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CHANGING PATTERN OF FASCIOLIASIS PREVALENCE EARLY IN THE 3RD MILLENNIUM IN DAKAHLIA GOVERNORATE, EGYPT: AN UPDATE

By

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Abstract

Fascioliasis is an important food- and water-borne parasitic zoonosis caused by liver flukes of genus *Fasciola* (Digenea: Fasciolidae) of worldwide distribution.

In Egypt, fascioliasis was encountered in nearly all Egyptian Governorates, particularly in the Nile Delta and specifically in Dakahlia. All enrolled cases were subjected to complete history taking, clinical examination, routine investigations and abdominal ultrasonography. Stool analysis, IHA and ELISA were used for fascioliasis diagnosis. Rural areas showed highest prevalence of fascioliasis than urban areas, however, but without significance (x^2= 0.042 & P= 0.837). Regarding human fascioliasis in examined the centers, no statistically significant difference (x^2 =2.824 & P=0.243) was detected. Regarding gender variation, the difference was statistically insignificant (x^2= 0.166 & P= 0.683). The difference between the age groups was statistically insignificant (x^2= 3.882 & P=0.274).

Clinically, 7 cases (35%) were asymptomatic and another 13 cases (65%) had different clinical pictures. Abdominal pain, anemia, eosinophilia, and tender hepatomegaly were seen in 70%, 80%, 70%, and 10%; respectively. Of them, 11cases showed positive abdominal ultrasonographic findings suggestive of fascioliasis.

Keywords: Egypt, Dakahlia Governorate, Zoonotic fascioliasis, Clinical outcome

Introduction

In human fascioliasis, the clinical picture may be suggestive of the disease. In support of clinical diagnosis, knowledge of the epidemiology of the disease is important with a history of ingestion of raw, wild or cultivated watercress or other vegetables or any contaminated water or food, may be suggestive of infection (Chen and Mott, 1990). Also, El-Shazly *et al*, (1991) stated that a high index of suspicion is needed as a first step in the diagnosis. Acute fascioliasis must be suspected in patients with tender hepatomegaly, fever, and eosinophilia (Neva and Brown, 1994). In chronic phase, the clinical picture is attenuated and can be easily confused with other disease. The classic pattern includes; vague gastrointestinal complaints, pain in the right hypochondrium or epigastrium, cholecystitis, cholangitis, en-

larged liver with and without pain on palpation, and ascites may appear in advanced cases (Osman and Helmy, 1994; Seif El-Nasr *et al*, 2006).

Direct parasitological examination is not a dependable test due to misdiagnoses or false negative results in chronic cases and in cases with scanty egg deposition. Serological diagnosis is the alternative method for such cases, especially during early stages of infection, in cases of ectopic fascioliasis and to assess treatment efficacy (Cook and Zuml, 2003). Nevertheless, cross reactions and false positivity in some serological tests constitute a major problem especially in a country like Egypt with endemic schistosomiasis (Hasseb *et al*, 2003).

This study aimed to detect prevalence and the intensity of infection in some rural and urban localities of Dakahlia Governorate, Egypt, for evaluating present situation of zoonotic fasciolaiasis.

Subjects, Materials, and Methods

A total of 1768 cases of different ages with a written consent from them or their gardeners were collected from Centers of Mansoura, Talkha, Aga and Belqas to study the fasciolisis prevalence from May 2010 to August 2012. Lab. work was accomplished in Departments of Parasitology of Mansoura and Al-Azhar Universities.

All cases were subjected to: 1. Questionnaire with special stress on occupation, age, sex, education, family income, and dietary habits as eating green vegetables, and/or drinking untapped water, fever history, abdominal pain, abdominal distension, anorexia, vomiting, diarrhea, dysentery, jaundice, itching, fatigue, chest symptoms as cough and pain. 2. Thorough clinical examination for fever, pallor, jaundice, abdominal distension, palpable liver, spleen and any abdominal mass. Chest and heart examination were performed.

