

***Sarcocystis heydorni*, n. sp. (Apicomplexa: Protozoa) with cattle (*Bos taurus*) and human (*Homo sapiens*) cycle**

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Abstract

Cattle (*Bos taurus*) are intermediate hosts for four species of *Sarcocystis*, *S. cruzi*, *S. hirsuta*, *S. hominis*, and *S. rommeli*. Of these four species, mature sarcocysts of *S. cruzi* are thin-walled (< 1µm) whereas *S. hirsuta*, *S. hominis*, and *S. rommeli* have thick walls (4 µm or more). Here we describe a new species of *Sarcocystis* with thin-walled sarcocysts in cattle. Two newborn calves were fed sporocysts from the feces of a human volunteer who had ingested raw beef. The calves were killed 111 and 222 days later. In addition to thick-walled sarcocysts of *Sarcocystis hominis*, both calves were coinfecting with a *Sarcocystis* species that had a thin-walled sarcocysts, distinct from *Sarcocystis cruzi*. The sarcocysts were mature, microscopic, up to 80 µm wide and up to 1060 µm long. By light microscopy, the sarcocyst wall was thin (< 1

µm thick) and had minute protrusions. By transmission electron microscopy, the sarcocyst wall had short, conical villar protrusions (vp), that were up to 0.5 µm long, up to 0.5 µm wide, similar to type 29. The vp on the sarcocyst wall lacked microtubules but had six or more disc-shaped plaques. The ground substance layer was smooth, approximately 0.5 µm thick, and without microtubules. The bradyzoites were 8-11 µm long. The structure of the sarcocyst wall was distinct from any species of *Sarcocystis* reported from livestock. This unique species is named in honor of Dr. Alfred Otto Heydorn who provided the sporocysts.

Keywords

Cattle. *Sarcocystis*. Ultrastructure. Human volunteer. Beef.

Introduction

Species of *Sarcocystis* usually have a two-host, prey-predator life cycle, with herbivores as intermediate hosts and carnivores as definitive hosts (Dubey et al. 2015a). The intermediate host becomes infected with *Sarcocystis* species by ingesting sporocysts excreted in the feces of the definitive host. After a brief period of schizogony, the parasite encysts in tissues and forms sarcocysts. The definitive host becomes infected by ingesting sarcocysts encysted in tissues of intermediate hosts.

Cattle (*Bos taurus*) are intermediate hosts for four named species of *Sarcocystis*, *S. cruzi*, *S. hirsuta*, *S. hominis*, and *S. rommeli* (Dubey et al. 2015b). Of these four species, sarcocysts of *S. cruzi* are thin-walled (<1 µm) whereas *S. hirsuta*, *S. hominis*, and *S. rommeli* have thick-walled sarcocysts. These species are easily distinguished by the ultrastructure of their cyst walls.

Of these four bovine *Sarcocystis* species, felids are definitive hosts for *S. hirsuta*, canids are hosts for *S. cruzi*, and humans are definitive hosts for *S. hominis*; the definitive hosts for *S. rommeli* are unknown. Here, we report the finding of a new *Sarcocystis* species in cattle experimentally inoculated with sporocysts from human feces.

Materials and methods

. In a study, five newborn calves in 1983 were fed with 10^5 to 10^6 *Sarcocystis* sporocysts from a human volunteer (Dubey et al., 1988). The isolate of *S. hominis* was originally derived from the feces of the human volunteer (Dr. Heydorn) who ate raw minced beef while traveling in Turkey in 1973; subsequently this strain was cycled every few years between experimentally infected cattle and Dr. Heydorn. The sporocysts used by Dubey et al. (1988) were from had been collected in 1982 (Heydorn, 1977; personal communication to JPD July, 2015). The calves were euthanized 13, 18, 24, 111 and 222 days post-inoculation (p.i.) (Dubey et al. 1988). One uninoculated calf served as control. The calves were born at the Beltsville Agricultural Research Center Dairy, and were housed indoors immediately after birth and throughout during the study (Dubey et al. 1988). Findings related to *S. hominis* in these calves were reported previously (Dubey et al. 1988; Dubey et al. 2015a).

