimens collected from patients were inoculated onto sorbitol MacConkey agar and common agar cultures for enteric bacterial pathogens. Non-sorbitol-fermenting strains were identified as *E. coli* by the API system (bioMérieux, Marcy l'Etoile, France) and were then tested with O157 antisera by latex agglutination assay (Oxoid, Basingstoke, Hants, UK). PCR for *stx* and *eae* gene detection was performed directly on stool samples and on *E. coli* O157:H7 isolates [6]. Serum samples from patients were examined for IgM and IgA antibodies to the lipopolysaccharide of eight major STEC serogroups (O26, O55, O91, O103, O111, O128, O145 and O157) by a line blot immunoassay technique [6].

The detection of *E. coli* O157:H7 from 25-g samples of beef burgers removed from patients' homes, the implicated supermarket chain and production plant was performed using the French Association for Standardization (AFNOR)-validated methodology based on the VIDAS kit (bioMérieux) as previously described [7]. The genetic relatedness of isolates obtained from patient stool samples and from beef burgers was studied by pulsed-field gel electrophoresis (PFGE) as previously described [8].

### Environmental and veterinary investigations

A trace-back investigation was carried out to identify the source of contamination of the beef burgers. This involved identification of the batch number of beef burgers consumed by patients, sampling of the beef burgers from patients' homes, the supermarket chain and the production plant, and identification of the source herds of the ground beef.

The implicated abattoir and production plant, both located on a single site, were investigated. A physical inspection of the site, sampling of the stored production samples, the production line and environment, and an inspection of the plant's hygiene and quality control procedures were carried out. Plant

Table 1. Demographical and infection confirmation information for identified *E. coli* O157:H7 outbreak cases stratified severity of symptoms, southwest France, October–November 2005 (*n* = 69)

	HUS cases ( <i>n</i> = 17)		Non-HUS cases ( <i>n</i> = 52)		Total cases ( <i>n</i> = 69)	
Demographic characteristics:						
Median age (age range)	3.5 (2–49) yr		6 (1–98) yr		5 (1–98) yr	
Male/female sex ratio	1.1		1.4		1.2	
	<i>n</i>	(%)	<i>n</i>	(%)	<i>n</i>	(%)
Confirmation of <i>E. coli</i> O157 infection						
Number of confirmed infections	17	(100)	46	(88)	63	(91)
By serology only	6	(35)	5	(11)	11	(17)
By microbiological isolation of the pathogen (± serology)	11	(65)	41	(79)	52	(75)
Isolation of the strain <i>E. coli</i> O157:H7 (carrying <i>stx1 stx2 eae</i> virulence genes)	11	(65)	35	(67)	46	(67)

HUS, Haemolytic uraemic syndrome.

records were investigated to determine the distribution chain of the implicated batches.

### RESULTS

The outbreak investigation identified 69 individuals meeting the case definitions. The median age of patients was 5 years (range 1–98 years) and 36 (61%) were children aged ≤5 years (Table 1). The male/female sex ratio was 1.2. All patients reported diarrhoea, 33 (48%) reported bloody diarrhoea. Seventeen patients (25%) developed HUS (median age 3.5 years, range 2–49) and all were hospitalized. No deaths occurred. A microbiological or serological confirmation of *E. coli* O157 infection was obtained for 63 patients (91%); 100% of HUS cases and 88% of non-HUS cases (Table 1). Microbiological isolation of an *E. coli* O157 strain was possible for 52 patients (75%) and an *E. coli* O157:H7 carrying the *stx1*, *stx2*, and *eae* virulence genes was isolated from two-thirds of HUS and non-HUS patients (Table 1).

Patients reported onset of diarrhoeal illness between 5 October and 3 November 2005 (Fig. 1). Patients were identified in six neighboring administrative districts in southwest France (Fig. 2). The highest attack rate, 8.9/100 000 population, was reported in the administrative district of Landes, while four of the remaining five districts had an attack rate of 1–5/100 000 population. One hundred percent of patients had consumed a single brand of beef burgers purchased in frozen uncooked form from a single supermarket chain in the 7 days before onset of symptoms.