Laboratory investigations included; a). Stool examination was done by direct smear, formol ether sedimentation (Garcia and Bruckner, 1997) and Kato-Katz technique (Katz *et al*, 1970). The slides were prepared for each individual fecal sample, utilizing a template of 41.7 mg. The number of eggs per slide was calculated as eggs per gram of feces (EPG) applying a multiplication factor of 24. The intensity of fascioliasis infection was classified according to EPG feces into: light infection up to 40, moderate infection for 41- 80 and heavy infection for more than 80 EPG (El-Shazly *et al*, 1991). Sensitivity of stool examination for *Fasciola* ova was increased by repeated analysis (Chen and Mott, 1990) and by fluke finder technique (Welch *et al*, 1987). The subjects were asked to be on liver-free diet one week prior to stool analysis (Markell *et al*, 1992). b). Serodiagnosis for fascioliasis by IHA (Fumose, France) and ELISA (E/S) antigen (Cypress diagnostics, Belgium was done (Haseeb *et al*, 2002; 2003). c). Complete and differential BC. d). Renal function tests (urea and creatinine). e). Liver function tests; Aspartate aminotransferase, Alanine aminotransferase, Alkaline phosphatase, gamma-glutamyl transferase, and total bilirubin, were all estimated by caliometrically (Tiedz, 1995). 4. Abdominal ultrasonography.

Statistical analysis: Statistical Packa-

ge for Social Sciences (SPSS) 15.0 (SPSS, Chicago, IL, USA) was used for statistical analyses. X^2-test was used to assess difference between the studied groups. A p-value ≤ 0.05 was considered significant.

Results

The results are shown in tables (1, 2, 3, 4, 5, 6, 7 & 8).

Table 1: Fascioliasis by stool examination, IHA and ELISA in selected centers

Detection Methods	Mansoura* (n=442)	Talkha* (n=663)	Belqas* (n=244)	Aga* (n=387)	Total* (n=1868)
Fresh stool* *	2(0.45%)	5(0.75%)	2(0.68%)	2(0.52%)	11(0.62%)
IHA**	3(0.68%)	6(0.90%)	3(1.02%)	2(0.52%)	14(0.79%)
ELISA**	5(1.13%)	8(1.21%)	4(1.36%)	3(0.78%)	20(1.13%)

* X^2= 2.824, p=0.243, **X^2= 10.07, p= 0.042

Table 2: Fascioliasis intensities by stool examination (epg) in different centers

Parameter	Mansoura (n=442)		Talkha(n=663)		Belqas(n=294)		Aga(n=3870		total
Intensity	n	epg	n	epg	n	epg	n	epg*	
Light	2	23,35	2	20,34	1	30	1	25	6
Moderate	0		2	61,69	1	77	0	0	3
Heavy	0		1	83	0	0	1	95	2

N=11, EPG* = egg per gram stool.

Table 3: Relation of various techniques for fascioliasis diagnosis to residence

Parameter	Stool	IHA	ELISA
Residence: Urban(581)	4(0.89%)	6(1.3%)	7(1.20%)
Rural(1178)	7(0.59%)	8{0.67%)	13(1.10%)
Total(1768)	11(0.62%)	14(0.79%)	20(1.13%)

ELISA p-value= 10.07 and x^2 = 0.042

Table 4: Distribution of fascioliasis according to sex

Parameter	Stool	IHA	ELISA
Males (912)	5(0.55%)	6(0.66%)	11(1.12%)
Females (856)	6(0.70%)	8(0.93%)	9(1.05%)
Total (1768)	11(0.62%)	14(0.79%)	20(1.13%)

ELISA X^2= 1.263, p-value=0.737.

Table 5: Clinical presentation among fascioliasis patients

Clinical Presentations	Numbers	(%)
Asymptomatic	7	35
Symptomatic	13	65
Abdominal pain:		
Rt Hypochondial pain	5	25
Diffuse abdominal pain	3	15
Lt side abdominal pain	2	10
Colicky abdominal pain	1	5
Epigastric pain	1	5
Loin pain	2	10
Jaundice	3	15
Nausea	6	30
Vomiting	2	10
Constipation	2	10
Diarrhoea	1	5
Pallor	14	25
Joint pain	2	10

Table 6: Age distribution of fascioliasis according to age groups

Parameter	Detection Method		
	Stool	IHA	ELISA
Age (years): Up to 5 years(324)	1(0.31%)	2(0.62%)	2(0.62%)
> 5-20 years(566)	3(0.53%)	3(0.53%)	4(0.71%)
>20-40years(559)	5(0.89%)	6(1.07%)	10(1.79%)
> 40 years(319)	2(0.63%)	3(0.49%)	4(1.25%)
Total(1768)	11(0.62%)	14(0.79%)	20(1.13%)

