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Sero-epidemiological survey for alveolar echinococcosis (by Em2-ELISA) of blood donors in an endemic area of Switzerland

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Abstract

Sera from 17 166 blood donors living in 10 cantons of northern Switzerland in an area endemic for *Echinococcus multilocularis* were investigated by serological survey for alveolar echinococcosis (AE). A highly species-specific antigen (Em2) and a commonly used *E. granulosus* hydatid fluid antigen (EgHF) were compared for their suitability in seroepidemiology. EgHF showed a degree of nonspecificity which did not allow direct detection of AE cases. Antibody reaction with Em2 resulted in the detection of 2 asymptomatic clinical cases of AE (seroprevalence 0·01%) within this population of blood donors. A further 4 persons were positive in Em2-ELISA. These 4 persons had negative imaging studies and will be followed serologically and clinically.

Introduction

Alveolar echinococcosis (AE) is a zoonotic disease caused by the larval stage (metacestode) of *Echinococcus multilocularis*. There is evidence that the infection is acquired many years before clinical manifestation of the disease. At the time when symptoms occur, pathological lesions in the liver, and sometimes other organs, have progressed so far that 42 to 74% of the patients are inoperable (SCHANTZ *et al.*, 1982; SCHANTZ & GOTTSTEIN, 1986). In these cases, and also after partial resection of liver lesions, the prognosis is unfavourable. In patients not submitted to specific chemotherapy lethality is very high, reaching 94% within 10 years after diagnosis of the disease (SCHICKER, 1976).

In Switzerland echinococcosis is well known as a public health hazard. Between 1956 and 1969 an annual average of 8·7 new cases of AE was diagnosed, amounting to 122 cases (DROLSHAMMER *et al.*, 1973). The annual morbidity rate per 100 000 inhabitants was 0·14. Most cases were of Swiss nationality and in the age group between 51 and 60 years. The highest number of cases was found in the northern parts of the country. Recently, for the period 1970 to 1983, an annual average of 10 new cases of AE, with a total of 145 cases, has been registered (Gloor, unpublished observations).

In Switzerland the epidemiological situation is complicated by the fact that *E. multilocularis* and *E. granulosus* occur sympatrically (ECKERT, 1970; DROLSHAMMER *et al.*, 1973).

The aim of the present survey was to determine the prevalence and geographical distribution of AE in a population of blood donors and to attempt early diagnosis of the infection. Early diagnosis and treatment may reduce mortality of the disease. The epidemiological situation of low prevalence required a serological test system of high diagnostic sensitivity and species-specificity for the detection of infections with *E. multilocularis*.

The purification of a species-specific antigenic polypeptide (Em2) from *E. multilocularis* metacestodes by affinity chromatography and its subsequent evaluation by enzyme-linked immunosorbent assay (ELISA) demonstrated a high diagnostic sensitivity (93·6%) and specificity with regard to cross-reactions due to nematode and trematode infections (100%) and to cestode infections (95%) (GOTTSTEIN *et al.*, 1983; 1986a; GOTTSTEIN, 1985).

This paper summarizes the results of 2 seroepidemiological studies on AE in northern Switzerland carried out by 2 institutions, particularly regarding the relative potential of EM2 and EgHF as immunodiagnostic tools for sero-epidemiology of AE.

Materials and Methods

Sera from 12 000 healthy blood donors were obtained between September 1984 and March 1985 from 9 cantons of northern Switzerland (Aargau, 1495; Appenzell, 109; Basel, 2370; Bern, 279; Schaffhausen, 1158; Solothurn, 197; St Gallen, 369; Thurgau, 4932; Zürich, 1091) which are endemic areas of AE (DROLSHAMMER *et al.*, 1973) (Fig. 1). The sera were collected by mobile teams of the Central Laboratory of the Blood Transfusion Service (Swiss Red Cross) and by local blood donation centres at Frauenfeld and Münsterlingen. These sera were selected for direct testing with Em2 by ELISA. Further sera were obtained from 5166 healthy blood donors originating from 2 other cantons (Jura including Jura bernois, Neuchâtel) of the endemic area (Fig. 1). These sera were collected by the regional blood transfusion centre of La Chaux-de-Fonds and were pretested with EgHF for comparative purposes. All sera were stored at −80°C till required. The following information was obtained concerning 9000 blood donors: 65% males and 35% females aged between 20 and 75 years (mean age for both sexes, 40 years); 90% of all blood donors included in this study lived in rural or semirural areas.

