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Impact of Solid Wastes of Urban and Rural Egyptian Districts on Fly Density and Associated Bacteria

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Abstract: To asses the correlation between the type of solid waste, associated fly density and disseminating of bacterial diseases, sixteen sampling sites, resembling six different localities within Cairo governorate were visited for collecting samples of solid wastes and associated flies. The visited areas were EL-Demerdash hospital, Fifteenth of May city, Manshaat EL. Sadr, Manshaat Nasser, EL. Mokatam hill and EL. Obour market. Sites from Fifteenth of May city were recording the highest fly population density. Thirteen bacterial genera were identified from both waste samples and its associated flies which are belonging to four different families. Five pathogenic bacterial genera were found to be similar, on biochemical basis, when isolated from collected flies and their breeding solid waste types.

Key words: *Musca domestica* · breeding places · enteric diseases mechanical transmission

INTRODUCTION

Solid waste is the term used internationally to describe non-liquid waste materials arising from domestic, trade, commercial, industrial, agricultural, mining activities and public services. Flies are attracted to wastes of decomposed organic materials to breed. Different species of that flies are incriminated as mechanical vectors of many human diseases (diarrhoea, dysentery, typhoid, conjunctivitis and intestinal worms. [1-7] and as biological vectors [8]. Diarrhoeal diseases are the most important cause of childhood mortality (3.3 million death-world wide each year [9-11]. Little attention was paid in developing countries, regarding the influence of solid wastes in increasing fly density and on the transmission and dissemination of fly born disease agents. However the present study aimed to correlate between the type of waste and it's characteristics, the fly density and bacterial contaminants of wastes and associated flies. Mitigation measures will be assumed to asses the impact of solid wastes on dissemination of bacterial pathogens carried by associated flies.

MATERIALS AND METHODS

Sampling sites: Sixteen sampling sites, resembling six different localities within Cairo governorate were visited during this study to estimate fly density during different seasons all over the year. The visited areas were El-Demerdash hospital (Site1,2), fifteenth of May city (site 3, 4, 5, 6 and 7), Manshaat EL-Sadr (site 8), Manshaat Nasser (site 9, 10) El-Mokatam hill (site 11, 12, 13 and 14), Finally El-Obour market (site 15, 16).

Fly collecting traps: Sticky traps each of 25X21cm disposed X-ray film, covered with thin layer of commercial glue mixed with yeast and sugar were used. Six traps were put in each site as replicates. Traps were left 24hr in each site. The collected traps were used to calculate fly density and to isolate fly associated bacteria.

Collecting of solid wastes: An appropriate amount of solid wastes in each fly collecting site were collected in a sterile coded glass jars.

Bacterial Isolation, culturing and identification: Fly associated bacteria were isolated, cultured maintained as mentioned in [12].While samples of collected garbage was washed using sterile saline and the resulted fluid were used as the source of bacterial samples and treated as in case of isolation from flies. Nutrient broth, agar, MacConkey's agar, Eosin-methylene Blue (EMB) agar, Staphylococcus media were used as enriched and differential media while the prepared API 20 E and API

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50HC Staph strips (from bio Merieux) were used to identify gram negative Enterobacteriaceae, Staphylococcus and micrococcus species.

Prepared tubes permit 20 physiological and biochemical tests as Arganine dihydro lase (ADH), Citrate Tryptophane deaminase (TDA), Urase (URE), Indole (INO), Vogs Proskaure (VP), Gelatine Liquification (GEL), Sorpitol (SOR), Salicine (SAC), Nitrate test, D-Glucose (GLU), Mannitol (MAN) hydrolysis, Gas production (H_2S), Adonilol,Galactose, Maltose, Trehalose, Salicine, Esculine, Erythritol, Arabinose (ARA), Amylase (AM), Urinase test (URE) used according to the method described by Radi *et al.* [12].

RESULTS

Description of solid wastes: Solid wastes in visited urban areas were described for each site. The description is included in Table 1-6 Site 1 and 2 representing hospital solid wastes. Solid wastes in sites (3, 9) were described as industrial type. Slaughter house wastes representing in sites (15and16), while collected garbage in sites (5, 6, 8 and 10) representing house hold solid wastes.