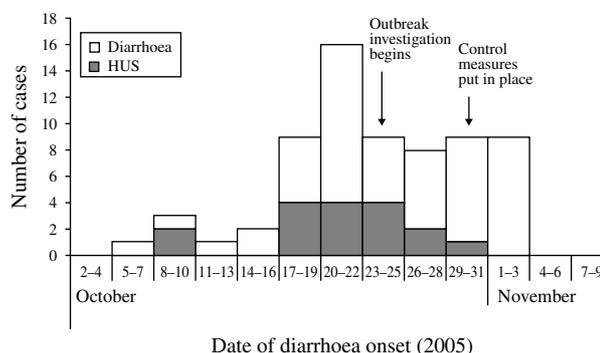
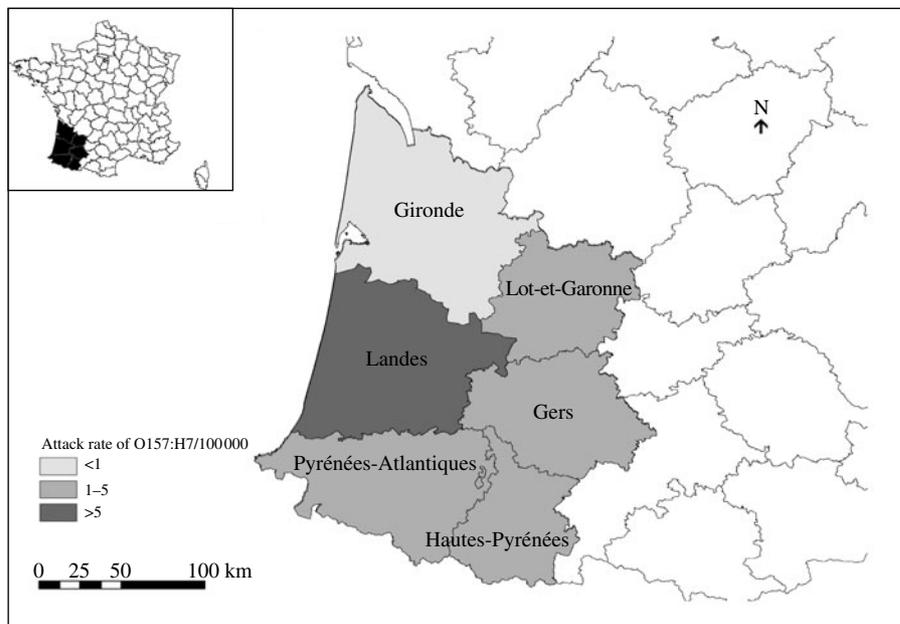


Fig. 1. Number of *E. coli* O157:H7 outbreak cases by date of diarrhoea onset and severity of symptoms, southwest France, October–November 2005 (*n* = 67). HUS, Haemolytic uraemic syndrome.

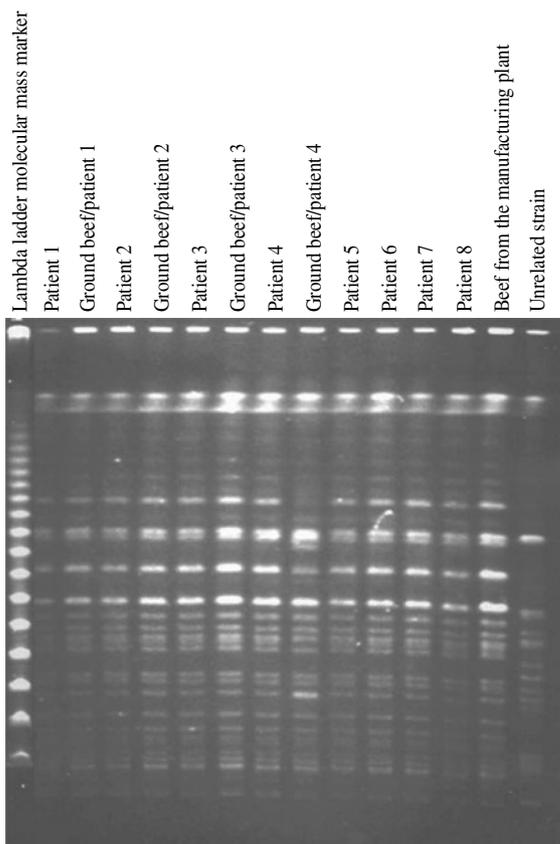
### Environmental and veterinary investigations

Fifty of the 60 beef burgers removed from the homes of 11 patients' families tested positive for *E. coli* O157:H7. Quantification of the bacterial load in these samples yielded values of <5–30 c.f.u./g. Fourteen samples of 100 g from recalled beef burgers from the supermarket chain and 27 samples from stored samples at the production plant tested positive for *E. coli* O157:H7, with bacterial loads of <5–20 c.f.u./g. PFGE analysis indicated that outbreak-related human isolates and isolates from beef burgers in patients' homes purchased from the supermarket chain, and from the production plant were genetically related (Fig. 3).