Table 7: Clinical signs among fascioliasis patients

Signs	Numbers	Percent
Hepatomegaly	2	10
Splenomegaly	1	5
Ascites	1	5
Anaemia	16	80
Eosinophilia	14	70
Cholecystitis	3	15
Cholangitis	4	20
Pancreatitis	1	5
Jaundice	5	25
Tender right upper quadrant	1	5
Tender colon	1	5
Tympanitic abdomen	2	10

Table 8: Ultrasonography of fascioliasis patients

Patients	Report
7 Cases	Normal size and wall thickness of gall bladder, with normal common bile duct (CBD) measure 3mm. no detected worms. No ascites.
3 Cases	Normal G.B., dilated C.B.D. = 1 cm, with multiple small hyperchondria mobile structures in C.B.D. each measures about 9 mm; impressive of parasitic worms. No ascites.
2 Cases	Minimal enlarged liver, with homogenous echopattern. Normal G.B. & C.B.D. No worms. No ascites.
4 Cases	Average sized coarse homogenous liver with mild PPF Thick-walled G.B. = 6 mm. with multiple variable echogenic structures in side. Normal C.B.D. = 5mm. No stones or worms. No ascites.
2 Cases	Hepatomegaly, C.B.D.dilatation, Splenomegaly, Intrahepatic biliary radical dilatation, Echogenic shadows in bile duct, Olympic game ring
2 Cases	PPF, Fatty liver, cirrhotic liver, ascites, cystic focal lesion on top of cirrhosis, Chronic calcular cholecystitis, bile duct stenosis.

Discussion

In the present study, 11 patients (0.62%) of the studied groups were positive for *Fasciola* infection by direct stool examination, while by serological examination using IHA, 14 (0.79%) patients were positive and 20 cases (1.13%) were positive by using ELISA. There was a significant difference between stool examination, IHA and ELISA (x^2=10.07, *p*-value=.042).

El-Shazly *et al.* (2001) recorded a prevalence of 7.4% in Dakahlia Governorate. In Beheira Governorate, the reported over-all prevalence was 21.8% by Esteban *et al.* (2003).

However, the prevalence of *Fasciola* of infection rates were reduced in the last decade due to the control measures undertaken by the Egyptian Governmental Authorities; including chemical molluscicides and Triclabendazole® administration (El-Shazly *et al*, 2009) and Mirazid® in the private hospitals and clinics (El-Shazly *et al*, 2002; 2006; Massoud *et al*, 2007).

Among the centers examined, the highest *Fasciola* infection prevalence was in Talkha: 5 cases (0.75%), 6 cases (0.90%) and 8 cases (1.21%) followed by Mansoura center: 2 cases (0.45%), 3 cases (0.68%) & 5 cases (1.13%), then followed by Belqas: 2 cases (0.68%), 3 cases (1.02%) and 4 cases (1.36%) and the lowest prevalence was detected in Aga: 2 cases 0.52%), 2 cases (0.52%) and 3 cases (0.75) by stool examination, IHA and ELISA, respectively (Tab. 1). There was no statistical significant difference of prevalence among these centers (x^2 =2.824 & P=0.243).

The heaviness of *Fasciola* infection in each center was Mansoura (mild, moderate, and heavy in 2, 0, 0; respectively), in Talkha 2, 2, & 1 and in Belqas 1, 1, & 1, respectively. The classification was adopted by El-Shazly *et al*. (1991) who classified that mild infection to be from one to 40 EPG, moderate ranged from above 40 to 80 and heavy above 80. Accordingly, El-Shazly *et al*. (2009) reported that 75% of the infected cases, in the rural areas, had light infection, and 25% had moderate infection while in urban ones all cases had light infection.

Comparison between urban and rural areas in our study revealed that increased infection with fascioliasis in rural areas; 7 cases (0.59%), 8 cases (0.67%),13 cases (1.10%) more than in urban ones; 4 cases (0.89%), 6 cases (1.03%) and 7 cases (1.20%) by stool examination, IHA, and by ELISA; respectively (Tab. 3). Rural areas showed higher prevalence of fascioliasis than urban areas with a statistical significant difference (x^2= 0.837 & P= 0. 042).