Estimation of house fly population density: Results in Table 1 showed the mean value of fly population density collected from 2 different sites within El-Demerdash

Table 1: Impact of solid wastes in EL-Demetrdash hospital on fly density

			No. of collected flies									
Location	Sites	Sorting of solid wastes	R1	R2	R3	R4	R5	R6	Total	Mean	±SD	P-value
El-Demerdash hospital	Hospital main disposal garbage area (1)	Papers, food wastes, gauze, bloody cotton and syringes	175	163	107	148	105	99	797	132.83	33.18	0.001***>
	Incinerator area (2)	Papers, food remainders, gauze, bloody cotton and syringes	208	98	112	218	97	102	835	139.17	57.52	0.001***>
	Garage area (control)	Sandy ground	15	3	22	18	9	14	81	13.50	6.72	
	F= 20.25	P-value<0.0001	***Extremely significant									

Table 2: Impact of solid wastes in Fifteenth of May city on fly density

Fifteenth of may	Quarry of cement company(3)	Broken furniture, plants and food remainders	351	430	374	281	394	357	2187	364.50	49.92	0.001***>
	Herbaceous area (4)	Wild vegetation, household, wastes and sewage	551	395	486	389	411	498	2730	455.00	66.21	0.001***>
	Bedouin area (5)	Deeaying materials, sheep duny, poultry manure and food remainders.	253	111	124	128	120	115	851	141.83	54.80	0.05*>
	Pig farm(6)	Food remainders, papers, metals, plastic, pig and donkeys dung and human excreta	383	349	366	363	358	297	2116	352.67	29.48	0.001***>
	Public plant nursery (Mashtal) (7)	Plastic sheets, pottery, decayed, plant leaves, heaps of dung and sheep dung.	166	5	10	107	54	32	365	62.33	62.86	>0.05 ns
	An area near the plant nursery (Control)	sand, dry plant leaves and some papers.	3	3	66	46	18	2	138	23.00	27.00	
F= 75.80	P-value<0.0001	***Extremely significant	*Significant			ns Not significant						

Table 3: Impact of solid wastes in Manshaat EL-Sadr on fly density

Manshaat EL-Sadr	Open fenced area between building (8)	Shaved hair, manure, food wastes, children diapers and papers	121	517	148	153	478	157	1574	262.33	183.01	0.005**>
	Control		0	3	0	0	3	0	6	1	1.55	
F= 12.233	P-value=0.0058	**Very significant										

Table 4: Impact of solid wastes in Manshaat Nasser on fly density

Manshaat Nasser	Plastic sorting area (9)	Secondhand plastic bottles	1	0	0	0	1	0	2	0.33	0.31	p>0.05 ns
	Sorting household solid wastes area (10)	Plastics, papers, glass and food remainders	235	366	215	216	384	211	1627	271.17	81.06	p<0.001***
	Control		0	1	0	2	1	0	4	0.66	0.62	
F= 66.89	P-value<0.0001	*** Extremely significant	ns Not significant									

Table 5: Impact of solid wastes in EL-Mokatam hill on fly density

EL-Mokatam hill	Empty area between buildings (11)	Food remainders, metals, glasses, plastics and papers	62	65	61	63	59	62	372	62.00	2.00	p<0.001***
	An area on the ramp of El-Mokatam hill(12)	Wood bars, sand, bricks, cement, plastics, metal containers and plant leaves	7	23	17	9	19	21	96	16.00	6.54	p<0.001***
	Inside a private garden of palace (13)	pottery, mud, coal and some birds manure	36	24	21	29	31	18	159	26.50	6.72	p<0.001***
	Edge of El-Mokatam hill(14)	Dust, stones and tiles	0	3	0	0	2	1	6	1.00	1.26	p>0.05 ns
	Control		2	0	0	0	1	0	3	0.50	0.84	
F= 203.527	P-value<0.0001	*** Extremely significant	ns Not significant									

Table 6: Impact of solid wastes in EL-Obour market on fly density

EL-Obour market	Fish store (15)	Fish viscera and shrimps scales	14	20	13	19	11	16	93	15.50	3.51	p>0.05 ns
	Fruit market (16)	Rotten fruits such as dates and banana	21	20	18	24	18	16	117	19.50	2.81	p<0.01**
	Empty area in front of the fruit market (control)	Empty cages and papers	17	5	11	14	7	12	66	11.00	4.43	
F= 8.18	P-value<0.0040	** Very significant	ns Not significant									