The ELISA using Em2 was performed as described by GOTTSTEIN (1985), using the criteria for interpretation of seropositivity reported earlier (GOTTSTEIN *et al.*, 1984). Briefly, the results are expressed as percentages of the maximum absorbence at 404nm of a positive reference serum minus mean value ($\bar{x}$) plus 3 standard deviations (SD) of sera from healthy blood donors living in an area not endemic for *E. multilocularis* (canton of Ticino). The ELISA using EgHF (fertile hydatid fluid of bovine origin) was carried out according to VOLLER *et al.* (1981) and positive sera were tested in a second step with Em2 for specificity confirmation. In both tests a result was considered "positive" (specific antibodies detectable) if its corresponding absorbance value was higher than $\bar{x}$ + 3 SD determined as described above. In order to exclude any error during sampling and processing of the sera, a second blood sample was collected from all blood donors demonstrating antibody activity to Em2.

The overall sensitivity (93·6%) and specificity (100%) of the ELISA for Em2 has been reported earlier (GOTTSTEIN, 1985). For EgHF-ELISA, the sensitivity was 91% (GOTTSTEIN *et al.*, 1986b), and the specificity with regard to an adult healthy Swiss population was 93·6% (STÜRCHLER *et al.*, 1986). The predictive values of positive and negative results were calculated for a hypothetical population of 10 000 with 0·01% infected persons, which corresponds approximately to the clinical prevalence in the canton of Thurgau (JÄGER *et al.*, 1979), as follows.

		Alveolar echinococcosis present	Alveolar echinococcosis absent
Test result	+	*a*	*b*
Test result	−	*c*	*d*

where a = expected number of cases × sensitivity/100, b = expected number of healthy blood donors × (100 − specificity)/100, c = expected number of AE cases × (100 − sensitivity)/100, and d = expected number of healthy blood donors × specificity/100. Then, positive predictive value = $a/(a + b)$; negative predictive value = $d/(c + d)$.

Patients with a positive result in Em2-ELISA were further investigated by ultrasound (US) and/or computer assisted tomography (CT) of the intra-abdominal and retroperitoneal organs. Both methods are very sensitive in diagnosing liver echinococcosis (HAERTEL *et al.*, 1980; OTTO *et al.*, 1982). US, however, is slightly inferior to CT when imaging lesions of small size in the liver (OTTO *et al.*, 1982).

Results

Predictive values

The predictive value of positive results in Em2-ELISA was calculated to be >99%, based on specificity, sensitivity and prevalence data listed above. For EgHF-ELISA, the positive predictive value was only 0·14%. The predictive value of a negative result was >99% for both Em2-ELISA and EgHF-ELISA.

Sero-epidemiology

The results of the present survey with sera directly tested in Em2-ELISA are shown in Table 1. Sera from 5 blood donors had antibodies reacting with Em2. All these persons were submitted to imaging studies with either US or CT, or both.

In 1 patient lesions were detected by imaging studies (see case histories). 4 persons had negative imaging studies and will be followed closely with periodic serology and US/CT studies. None of these 4 persons' serum showed arc-5 precipitation in immunoelectrophoresis. Antibody concentrations in Em2-ELISA were 14%, 16%, 35% and 21%, respectively. In contrast, antibodies were not detected by ELISA using EgHF.

The results of sera pretested by EgHF-ELISA with subsequent re-examination of the positive reactions by Em2-ELISA are shown in Table 2. Serum

Table 1—Prevalence of antibodies to species-specific polypeptide (Em2) antigen by ELISA in Swiss adult blood donors (sampled between September 1984 and March 1985)

Group No.	Serological result[1]	Clinical finding	Blood donors No.	Blood donors %
I	Positive	AE confirmed clinically	1	0·01
II	Positive	No liver lesion detected by US/CT	4	0·03
III	Negative	No investigation made	11 995	99·96
Total			12 000	100·00