Recently, all histological sections of tissues from the five inoculated calves stained with hematoxylin and eosin (H and E), and one paraffin block of tongue from the calf killed day 222 p.i. were located. The paraffin block of tongue was deparaffinized and processed for transmission electron microscopy as described (Dubey et al. 2015c). All H and E sections from the five calves were reexamined for the present study, including sections from heart, esophagus, and skeletal muscle.

Results

No sarcocyst was found in tissues of calves killed at 13, 18, and 24 days p.i. Two types of sarcocysts (thick-and thin-walled) were found in calves killed 111 days p.i. (calf 1) and 222 days p.i. (calf 2); most sarcocysts found were thick-walled (Table 1). The thick-walled sarcocysts were *S. hominis* as described before (Dubey et al. 1988); the esophagus was the most heavily infected tissue for *S. hominis*. Thin-walled sarcocysts were found in the tongue, diaphragm, and skeletal muscle but not in the heart (Table 1). More tissue cysts were seen in calf 1 than in calf 2. Sarcocysts were up to 80 μm wide and up to 1060 μm long (Fig. 1a,c). By light microscopy of 6 μm thick sections the cyst wall appeared thin (Fig. 1c). On higher magnification minute protrusions were recognized on the cyst wall of some but not all (Fig. 1d-f) . Most sarcocysts examined contained bradyzoites.

Table 1 : Distribution of sarcocysts in muscles of 2 calves after oral inoculation with sporocysts from human feces.

Calf no.	p.i. day	Tissue	Total cysts examined	Thick walled (<i>S. hominis</i>)	Thin walled (<i>S. heydorni</i>)
1	111	Esophagus	365	343	22
		Heart	2	2	0
		Diaphragm	8	4	4
		Limb muscle	80	65	15
		Tongue	240	193	47
		Subtotal (%)	695	607 (87.4)	88 (12.6)
2	222	Esophagus	2	1	1
		Heart	0	1	0
		Diaphragm	7	5	2
		Limb muscle	2	1	1
		Tongue	84	65	19
		Subtotal (%)	96	73 (76.0)	23 (24.0)

Five sarcocysts were found in H and E stained section cut from the paraffin block of the tongue of calf 2; all five sarcocysts were removed from the paraffin block and were processed for TEM. Three of the five sarcocysts were thick walled *S. hominis* and two sarcocysts were thin walled. The thin-walled sarcocysts are described here.

In 1- μm thick Toluidine blue stained sections minute villar protrusions were recognized (Fig. 1b). Ultrastructurally, the sarcocyst wall had short conical villar protrusions (vp) (Fig. 2), similar to type 29 (Dubey et al. 2015b). The vp were up to 0.5 μm wide at the base, 0.25 μm wide at the tip, and up to 0.5 μm long (Fig. 2b). The parasitophorous vacuolar membrane (pvm) covering the vp was lined with an electron dense layer, 10 nm thick (Fig. 2c). The vp were without granules but had 6 or more disc-shaped plaques (Fig. 2 c, d). The ground substance layer (gs) was 0.5 μm thick and lacked microtubules. The total cyst wall (cw), including vp and the gs was 1 to 1.5 μm thick, depending on the plane of section, being thickest at the point of origin of septa (Fig. 2b)

Both sarcocysts were mature and contained only a few merozoites. The bradyzoites were elongated and packed, so it was difficult to measure them. They were 8-11 μm long and 2.0-2.5 μm wide (Fig. 3 a-c). They contained a conoid (co), two rhoptries (rh 1, rh 2), numerous micronemes (mn), a long twisted mitochondrion (mc), numerous amylopectin granules (am), and a micropore (mp) (Fig. 3d).