The present results were in accordance with El-Shazly *et al*. (1999) in the same rural districts screened herein and reported a prevalence of 0.4% of fascioliasis, However, the results were much less than in Kafr El-Hessa village, where fascioliasis prevalence was 7% (El-Gilany *et al*, 2001). In some Nile Delta villages prevalence rates ranged from 2% to 17% (WHO, 1995).

Man is an accidental host, but depending on dietary habits, there can be very high prevalence of fascioliasis in populations of particular areas. Infection results from eating uncooked and unwashed aquatic vegetables on which larval parasite are encysted, with majority of human infections reported specially from rural areas (Dobrucali *et al*, 2004).

Regarding sex's variation (Tab. 4) the prevalence of *Fasciola* showed relative increase among female cases by stool examination; 6 cases (0.7%) in females and 5 cases (0.55%) in males. But, infection was higher in males (9 cases (1.01%) than females and 11 cases (1.21%) in males, without difference in significance (x^2=0.683 & P= 0.166).

Qureshi *et al.* (2005) in Pakistan found that females had a slightly higher infection (0.31%) than males (0.28%) but without a significant level. Abdel Aal *et al.* (1999) and Curtale *et al*. (2003) also reported non-significant in females than males. On the other hand, El-Shazly *et al*. (2006) reported a significantly higher prevalence among females. This fact was reported by several authors (Garcia *et al*, 1985; Haseeb *et al*, 2002; 2003). This could be attributed to the fact that females are

more exposed to infection during families' food preparation, by consuming or tasting improperly washed vegetables while cooking.

In the present study, fascioliasis was relatively higher in the age group between 20 and 40 years old, 5 cases (0.89%) by stool examination and 10 cases (1.79%) by ELISA followed by age group 5 and 20 years old, 3 cases (0.63%) by stool examination and 4 cases (0.71%) by ELISA. The lowest prevalence was among age group less than 5 years old (0.31%) by stool examination and 2 cases (0.62%) by ELISA (Tab. 5). The difference between the groups was statistically insignificant (x^2= 3.882 & P=0.274). El-Shazly *et al*. (2002) studied the distribution of cases by age and found that all age groups were infected with *Fasciola* and the lowest infection was evident among those less than five years old.

Qureshi *et al*. (2005) showed that all infected individuals with *Fasciola* were below 20 years. Curtale *et al.* (2003) also found that individuals below 19 years were more susceptible while Abdel-Aal *et al.* (1999) and Moghaddam *et al.* (2004) reported that all ages were equally susceptible to fascioliasis. This may be due to difference in living habits and hygienic conditions in a particular area. El-Shazly *et al.* (1999) in the same rural districts screened reported a prevalence of 6.4% in the age group of 5-15 years. Infection was more prevalent in females, illiterates, farmers and in age group of 30-45 years. In endemic areas, children are most affected age-group. There was no significant difference between males and females which is explained by similarity of diet in both sexes.

Prevalence of fascioliasis in Bolivian Altiplano fluctuated from 27.6% to 55.6% in school children (Esteban *et al*, 1997). An estimated prevalence of 20% was in a population of 1.8 million Bolivian farmers (Bjorland *et al*, 1996). El-Shazly *et al.* (2006) reported that the incidence of fascioliasis was 0.4% in Gogar and 0.2% in Mansoura, in Gogar, 75% of cases had light infection and 25 % of cases had moderate infection, but in Mansoura, all had light infection. Motawea *et al.* (2001) reported that the prevalence in Kafr El-Hessa was 7%. This agreed with Husein *et al.* (2000) who reported 7.3% in Abis 7 and Abis 8. Curtale *et al.* (2005) reported that the prevalence in endemic area was reduced from 5.6% to 1.2% after selective treatment carried by the Egyptian Ministry of Health and Population targeting high risk age groups at villages from 1998 to 2002. Also, El-Shazly *et al.* (2001; 2005a) found light infection in 60.4% of cases, moderate in 27.1% and severe infection in 12.5% of cases.