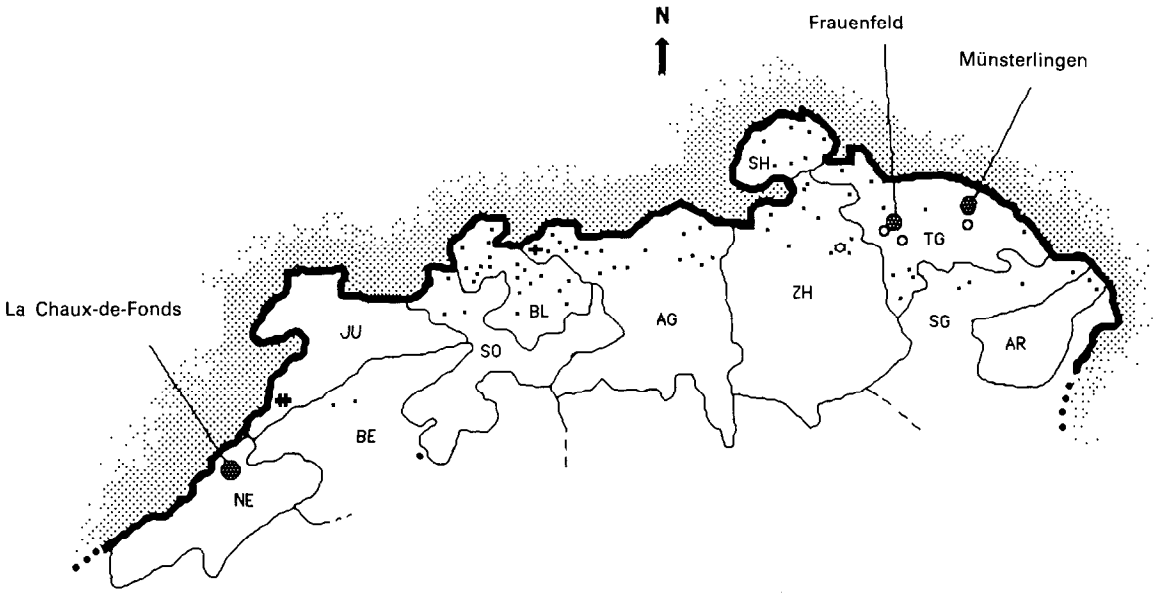
[1]Positive: antibodies detected with Em2-antigen.
Negative: no antibodies detected with Em2-antigen.
Abbreviations. AE: alveolar echinococcosis, US: ultrasound examination, CT: computer assisted tomography.

Table 2—Prevalence of antibodies to *E. granulosus* hydatid fluid (EgHF) antigen by ELISA in Swiss adult blood donors (sampled between October 1984 and May 1985)

Group No.	Serological result[1]	Clinical finding	Blood donors No.	Blood donors %
I	Positive (EgHF-antigen), negative (Em2-antigen)	No liver lesion detected by US/CT	16	0·31
II	Positive (EgHF-antigen), positive (Em2-antigen)	AE confirmed clinically by US/CT	1	0·02
III	Negative (EgHF-antigen)	No investigation made	5149	99·67
Total			5166	100·00

[1]Positive: antibodies detected with EgHF or Em2 antigen.
Negative: no antibodies detected with EgHF or Em2 antigen.

Abbreviations: see Table 1.



‡ Home of AE patient W.C. + Home of AE patient G.A. ○ Homes of 4 persons positive in Em2-ELISA; no liver lesion (US/CT), no sign of AE. • Villages screened by mobile équipes for blood donation performances. ● Regional blood donation centres.

Fig. 1. Map showing blood sampling areas and homes of Em2-positive blood donors and patients with alveolar echinococcosis detected serologically.

antibodies from 17 persons reacted in EgHF-ELISA, but only one serum was confirmed for specificity by Em2-ELISA. This Em2-positive serum was from a 22-year-old man (W.C.) with positive imaging studies (see case histories). All 16 persons "positive" by EgHF-ELISA but "negative" by Em2-ELISA showed no clinical sign of the presence of AE and will not be followed in this study. Fig. 1 shows the geographical location of all villages and areas screened in the present study, location of detected AE patients and healthy persons with antibodies to Em2 but negative imaging studies. Overall, the investigation of 17 166 blood donors' sera resulted in 2 patients positive by Em2-ELISA with clinically confirmed AE, a seroprevalence of clinically confirmed AE of 0·01%. Furthermore, 4 persons were detected with antibody to Em2 but without clinical evidence of AE at the time of investigation, giving a total seroprevalence of 0·03%.