Table 7: Physiological characters of isolated bacteria

Test	Result	Test	Result	Test	Result	Test	Result
Citrobacter freundii							
ONPG	+	H2S	+	GEL	-	RHA	+
ADH	-	URE	-	GLU	+	SAC	+
LDC	-	TDA	-	MAN	+	MEL	-
ODC	-	IND	-	INO	-	AMY	-
CIT	-	VP	-	SOR	+	ARA	+
OX-							
Klebsiella pneumoniae							
ONPG	+	H2S	-	GEL	-	RHA	+
ADH	-	URE	+	GLU	+	SAC	+
LDC	+	TDA	-	MAN	+	MEL	+
ODC	-	IND	-	INO	+	AMY	+
CIT	+	VP	+	SOR	+	ARA	+
OX	-						
Enterobacter cloacae							
ONPG	+	H2S	-	GEL	+	RHA	+
ADH	+	URE	-	GLU	+	SAC	+
LDC	+	TDA	-	MAN	+	MEL	+
ODC	+	IND	-	INO	-	AMY	+
CIT	+	VP	+	SOR	+	ARA	+
OX	+						
Enterobacter agglomerans							
ONPG	+	H2S	-	GEL	-	RHA	+
ADH	-	URE	-	GLU	+	SAC	+
LDC	-	TDA	-	MAN	+	MEL	+
\ODC	-	IND	-	INO	+	AMY	+
CIT	-	VP	+	SOR	+	ARA	+
OX	-						
Enterobacter sakazakii							
ONPG	+	H2S	-	GEL	-	RHA	+
ADH	+	URE	-	GLU	+	SAC	+
LDC	+	TDA	-	MAN	+	MEL	+
ODC	+	IND	-	INO	+	AMY	+
CIT	+	VP	+	SOR	+	ARA	+
OX	-						
Enterobacter aerogenes							
ONPG	+	H2S	-	GEL	-	RHA	+
ADH	-	URE	-	GLU	+	SAC	+
LDC	+	TDA	-	MAN	+	MEL	+
ODC	+	IND	-	INO	+	AMY	+
CIT	+	VP	+	SOR	+	ARA	+
OX	-						
Serratia marcescens							
ONPG	+	H2S	-	GEL	+	RHA	+
ADH	-	URE	+	GLU	+	SAC	+
LDC	+	TDA	-	MAN	+	MEL	+
ODC	-	IND	-	INO	+	AMY	+
CIT	+	VP	-	SOR	+	ARA	+
OX	-						
Proteus mirabilis							
ONPG	-	H2S	+	GEL	+	RHA	-
ADH	-	URE	+	GLU	+	SAC	-
LDC	-	TDA	+	MAN	-	MEL	-
ODC	+	IND	-	INO	-	AMY	-
CIT	-	VP	-	SOR	-	ARA	-
OX	-						

Table 7: Continue

Test	Result	Test	Result	Test	Result	Test	Result
Erwinia spp.							
ONPG	+	H2S	-	GEL	-	RHA	+
ADH	-	URE	-	GLU	+	SAC	+
LDC	-	TDA	-	MAN	+	MEL	+
ODC	-	IND	-	INO	-	AMY	+
CIT	+	VP	-	SOR	-	ARA	+
OX	-						
Aeromonas hydrophila							
ONPG	+	H2S	-	GEL	-	RHA	-
ADH	+	URE	-	GLU	+	SAC	+
LDC	-	TDA	-	MAN	+	MEL	-
ODC	-	IND	-	INO	-	AMY	+
CIT	-	VP	+	SOR	-	ARA	-
OX	-						
Aeromonas salmonicida							
ONPG	-	H2S	-	GEL	+	RHA	-
ADH	-	URE	-	GLU	-	SAC	+
LDC	-	TDA	-	MAN	+	MEL	-
ODC	-	IND	+	INO	-	AMY	-
CIT	-	VP	-	SOR	-	ARA	+
OX	-						
Pseudomonas aeruginosa							
ONPG	-	H2S	-	GEL	+	RHA	-
ADH	+	URE	-	GLU	+	SAC	-
LDC	-	TDA	-	MAN	-	MEL	-
ODC	-	IND	-	INO	-	AMY	-
CIT	+	VP	-	SOR	-	ARA	-
OX	+						
Pseudomonas fluorescens							
ONPG	-	H2S	-	GEL	+	RHA	-
ADH	+	URE	-	GLU	+	SAC	+
LDC	-	TDA	-	MAN	-	MEL	-
ODC	-	IND	-	INO	-	AMY	+
CIT	+	VP	+	SOR	-	ARA	-
OX	-						
Kluyvera sp.							
ONPG	-	H2S	-	GEL	-	RHA	+
ADH	-	URE	-	GLU	+	SAC	+
LDC	-	TDA	-	MAN	+	MEL	+
ODC	+	IND	+	INO	-	AMY	+
CIT	+	VP	-	SOR	+	ARA	+
OX	-						
Escherichia coli							
ONPG	+	H2S	-	GEL	-	RHA	+
ADH	-	URE	-	GLU	+	SAC	-
LDC	+	TDA	-	MAN	+	MEL	+
ODC	-	IND	+	INO	-	AMY	-
CIT	-	VP	-	SOR	+	ARA	+
OX	-						