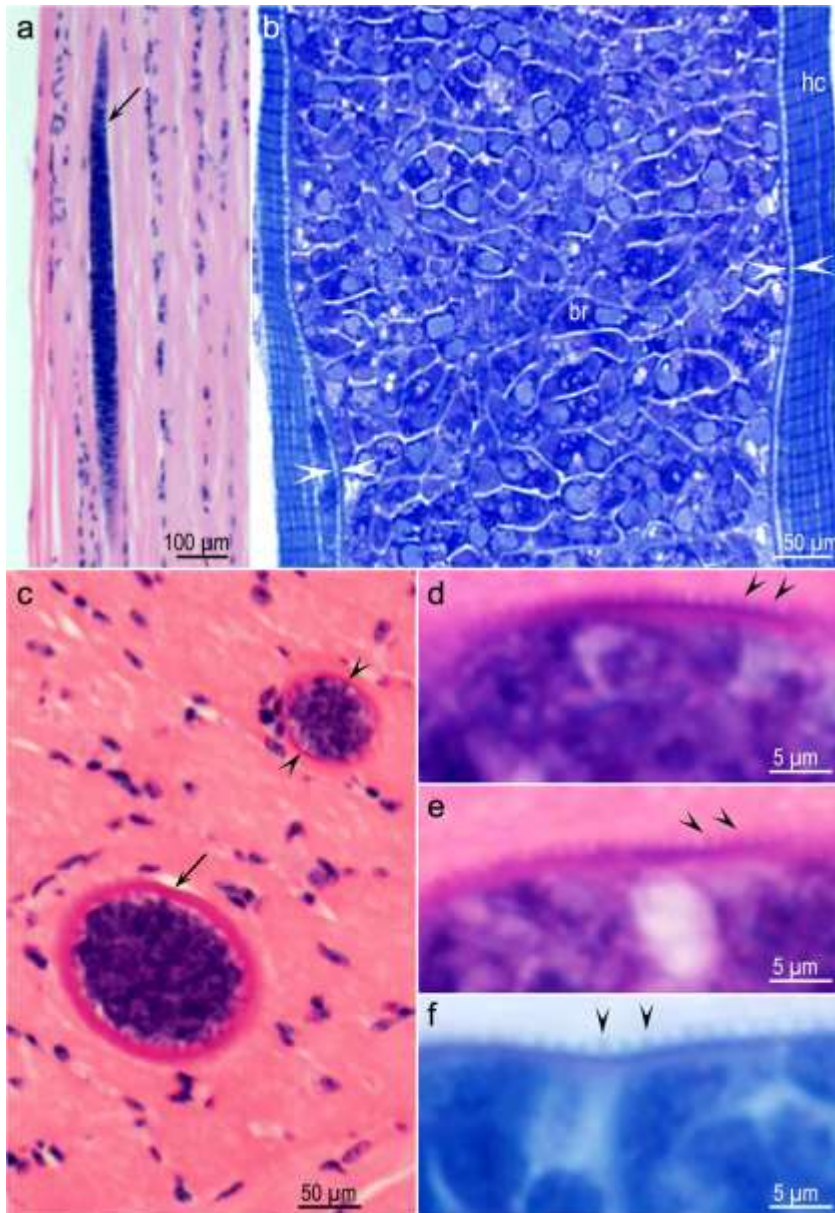


Fig. 1 *Sarcocystis heydorni* n. sp. sarcocysts from skeletal muscle of calves after 111 (calf 1), and 222 (calf 2) days post inoculation with sporocysts from the feces of a human volunteer who ate raw beef. Fig. 1a, c-e. Hematoxylin and eosin stain of 6 μm thick sections from calf 1. Fig.1b Toluidine blue stained 1 μm thick section of tongue of calf 2. **a**, An elongated sarcocyst. **b**, Note a thin-walled (opposing arrowheads) sarcocyst packed with bradyzoites (br). There is no cellular inflammatory reaction around the infected host cell (hc). **c**, A thin-walled (arrowheads) *S. heydorni* and a thick-walled *S. hominis* (arrow) sarcocysts. **d-f**, Note minute villar protrusions (arrowheads) on the cyst wall.