A batch number for the consumed beef burgers was identified for 31 of the 56 patients' families (55%) and all those families had consumed burgers from



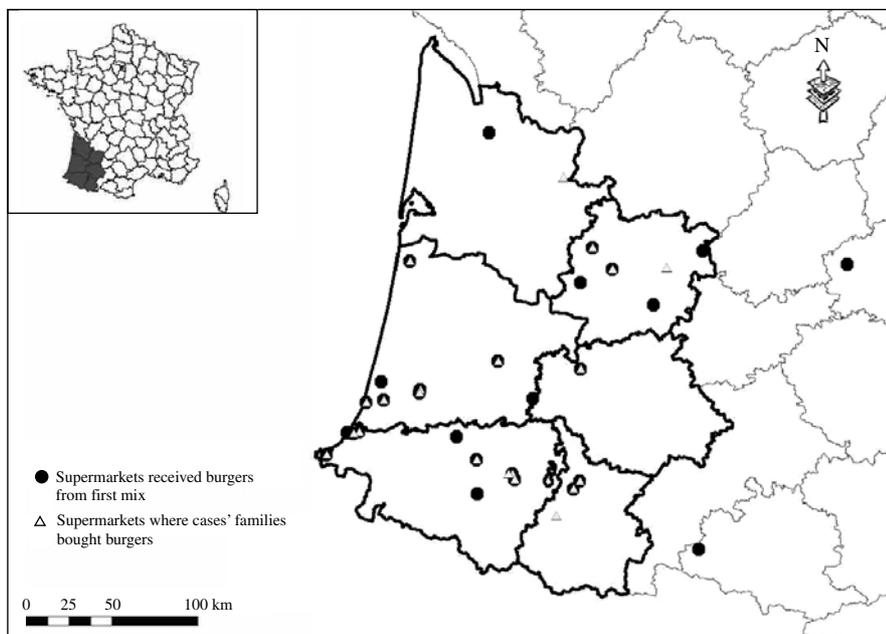
**Fig. 2.** Attack rate of *E. coli* O157:H7 per 100 000 population in affected administrative districts, southwest France, October–November 2005.



**Fig. 3.** Representative *Xba*I PFGE patterns of *E. coli* O157:H7 strains isolated from patients’ stool with strains isolated from ground beef taken from patients’ homes and from the manufacturing plant, southwest France, October–November 2005.

a single batch. This batch, produced on 22 August 2005, comprised 178 680 frozen beef burgers made from eight individual mixes of ground beef. Each mix contained at least 1 tonne of ground beef. Stored production samples from the first, second, third and fifth mixes produced on 22 August tested positive for *E. coli* O157:H7. Quantification of the bacterial load showed the first mix was heavily contaminated, <math>< 10\text{--}20\text{ c.f.u./g}</math>, and that subsequent mixes were contaminated to a lesser degree, absence to <math>< 5\text{ c.f.u./g}</math>. The source mix of beef burgers consumed by eight patients’ families was identified, seven (88%) of whom consumed burgers made from the first mix and one from the second mix of ground beef produced that day.

The contaminated batch was distributed as 17 868 boxes of ten frozen uncooked 100-g patties of ground beef to 81 stores of a single supermarket chain in 18 administrative districts in southwest France in late September 2005. Boxes of beef burgers produced from the first mix of the batch were distributed to 26 stores in eight districts (Fig. 4). The product had an expiration date of 22 June 2006. The precise supermarket store where patients’ families purchased their contaminated beef burgers was identified for 51 (91%) of the 56 families. Forty-eight families (94%) reported buying their beef burgers from a supermarket store that received burgers made from the first mix of the contaminated batch of ground beef (Fig. 4).