Test	Result	Test	Result	Test	Result	Test	Result
Staphylococcus aureus							
O	-	LAC	+	NTT	+	SAC	+
GLU	+	TRE	+	PAL	+	MDG	-
FRU	+	MAN	+	VP	+	NAG	+
MNE	+	XLT	-	RAF	-	ADH	-
MAL	+	MEL	-	XYL	-	URE	-
Staphylococcus epidermidis							
O	-	LAC	+	NTT	-	SAC	+
GLU	+	TRE	-	PAL	+	MDG	-
FRU	+	MAN	-	VP	+	NAG	-
MNE	+	XLT	-	RAF	-	ADH	+
MAL	+	MEL	-	XYL	-	URE	-
Staphylococcus xylosus							
O	-	LAC	+	NTT	-	SAC	+
GLU	+	TRE	-	PAL	+	MDG	-
FRU	+	MAN	-	VP	+	NAG	-
MNE	+	XLT	-	RAF	-	ADH	+
MAL	+	MEL	-	XYL	-	URE	+
Staphylococcus cohnii							
O	-	LAC	-	NTT	-	SAC	+
GLU	+	TRE	+	PAL	+	MDG	-
FRU	+	MAN	+	VP	+	NAG	-
MNE	+	XLT	-	RAF	-	ADH	-
MAL	+	MEL	-	XYL	-	URE	-
Staphylococcus saprophyticus							
O	-	LAC	+	NTT	+	SAC	+
GLU	+	TRE	+	PAL	+	MDG	-
FRU	+	MAN	+	VP	+	NAG	-
MNE	-	XLT	-	RAF	-	ADH	-
MAL	-	MEL	-	XYL	-	URE	+
Gaffkya tetragena							
O	+	LAC	-	NTT	+	SAC	-
GLU	-	TRE	+	PAL	+	MDG	-
FRU	-	MAN	+	VP	-	NAG	+
MNE	-	XLT	-	RAF	-	ADH	-
MAL	-	MEL	-	XYL	-	URE	-
Micrococcus spp.							
O	-	LAC	-	NTT	-	SAC	+
GLU	+	TRE	-	PAL	+	MDG	-
FRU	-	MAN	-	VP	+	NAG	+
MNE	-	XLT	-	RAF	-	ADH	-
MAL	-	MEL	-	XYL	-	URE	-

hospital (site 1,2) to be 132.83±33.18, 139.17±57.52 respectively, these data representing 48.8 and 51.2% of the total number of collected flies in these sites. While the data in (Table 2) revealed fly density collected from different sites in fifteenth of May city. The Herbaceous area showed the highest fly density (455.00±66.21) representing 33.1% of total collected flies from this city.