In Egypt, the pattern of *Fasciola* species infection appears to be changing in some areas of the Nile Delta. The infection was spreading from the original situation of human sporadic cases in known animal endemic foci to a present human endemicity, which may be catalogued as a meso-endemic zone including a few human hyper-endemic foci (Mas-Coma *et al*, 1999). Human fascioliasis in Egypt was known on an individual basis. The first confirmed cases were diagnosed in 1978 in Abis area; Alexandria district which was de-

clared as an endemic focus (Farag *et al,* 1979). Since then, the problem has received an increasing attention. Human fascioliasis was reported from different governorates. For example, it has been reported in Alexandia (Abou Basha *et al,* 1989), in Qalyoubia (Magdi *et al,* 1993), in Menoufia (Abdel Rahman *et al,* 1995), in Kafr El-Sheikh (El Bahy, 1997), in Cairo (Abel Aal *et al,* 1999), in Dakahlia, (El-Shazly *et al,* 2001; 2005b; Wahib *et al*, 2006), in Sharkia (Hassan *et al,* 1995), in Al-Fayoum (Abo-Madyan *et al*, 2004). El-Shazly *et al*. (2005a) diagnosed coprologically twenty five patients passing eggs, in period from April 2004 to April 2005, complaining from calcular and non-calcular cholecystitis, dilated intrahepatic biliry radicles and ascites in 32%, 24%, 20% & 4%; respectively.

Regarding the clinical pictures of fascioliasis cases (Tab. 6); seven cases (35%) were asymptomatic, while 13 cases (65%) were presented with many complaints: pain in the right hypochondrium 5 cases (25%), left-sided abdominal pain 2 cases (10%), colicky abdominal pain one case (5%), epigastric pain one cases (5%), loin cases 2 cases (10%), jaundice 3 cases(15%), nausea 6 cases (30%), vomiting 2 cases (10%), constipation 3 cases (15%), pallor 14 cases (70%), and diarrhea one case (5%). Physical and laboratory examinations (Tab. 7) revealed hepatomegaly in 2 cases (10%), splenomegaly in one case (5%), ascites in one case (5%), anemia in 18 cases (80%), eosinophilia in 14 cases (70%), cholecystitis in 3 cases (15%), cholangiolitis in 4 cases (20%), pancreatitis in one case (5%), jaundice in 5 cases (25%), tender right upper quandrant in one case (5%), tender colon in one case (5%), and tympanitic abdomen in 2 cases (10%).

In the present study, 35% of cases were asymptomatic. The asymptomatic patients were reported (Abdel Aal *et al,* 1999; Motawea *et al,* 2001; Curtale *et al*, 2005; Mas-Coma *et al*, 2005; Le *et al*, 2007**;** El-Shazly *et al,* 2009). Many asymptomatic cases were spontaneous cured (Hiller and Galanes, 1988). El-Shazly *et al.* (2001) found 382 fascioliasis patients in association of other manifestations, pallor (100%), abdominal pain (92.67%), fever (85.6%), fatigue (83.25%), hepatomegaly (81.94%), nausea (67.53%), splenomegaly (13.4), ascites (13.1%), jaundice (11.5%), urticarial rash (1.3%) and itching (1.04%).

Abdel Wahab *et al.* (1996) reported fever, diarrhea, hepatomegaly, right hypochondrial pains and tenderness, anorexia, but nausea, vomiting, and icterus were not so common in patients. Ashton *et al.* (1970) reported vague clinical picture, in many fasciolaisis patients, due to unexplained lack of development of larvae into adult worms. Prince *et al.* (1993) stated that signs and symptoms of various stages of fascioliasis infection are quite different and no definite clinical picture can be given.

Infection with *F. hepatica* has two clinical phases with different signs and symptoms. The appearance of symptoms of fascioliasis and the disease severity depend on the intensity of infection. Symptoms may appear in few days after ingestion of larvae, when the immature forms reach the abdominal cavity and migrating across the or in