**Fig. 4.** Distribution of *E. coli* O157:H7 contaminated frozen beef burgers produced from the first ground beef mix of 22 August 2005 to supermarket stores and supermarket stores where patients' families reported purchasing their beef burgers, southwest France, October–November 2005.

The 50 carcasses used to produce the first mix came from 30 different herds of cattle. At the time of submission of this paper, the results of the inspection of the production plant, the abattoir and the source farms remain under legal embargo because of multiple judicial actions resulting from this outbreak. The owners of the production plant did, however, report that the beef burger production line was not routinely disinfected between mixes, only at the end of the production day. They also reported hiring temporary staff during August 2005 to cover holiday leave of full-time staff.

The implicated supermarket chain issued a voluntary full national recall of the brand of beef burgers on 30 October 2005. The general public was informed through media channels and posters in the supermarket chain. Using supermarket loyalty cards, the supermarket identified customers that had purchased the implicated brand of beef burgers since late September 2005. The contact details of certain customers, not in possession of a loyalty card that had purchased the beef burgers and paid by cheque or debit card, were obtained from their personal bank. A team of 750 supermarket staff contacted 22 000 customers following the product recall informing them about the contaminated product, advising non-consumption of the beef burgers and encouraging customers to return the product to the supermarket.

Following the product recall, 3120 boxes of beef burgers (17% of the implicated batch), were returned by customers to the supermarket chain and another 3596 boxes (20% of the implicated batch), were removed either from supermarket shelves or stocks. Sixty-three percent ( $n=11\,152$ ) of boxes of the contaminated batch were thus either consumed by customers or disposed of in customers' homes.

## DISCUSSION

This investigation identified the largest and first community-wide *E. coli* O157:H7 outbreak recorded in France to date. The French HUS surveillance system has previously identified two familial clusters of *E. coli* O157:H7 linked to the consumption of non-pasteurized goat's cheese and lamb sausage [9, 10]. Analysis of data from this surveillance system has identified the consumption of undercooked beef burgers as a principal risk factor for sporadic HUS in children aged <15 years [11].

This outbreak represents the first time that frozen uncooked beef burgers have been identified as a vehicle of transmission for *E. coli* O157:H7 in an outbreak setting in France. Ground beef, and particularly beef burgers, are globally recognized as an important transmission vehicle for this organism [12–14]. Frozen beef burgers have previously been

implicated in reported outbreaks of *E. coli* O157:H7 in the United States [15, 16] and have also been implicated in outbreaks of *Salmonella* in France [17].

The microbiological and epidemiological evidence strongly suggest that a contamination of the batch of beef burgers produced on 22 August 2005 was responsible for this outbreak. All patients' families with an identified batch number had purchased beef burgers belonging to the batch produced on that date. Further evidence is provided by the *E. coli* O157:H7 positive samples of beef burgers from the batch of this date that were retrieved from patients' homes, the production plant and the product recall. The microbiological evidence of heavy contamination of production samples from the first mix produced on 22 August 2005 and the lesser contamination of samples from subsequent mixes produced that day favours a hypothesis of *E. coli* O157:H7 introduction into the first mix of ground beef of 22 August with cross-contamination of successive mixes. The plant owners' statement that the production line was not routinely disinfected between mixes would support such a hypothesis. Further epidemiological evidence is provided by the fact that 94% of patients' families purchased beef burgers from supermarkets that received burgers produced from the first mix of the batch.

The exact source of the contamination of the beef introduced into the burger production line on 22 August 2005 is not known but it is probable that a significant contamination of cattle carcasses with *E. coli* O157:H7 occurred in order to result in the heavily contaminated ground beef observed in production samples from the first mix. *E. coli* O157:H7 lives in the intestinal tract of healthy cattle and is transferred to meat during slaughter and processing [18, 19]. Contamination of food products by this organism can additionally result from the contamination of farm animal hides and surface contamination of abattoirs linked to storage pools of liquid manure [20]. It is possible that the hiring temporary staff at the production plant during August 2005 to cover holiday leave of full-time staff could have resulted in less rigorous routine hygiene practices on the production line, which facilitated the contamination event. However, no evidence exists to support such a hypothesis. The role of seasonal workers in other foodborne outbreaks has not previously been described in France.