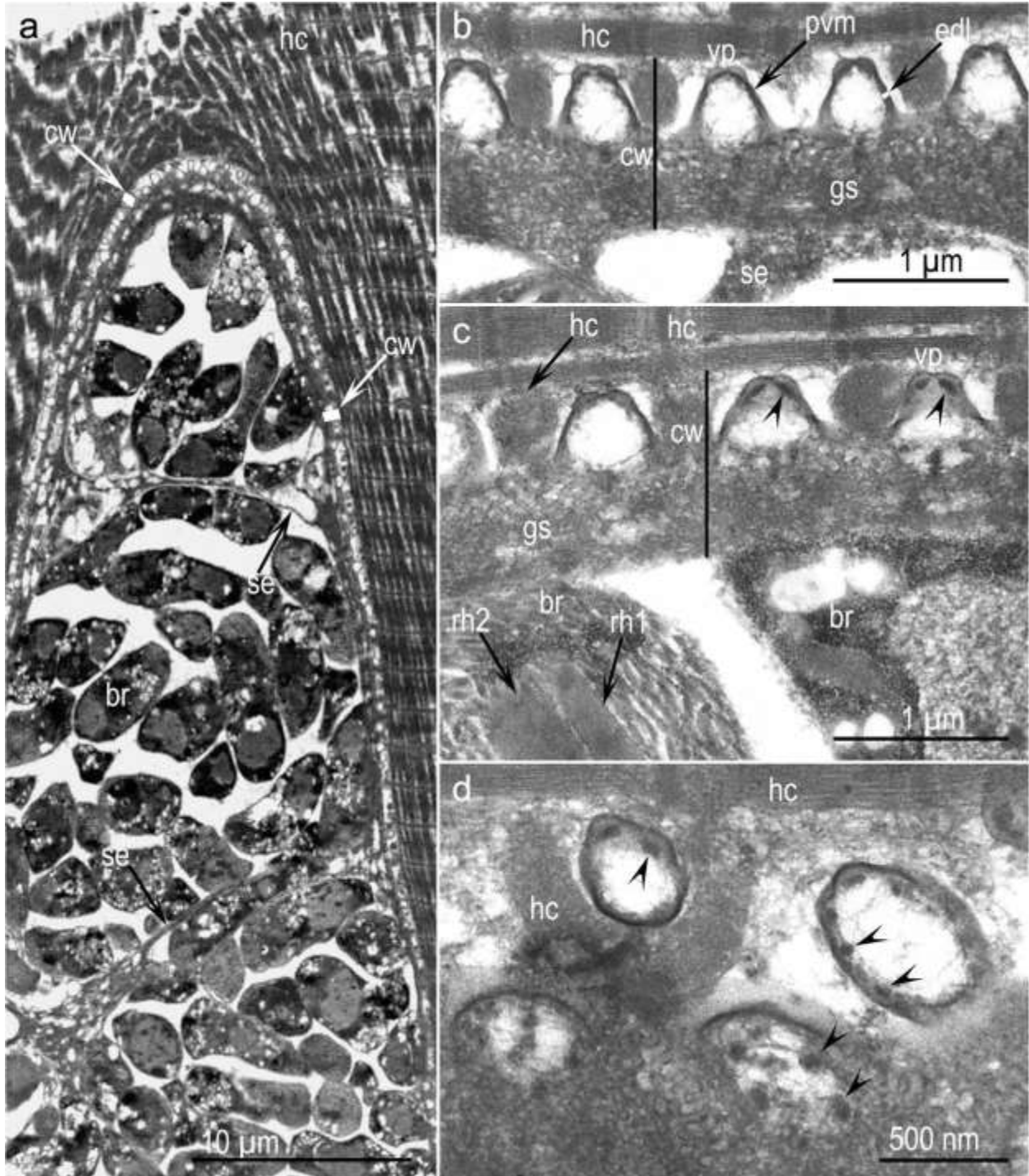


Fig 2 TEM of *S. heydorni* sarcocyst walls in tongue of calf 2. Note cyst wall (cw), septa (se), parasitophorous vacuolar membrane (pvm), electron dense layer (edl), villar protrusions (vp), bradyzoites (br), two rhoptries (rh1, rh2) in bradyzoites, ground substance (gs), disc-shaped plaques (arrowheads) in vp, and host cell (hc). **a**, low magnification of the entire width of the sarcocyst, **b-d**, higher magnification of the cyst wall.