Case histories

Patient G.A. (Table 1). A 41-year-old woman of Austrian origin, but living in Switzerland for 19 years. The ELISA revealed an antibody concentration of 69% using Em2 and 77% using EgHF. Furthermore, arc-5 was precipitated in immunoelectrophoresis. US and CT imaging demonstrated a lesion involving both lobes of the liver with a maximum diameter of about 10 cm, compatible with AE (Fig. 2). A second lesion was found in the head of the pancreas. Due to the localization and extent of the lesions the patient was considered to be primarily inoperable and continuous chemotherapy with mebendazole (2·5 g/d) was started.

Patient W.C. (Table 2). A 22-year-old Swiss man. Antibody concentrations of 61% and 51% were obtained by Em2-ELISA and EgHF-ELISA respectively. No arc-5 was detectable by immunoelectrophoresis. US showed a lesion in the right lobe of the liver with a maximum size of 6×7 cm (Fig. 3). In this patient a complete resection of the parasite lesion was performed by right side hemihepatectomy. Pathological examination confirmed AE.

4 further persons, 3 men and a woman of Swiss nationality aged between 20 and 43 years, showed anti-Em2 antibody concentrations between 14% and 35%. Detection of arc-5 in immunoelectrophoresis failed, one serum precipitating an arc which was not arc-5. US, CT, or both, did not demonstrate liver lesions. In order to ascertain whether the anti-Em2 antibodies are related to early AE, these persons will be examined every 6 months by serology and every year by US.

Discussion

Experience with AE in Switzerland points to a fundamental need for a reliable method permitting an early diagnosis during the preclinical stage. If an organ lesion could be identified in an initial, small stage and before infiltrating bile ducts or major vessels, or before the formation of distant metastases, the surgical resectability rates and the prognosis of the disease should considerably improve.

The use of Em2 for immunodiagnosis of AE has been assessed in a pilot study in Alaska (GOTTSTEIN *et al.*, 1985; SCHANTZ & GOTTSTEIN, 1986), which showed that lesions were detectable by Em2 which were not indicated by EgHF. This Alaskan study was designed for an area in which *E. multilocularis* is

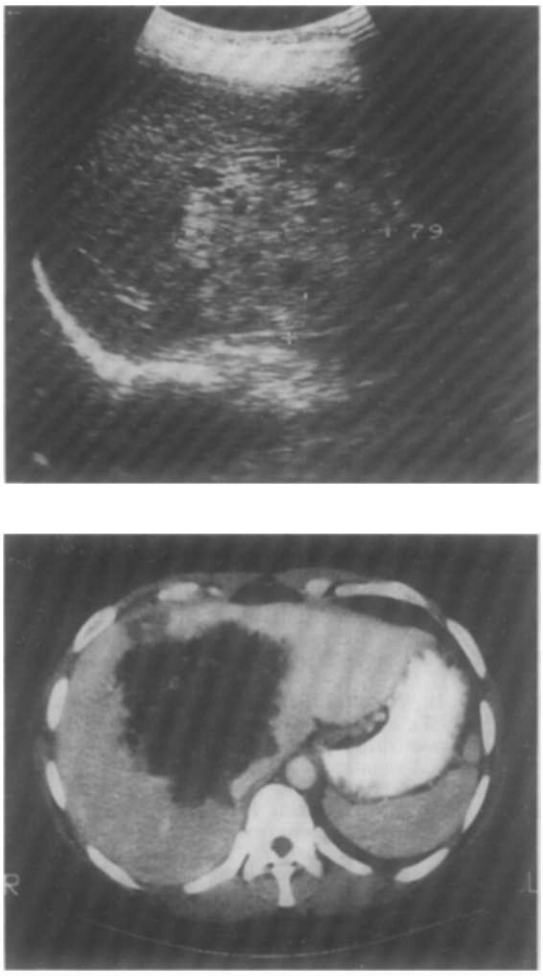


Fig. 2. (a) Ultrasound (US) and (b) computer-assisted tomography (CT) findings in patient G.A. CT imaging clearly demonstrates the irregular lesion in both lobes of the liver with a maximum diameter of 10 cm. In US the contour of the margin of the lesion appears more sharply. In the section shown the diameters of the lesion (marked by + signs) were 6·6 cm and 7·9 cm, respectively.