Table 8: Sources and medical importance of isolated bacteria

Bacterial taxon	Medical importance	Site and source of isolation
E.cloi	It is a coliform bacteria, it may contaminate urinary tract	Sites 2, 5 (house fly)
		2, 8, 15 (garbage)
Citrobacter freundii	Cause urinary tract, gall bladder middle ear and meninges infections and wound sepsis.	3, 6, 7, 8 (house flies)
		10, 15, 16 (garbage)
Klebsiella pneumonia	It is associated with respiratory infection and	5, 8 (house fly)
	both endemic and epidemic infections in hospital	1, 2, 7 (garbage)
Enterobacter cloacae	It is usually isolated from urinary tract infections	6, 7 (house fly)
		3 (garbage)
Enterobacter agglomerans	As Ent. cloacae	20 (house fly)
		7 (garbage)
Enterobacter sakazakii	It cause bacterimia	15 (house fly) 22 (garbage)
		24 (house fly & garbage)
Enterobacter aerogenes	It is found in faeces of man and animals, sewage, soil, water and dairy product	6 (house fly)
		3, 16, 23 (garbage)
Serratia marcescens	It is isolated from urinary infections, wound infections and cerebrospinal fluid	16 (house fly)
		2, 23 (garbage)
Proteus mirabilis	It is found in fecal parts of animals, sewage and soil. It is isolated from gastrointestinal	
	and urinary tract infections. Pneumonia and septicemia can also be due to this organism.	2, 5, 6, 16 (house fly)
Erwinia spp.	Different species are associated with plants.	
	One species is isolated from animal and human host	3, 7 (house fly)
Aeromonas hydrophila	Causes red Leg disease in frogs. Pathogenic for snakes	2 (house fly)
	causing septicemia. It may cause fish infections in fresh water.	6, 15 (garbage)
Aeromonas salmonicida	Cause furunculosis to fishes	15 (house fly)
		10 (garbage)
Pseudomonas aeroginosa	It is isolated from wide varieties of environmental sources	1, 10 (house fly)
	including earth, water animals, insects, kitchens, bathrooms,	2, 6, 7 (garbage)
	hospital stuff. It may be pathogenic under certain circumstances.	3 (house fly & garbage)
Pseudomonas fluorescens	It is common associated with spoilage of food and clinical specimens.	10, 15 (garbage)
Kluyvera spp.	It is isolated from sputum, urine, stool and blood and	12 (house fly)
	considered potentially dangerous pathogen to human.	6 (garbage)
Staphylococcus aureus	It is the cause of many human infections as boils, abscesses,	3 house flies)
	osteomyelites and bronchopneumonia. It is also cause food poisoning.	1, 8(garbage)
Staphylococcus epidermidis	It is isolated from abscesses and wounds it is considered secondary invaders to human.	6, 10 (garbage)
Staphylococcus xylosus	It is isolated from polluted water, dairy products and different cheeses	8, 16 (garbage)
Staphylococcus cohnii	No medical importance.	16 (house fly)
		3 (garbage)
Staphylococcus saprophyticus	It is able to agglutinate human, bovine & sheep erythrocytes.	15 (house fly)
Gaffkya tetragena	It can contaminate wounds and cause carbuncle and pyemia.	10 (house fly)
Micrococcus spp.	No medical importance in the available references	13 (house fly) 5 (garbage)

Table 9: Bacterial species sharing both solid wastes and their fly samples

Taxon name	Location	Site	Type of solid waste
E.coli	EL-Demerdash hospital	Incinerator area (site 2)	food remainders, gauze, bloody cotton and syringes
			plastic and blood bags
Klebsiella pneumonia	Manshaat EL-Sadr	fenced area between building (site 8)	Shaved hair, manure, food wastes, children diapers and papers
Enterobacter zakazaki	EL-Obour market	Fish store (site 15)	Fish viscera and shrimps scales
Staphylococcus xylosus	EL-Demerdash hospital	main disposal garbage area (site 1)	food stuffs, bloody cotton and hazardous, solid wastes
Pseudomonas aeruginosa	Fifteenth of may city	Quarry of cement company (site 3)	Broken furniture, plants and food remainders

The quarry of cement company (site-3) and pig farm (site-6) which classified as industrial and household types respectively appeared to be the most attractive sites to flies, representing 26.5 and 25% of the total collected flies from that area. One site from Monshaat El-Sadr representing house hold type of waste (Table 3) showed mean fly density of 262.33±183.01. Table 4 clears that the highest mean of fly density from Manshaat Nasser accompanied with the site no (10), representing house hold wastes, to be 271.17±81.06. It is noticed that there is no significant differences between fly density (0.33±0.32) in site (10) and that of control (0.66±0.61). Four different sites from El-Mokatam hill were visited. The associated fly density are represented in Table 5, the highest mean fly density (62.00±2.00) was recorded in site (11) which represent house hold wastes. In El-Obour market, agricultural wastes in site (16, Table 6) was more attractive to flies than the slaughter house wastes (site 15) the fly density from previous sites are 19.50±2.81, 15.50±3.51 respectively. It is realized that, concerning means of the densities, there was significant differences between site 16 and control site, while the difference between site 15 and the control site was not significant.