The voluntary full national recall of the implicated brand of beef burgers by the supermarket chain,

issued before samples from the batch had tested positive for *E. coli* O157:H7, coupled with the high national media profile of the outbreak prevented further cases of infection. No identified cases reported consuming the contaminated beef burgers after the date of the recall, suggesting the control measures taken were effective. The frozen nature of the beef burgers and the associated expiry date 9 months after distribution to supermarket stores increased the risk of the contaminated product being consumed in the months following outbreak detection and made the product recall and communication to the public all the more necessary. It was not possible to assess the added benefit of the supermarkets' time- and resource-intensive efforts to personally inform by telephone customers who had purchased the product, as customers were not asked if they had already been aware of the outbreak and implicated food product prior to the supermarket's call.

The findings of this report are subject to at least two limitations. First, it is probable that our investigation has underestimated the true size of this outbreak. The high proportion of HUS cases observed, 25% compared to the figures of 5–8% reported in the literature [1, 21–23], suggests that this outbreak was either caused by a particularly virulent strain of *E. coli* O157:H7 [24], or that patients with a milder form of infection were not identified. The fact that most primary diagnostic French laboratories do not routinely screen stool specimens for STEC is likely have contributed to the underestimation of the number of people with milder forms of infection. Taking the number of HUS cases associated with this outbreak and extrapolating using the afore-mentioned percentage of STEC infections that develop HUS, we could roughly estimate the total number of cases in the outbreak to be in the range of 200–300, although such an estimation must be interpreted with caution. Second, the multiple ongoing judicial actions resulting from this outbreak prevent the investigation team from providing a more detailed account of the contamination of the carcasses used to produce the batch of beef burgers.

This outbreak was detected through surveillance of HUS in French children aged <15 years. While the surveillance of STEC infection through its post-infectious syndrome may seem counter-intuitive due to the delay of about 7 days between onset of prodromal diarrhoea and HUS [3, 25], the French system has proven itself to be reactive and capable of detecting clustered cases on multiple occasions [9, 10]. The

involvement of the National Reference Laboratory for *E. coli* means that isolates from HUS cases are routinely identified to at least serogroup level with characterization of virulence genes carried out on received isolates. The system is additionally capable of detecting non-O157 STEC strains and clusters, a task that most routine diagnostic laboratories undertake infrequently, due to the practical difficulties associated with identification of this group of diverse sorbitol-fermenting strains.

A feasibility study for extension of the HUS surveillance network to more generalized laboratory-based surveillance of STEC infections was carried out in 2003 as part of an evaluation of the system [26]. This study showed that few diagnostic laboratories routinely screen stools for EHEC and that only 36% of surveyed laboratories reported having carried out detection of *E. coli* O157 in 2003. Based on this information, the authors believed it unlikely that this outbreak could have been more rapidly detected via the current French laboratory network. The 2003 feasibility study concluded that a laboratory-based STEC surveillance system is currently not feasible in France [26].

This outbreak was large and severe and occurred despite the existence of hygiene and safety regulations in the ground beef industry in France. There is currently no mandatory testing of beef or beef products by producers for the presence of STEC in France. The undertaking of voluntary auto-controls during the production process is encouraged by the French Ministry of Agriculture and Fisheries and STEC-positive auto-controls, regardless of the level of contamination, should be reported to the Ministry for follow-up investigation. Following this outbreak, the French Agency for Food Safety and the French Ministry of Agriculture and Fisheries published documents that define the STEC serogroups considered pathogenic for humans, outline an annual surveillance plan for estimating the prevalence of STEC in ground beef in France, detail hygiene regulations for the production of ground beef, clarify the actions to take in case of an auto-control found to be positive for a pathogen such as STEC and describe the laboratory methods for detecting pathogenic STEC in food.

A national public health campaign was launched in January 2006 to raise awareness among the general population of the risk of STEC-related HUS in children and of preventative measures that can be taken. As beef burgers are commonly consumed

under-cooked in France, by adults and children alike, the prevention message emphasized the need to thoroughly cook ground beef before consumption, particularly when given to young children. A programme of re-education of all professionals involved in the production and preparation of beef burgers in France, from cattle breeders to restaurant owners, was additionally undertaken during 2006 via the production of information leaflets and letters of information explaining the public health risks of STEC contamination and methods of minimizing that risk.

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## DECLARATION OF INTEREST

None.

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