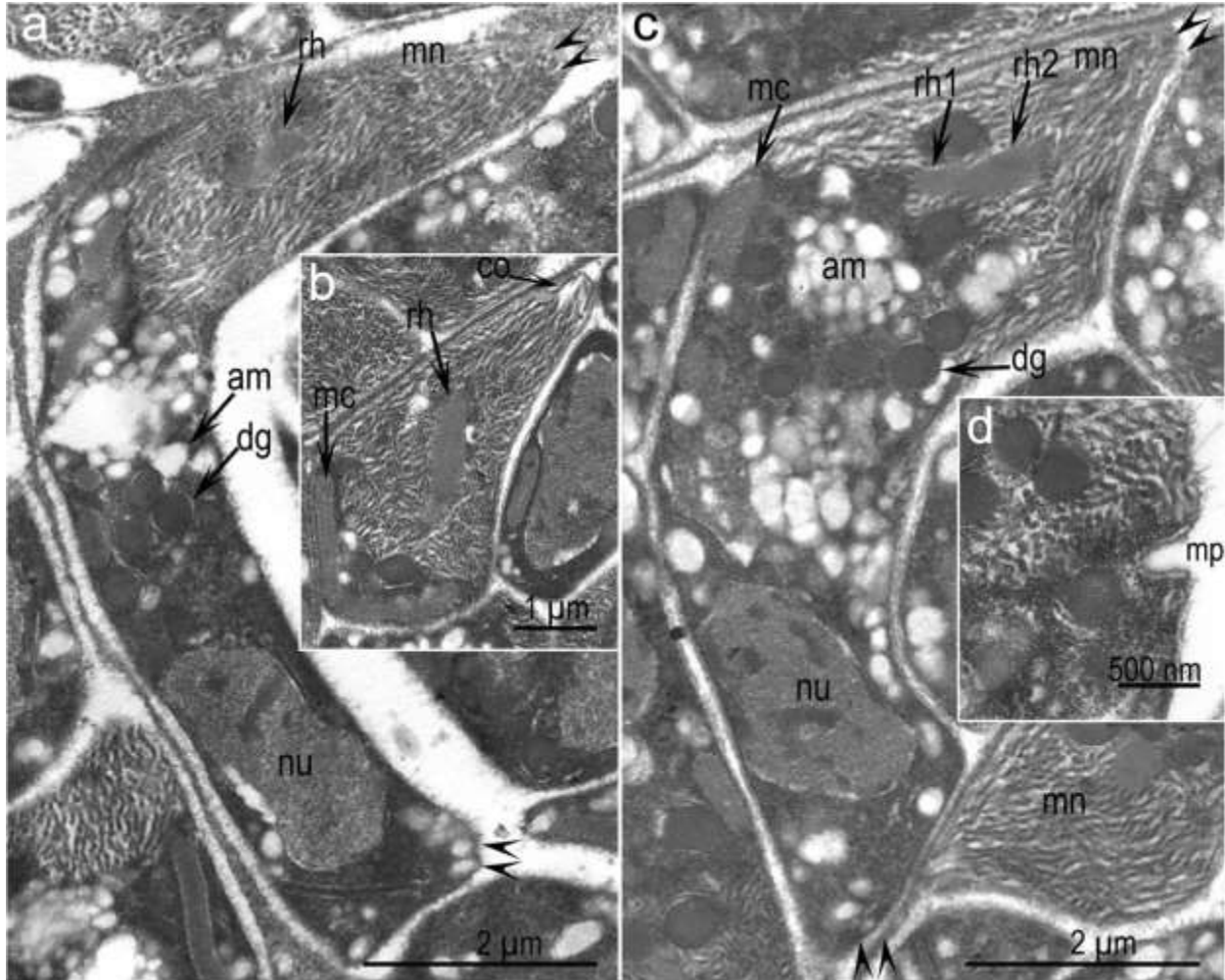


Fig. 3 TEM of *S. heydorni* bradyzoites in tongue of calf 2. **a, c.** Two longitudinal sections of bradyzoites (arrowheads at opposing ends). **b.** Conoidal end (co) of a bradyzoite. **d.** Micropore. Note convoluted mitochondrion (mc), two rhoptries (rh1, rh2), numerous micronemes (mn), amylopectin granules (am), and dense granules (dg).

Description of *Sarcocystis heydorni* n. sp. (Figs. 1-3)

Diagnosis: Sarcocysts microscopic, up to 1060 µm long, up to 80µm wide. Sarcocyst wall 1.0 to 1.5 µm thick with minute truncated pyramidal 0.5 µm long villar protrusions (vp), vp wide at the base and narrow at the tip and containing up to 6 or more disc-shaped plaques. The ground substance layer 0.5 µm thick, without microtubules. Bradyzoites 8-11 µm long.

Etymology: Species named after Dr. Alfred Otto Heydorn who, with Michel Rommel, Heinz Mehlhorn, and Jack Frenkel conclusively showed that there were three species of *Sarcocystis* in cattle (*S. bovicanis* now called *S. cruzi*, *S. bovifelis* now called *S. hirsuta*, and *S. bovihominis* now called *S. hominis*). Dr. Heydorn was the volunteer who ate the raw beef.

Intermediate host: Cattle (*Bos taurus*).

Distribution: Unknown.

Definitive host: Human.

Specimens deposited: Histological sections of muscles from calves 1 and 2, and Toluidine blue stained sections from calf 2 were deposited in the United States National Parasite Collection in the Division of Invertebrate Zoology and National Museum of Natural History, Smithsonian Institution, Washington, D.C under (USNM Nos. 1283129-1283132).

Discussion