Echeverria *et al.* [13] proved that the solid wastes was a favourite place for fly breeding while [14] and [15] concluded that the house fly *Musca* sp. was dominant than stable fly and *Fannia* sp. breeds on poultry faeces in farms.

Bacteria associated with different types of solid wastes and collected flies: According to morphological and physiological characteristics of isolated bacteria, provided by the API tubes (Table 7). Thirteen bacterial genera were isolated from both surveyed house flies and collected garbage. Bacterial isolates are belonging to the families Enterobacteriaceae (*Citrobacter, Esherichia, Klebsiella, Enterobacter, Serratia, Proteus, Erwinia and Kluyvera* spp.), Vibrionacea (*Aeromonas* spp.) Pseudomonadaceae(*Pseudomonas* spp), Micrococcaceae (*Staphylococcus*, *Gaffkya* and *Micrococcus* spp.) as shown in Table 7, although Twenty two bacterial species were isolated from garbage sites and flies (Table 8). Five bacterial genera only are found to be similar when isolated from both collected fly samples and their breeding solid waste type as shown in Table 9. [16-18]. Isolated the majority of these bacteria from collected flies from different breeding localities and illucidating its medical importance.

DISCUSSION

The importance of solid waste management imerged from its suitability for fly breeding and subsequently distribution of many microbial disease agents, especially entrics. Data proved that the waste constituents in different localities influence fly population density (Table 1 and 2). The duration of solid wastes (The period in which the solid wastes are left without disposing or getting rid off) is an important factor affecting the fly population.

For house-hold waste type (sites 5, 6, 8) (including decaying materials) poultry manure, food reminder, shaved hairs, plastics in Manshaat Naser and EL-Sadr where solid wastes are left for relatively long periods, The accompanied fly density was higher by 3-5 times than that sites of EL-Mokatam hill which considered of higher socioeconomic level with higher rate of disposing wastes(Table 2, sites 11, 12). While in rural areas, comparing solid wastes in EL-Obour market (site 16) and fifteenth of May sites (4, 7 Table 1) which could both considered agricultural wastes, fly population density was very higher in sites of fifteenth of May that of EL-Obour market. The slow disposing process of such wastes in suburban areas like fifteenth of may is a factor limiting fly density. This may be due to remaining of fermented matters for long time in such waste sites.

Regarding the correlation between solid wastes and the rate of fly contamination with pathogens, results revealed that, six bacterial species were similar when isolated from wastes and surrounding flies. This proved that fly bacterial contamination is mainly due to fly visits to those solid waste sites. On the other hand the dissimilarity of other bacterial species which are isolated from flies but not from breeding waste site could be considered natural contaminants from other visiting sites. The present results [16, 19-23] are in accordance with several authors In spite of the agreement with many authors in these conclusions. It still thought that this similarity of bacterial species between waste location and associated flies is not enough proof to incriminate house flies to disseminate and responsible for transmitting waste contaminants at the level of conventional bacterial identification techniques. So, using molecular biological techniques such as protein profiling and DNA finger print may help to assure these findings.

CONCLUSION

The achieved results cleared that the surveyed localities could resemble urban and rural sites and may have similar solid waste type. The duration of solid wastes before elimination is an important factor in controlling fly density and accordingly affact control planes. identical pathogens,isolated from flies and breading solid wastes incriminate house flies as vactor for such pathogens. The following recommendations may be helpful: Environmental health education of all people who handling these types of wastes, using antimicrobial agents for disinfection of the disposed materials. using appropriate containers before disposing hazardous materials. Increasing the role played by the municipal authorities and establishment of main garbage collecting areas at the out skirts of urban areas.

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