Fig. 3. Ultrasound imaging in patient W.C. showed a lesion caused by *E. multilocularis* in the right lobe of the liver. The extent of the lesion (+) was 4·8 cm. In this patient radical surgery was performed.

hyperendemic. Based on these results, and on the predictive values of Em2-ELISA, we designed the present study for an area of moderate endemicity. Despite the relatively low number of blood donors included in this study, the survey resulted in the detection of 2 asymptomatic, clinically confirmed cases of AE. One patient (G.A.), although asymptomatic, unfortunately had already reached an advanced stage of the disease and was considered to be inoperable. In the second patient (W.C.), however, the parasite lesion was small and completely resectable.

4 persons had positive Em2-serology but showed negative imaging studies (US and/or CT). Interestingly, 3 of these 4 persons lived in the canton of Thurgau, which has a slightly higher prevalence of human cases than the other cantons investigated (JÄGER *et al.*, 1979). Due to the high specificity of Em2, false positive reactions due to heterologous infections can be excluded with a high degree of probability. Such positive antibody responses in asymptomatic persons without liver lesions may be due to early or abortive *E. multilocularis* infections, or possibly to challenge with eggs which did not develop into metacestodes. The lesions caused by the parasite could also be located in tissues not examined by routine imaging studies. This, however, is not very likely because primary lesions of AE are located in the liver in 98% to 100% of cases (DROLSHAMMER *et al.*, 1973). Studies in Alaska have already shown that Em2-ELISA detected asymptomatic cases not detected by other serological techniques (especially using antigens derived from *E. granulosus*), in which the larval cestode was of very small size and had died out at an apparently early stage of infection (RAUSCH *et al.*, 1987). This was assessed through immunohistochemical tests and by inoculation of parasite material isolated from patients into susceptible rodents. The spontaneous death of larval *E. multilocularis*, which is known to occur in animals with high natural resistance against this parasite, like cattle and sheep (LUKASHENKO, 1971), was also postulated for humans but has never yet been demonstrated. Such spontaneous rejection of the infection may also have occurred in the Swiss patients. These persons will be followed carefully by clinical and serological means; long-term studies on them will provide more information about the possibilities listed above which may be responsible for Em2-seroconversion.

The comparison of the use of Em2 and EgHF in ELISA for seroepidemiology clearly shows that, for assessing an endemic area with a relative low prevalence of AE, there is an absolute need for a highly specific antigen like Em2. The use of a non-specific antigen like EgHF alone is of little value.

The detection of 2 cases of AE among 17 166 blood donors implies a prevalence rate of 11·6 per 100 000. The total number of inhabitants of the age allowing

blood donation in the whole area of the present survey was estimated to be about 1 million. Therefore, about 116 persons living in this area were expected to be infected with *E. multilocularis* at the time of the screening (1984/1985). JÄGER *et al.* (1979) observed a morbidity rate of 7·8 per 100 000 in the canton of Thurgau over a period of 10 years. Serological screening with Em2-ELISA will detect all persons infected with *E. multilocularis*, especially early asymptomatic AE cases, which, if not detected by serology in a preclinical stage, may develop into symptomatic clinical cases of AE. Comparison of the results of the present survey with the clinical prevalence data of JÄGER *et al.* (1979) is feasible only if we know the average number of years between infection and diagnosis of AE, based on clinical symptoms. Combining Jäger's data (annual morbidity rate per 100 000 persons = 0·78, based on clinical findings) with ours (incidence rate per 100 000 persons = 11·6, based on serological findings), on average 15 years may be presumed to pass between time of infection and onset of symptoms (11·6/0·78 = 15).

At present no reliable measures are available to control the disease in animal populations or to prevent infections of humans. Therefore, further sero-epidemiological surveys employing ELISA with Em2 antigen should be carried out in endemic areas to identify locations of high transmission risk. If this could be achieved, local sero-epidemiological surveys could be conducted in restricted groups of the population, to detect cases of asymptomatic AE at an early stage. However, the practical planning of such a screening system in areas of moderate endemicity requires more information, which should be obtained from various areas endemic for *E. multilocularis*.