Sarcocystis heydorni, proposed here, is structurally distinct from the other bovid sarcocysts. Its sarcocysts are thin-walled and have villar protrusions that have not been recognized previously in any species of *Sarcocystis* in livestock (Dubey et al. 2015b). The only other thin-walled sarcocyst in cattle is *S. cruzi* which has long villar protrusions that are folded on the cyst wall, giving it a thin walled appearance (Dubey et al. 2015b). For *S. cruzi*, the myocardium is the predilection site whereas *S. heydorni* was not found in the heart. *Sarcocystis heydorni* was not observed by TEM during the initial study that described the structure of *S. hominis*. This may be due to the fact that only the esophagus (calf 1) and skeletal muscle (calf 2) were processed for TEM (Dubey et al. 1988) and that thin walled sarcocysts were rare in these tissues.

The source of the *S. heydorni* described here is considered of human origin because the calves inoculated with sporocysts were taken at birth, raised in confinement, fed only the milk replacer and pelleted calf feed; therefore were not exposed to carnivores or feed contaminated with carnivore feces. Furthermore, sarcocysts were not found in the three calves killed 13, 18, and 24 days p.i., or in the control uninoculated calf raised under the same conditions. *Sarcocystis cruzi* is the most common parasite in cattle worldwide (Dubey et al. 2015b); its absence in the calves inoculated with human derived sporocysts is further indication that experimental calves had not been exposed to canine feces infected with *Sarcocystis* sporocysts.

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