the liver parenchyma (acute phase). The symptoms may be related lesions in the abdominal cavity and during acute phase including fever, right upper quadrant pain, nausea, anorexia, vomiting, eosinophilia, urticaria, hepatomegaly, jaundice, pruritus and weight loss, elevated liver enzymes, and hypergammaglobulinaemia. There may be as well hepatomegaly, anemia and jaundice. When the worms reach the bile ducts, mature and begin to produce eggs. According Alksoy *et al.* (2006) this stage can be complicated by mild hepatitis, hepatic necrosis, and subcapsular hemorrhage. The biliary (chronic) stage can remain asymptomatic for many years; and eosinophilia was the only prominent symptom (Abo-Madyan *et al*, 2004). Nevertheless, patient suffered from episodic, intermittent right upper quadrant pain, cholangitis, cholestsis, pancreatitis, bile duct stones, and biliary obstruction due to duct wall thickening or obstruction (Kabil *et al*, 1994). Rarely, extrabiliary migration can cause painful or itchy spontaneous nodules (up to 6 cm.), focal lesions (mass, abscess, or hemorrhage) in brain, orbit, lungs, or other body sites (Wilson, 1991). In a group of fascioliasis patients (24% of 187 cases of undetermined fever), Abel Wahab *et al.* (1996) reported diarrhea, hepatomegaly, right hypochodrial pain, tenderness, anorexia, leucocytosis and high eosinophilia.

During intraocular surgery of a 44-year-old woman from Guilan Province in the north Iran, a small flat parasite was removed from the anterior chamber angel of the left eye. According to the morphological characterization of the parasite, it was identified as *F. hepatica*. The route of entry of the parasite was not identified. Meanwhile, stool and serology examination of the patient for diagnosis of *Fasciola* infection was negative. In endemic areas, ocular involvement should be considered in cases of uveitis, despite no other system involvement (Dobrucali *et al*, 2004). Ragab and Farag (1978) and Kabil *et al.* (1994) reported hemophilia as a complication; this may explain the lower mean hemoglobin level and pallor in cases than control. Esteban *et al.* (2003) suggested that anemia of fasciolaisis could be due to the release of toxins by adult flukes which may have deleterious effects on blood cells. Other workers suggest that anemia was due to the hematophagous habit of the fluke; so causing a blood loss type of anemia. Kabil *et al.* (1994) reported eosinophilia (above7%) in (67%) of patients with a range of 10-18%

In the present work (Tab. 8), the ultrasonography of fascioliasis patients showed the following: 7 cases showed normal size and wall thickness of G.B., with normal common bile duct measured 3 mm, and no detected worms or ascites. Three cases showed normal G.B. and dilated CBD measures 1 cm, multiple small hypochondria mobile structures seen in CBD, each measures 9mm; impressive of parasitic worms and no ascites. Two cases showed minimal enlarged liver, with homogenous echopattern (normal G.B., normal G.B. and no ascites). Four cases showed average sized, coarse homogenous liver with mild periportal fibrosis (PPF),

thick walled G.B. measures 6 mm, with multiple variable echogenic structures inside, normal C.B.D. measures 5 mm, no detected stones nor worms or ascites. Two cases had hepatomegaly, CBD. dilatation, splenomegaly, intrahepatic biliary dilatation, echogenic shadows in bile duct, Olympic game ring. Two cases showed PPF, fatty liver, cirrhotic liver, ascites, cystic focal lesion on top of cirrhosis, chronic calcular cholecystitis and bile duct stenosis.

Motawea *et al*. (2001) reported ultrasonographic biliary abnormalities in 47.7% of *Fasciola* cases compared to 1.3% among controls; those specific for *Fasciola* were hyperechogenic crescent-shaped parasite in C.B.D. & G.B. without posterior acoustic shadowing in 8.2% & 3.1%, respectively. Spontaneous mobility of the parasites was found in 6.9% and 2.5%; respectively. Tender hepatomegaly on probing was found in 4.4% of *Fasciola* cases. Non-specific ultrasound findings, highly suggestive of fasciolaisis were C.B.D. dilatation, G.B. tenderness, calculi, wall-thickening and impaired G.B. contractility in 13.8%, 12.6%, 4.4%, 3.1%, and 1.9%; respectively.

Conclusion

In general, it is estimated that millions of people are infected worldwide and the number of those at risk exceeds 180 million. The outcome data showed that despite the observed marked reduction in the prevalence and intensity of fascioliasis in Dakahlia Governorate, clinicians should consider its possibility in the differential diagnoses in cases of hepatomegaly, anemia, eosinophilia, and abdominal pain. In spite of the fact that fascioliasis prevalence is decreasing, the activity of the Ministry of Health and the Ministry of Agriculture must not stop

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