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References

Drolshammer, I., Wiesmann, E. & Eckert, J. (1973). Echinokokkose beim Menschen in der Schweiz 1956-1969. *Schweizerische Medizinische Wochenschrift*, **103**, 1337-1341; 1386-1392.

Eckert, J. (1970). Echinokokkose bei Mensch und Tier. *Schweizer Archiv für Tierheilkunde*, **9**, 443-457.

Gottstein, B. (1985). Purification and characterization of a specific polypeptide from *Echinococcus multilocularis*. *Parasite Immunology*, **7**, 201-212.

Gottstein, B., Eckert, J. & Fey, H. (1983). Serological differentiation between *Echinococcus granulosus* and *E. multilocularis* infections in man. *Zeitschrift für Parasitenkunde*, **69**, 347-356.

Gottstein, B., Eckert, J. & Woodtli, W. (1984). Determination of parasite-specific immunoglobulins with the ELISA in patients with echinococcosis treated with mebendazole. *Zeitschrift für Parasitenkunde*, **70**, 385-389.

Gottstein, B., Schantz, P. M. & Wilson, J. F. (1985). Serologic screening for *Echinococcus multilocularis* infections with ELISA. *Lancet*, **i**, 1097-1098.

Gottstein, B., Schantz, P. M., Todorov, T., Saimot, A. G. & Jacquier, P. (1986a). An international study on the serological differential diagnosis of human and alveolar echinococcosis. *Bulletin of the World Health Organization*, **64**, 101-105.

Gottstein, B., Witassek, F. & Eckert, J. (1986b). Neues zur Echinokokkose. *Schweizerische Medizinische Wochenschrift*, **116**, 810-817.

Haertel, M., Fretz, C. & Fuchs, W. A. (1980). Zur computertomographischen Diagnostik der Echinokokkose. *Fortschritte auf dem Gebiet der Röntgenstrahlen*, **133**, 164-170.

Jäger, P., Hardmeier, T. & Preter, B. (1979). Die Echinokokkose beim Menschen unter besonderer Berücksichtigung des *Echinococcus alveolaris* im Kanton Thurgau. *Mitteilungen der Thurgauer Naturforschenden Gesellschaft*, **43**, 254-265.

Lukashenko, N. P. (1971). Problems of epidemiology and prophylaxis of alveococcosis (multilocular echinococcosis): a general review, with particular reference to the USSR. *International Journal for Parasitology*, **1**, 125-134.

Otto, R., Woodtli, W. & Ammann, R. (1982). Sonographie versus Computertomographie bei Lebermanifestationen der Echinokokkose. *Deutsche Medizinische Wochenschrift*, **107**, 1717-1721.

Rausch, R. L., Wilson, J. F., Schantz, P. M. & McMahon, B. J. (1987). Spontaneous death of *Echinococcus multilocularis*: cases diagnosed serologically (by Em2-ELISA) and clinical significance. *American Journal of Tropical Medicine and Hygiene*, **36**, 576-585.

Schantz, P. M. & Gottstein, B. (1986). Echinococcosis (hydatidosis). In: *Immunoserology of Parasitic Diseases*, Vol. I. *Parasitic Diseases*, K. Walls & P. M. Schantz (editors). Orlando, Florida: Academic Press, pp. 69-107.

Schantz, P. M., Van den Bossche, H. & Eckert, J. (1982). Chemotherapy for larval echinococcosis in animals and humans: report of a workshop. *Zeitschrift für Parasitenkunde*, **67**, 5-26.

Schicker, H. J. (1976). *Die Echinokokkose des Menschen. Stand von Diagnose, Therapie und Prognose bei Echinokokkenerkrankungen in Baden-Würtemberg in den Jahren 1960-1972*. Dissertation in Medicine, University of Tübingen.

Stürchler, D., Bruppacher, R. & Speiser, F. (1986). Epidemiologische Aspekte der Toxokariasis in der Schweiz. *Schweizerische Medizinische Wochenschrift*, **116**, 1088-1093.

Voller, A., Bartlett, A. & Bidwell, D. E. (1981). *Immunoassays for the 80's*. Falcon House, Lancaster, England: MTP Press.

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