Trypanosoma cruzi Infection in the Opossum *Didelphis marsupialis:* Absence of Neonatal Transmission and Protection by Maternal Antibodies in Experimental Infections

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The high rate of natural Trypanosoma cruzi *infection found in opossums does not always correlate with appreciable densities of local triatomid populations. One alternative method which might bypass the invertebrate vector is direct transmission from mother to offspring. This possibility was investigated in five* T. cruzi *infected females and their litters (24 young). The influence of maternal antibodies transferred via lactation, on the course of experimental infection, was also examined. Our results show that neonatal transmission is probably not responsible for the high rate of natural* T. cruzi *infection among opossums.*

In addition antibodies of maternal origin confer a partial protection to the young. This was demonstrated by the finding of a double prepatency period and 4,5 fold lower levels of circulating parasites, in experimentally infected pouch young from infected as compared to control uninfected mothes. On the other hand, the duration of patent parasitemia was twice as long as that observed in the control group.

Key words: *Trypanosoma cruzi* – *Didelphis marsupialis* – opossum – antibodies – protection – neonatal transmission

The opossum *Didelphis marsupialis,* one of the most important and ancient silvatic reservoirs of *Trypanosoma cruzi,* is also considered as a link between domestic and silvatic transmission cycles, because of its ability to adapt to human dwellings in deforested areas (Zeledon et al. 1970, Barretto & Ribeiro 1979). Opossums are omnivorous, feeding on eggs, fruits, small vertebrates and insects.

Depending on the strain of the parasite, experimental infections can be permanent or controlled and sometimes eliminated, in opossums from an early age (Thomaz et al. 1984, Deane et al. 1984). In addition, experimental infections can often result in multiplication and differentiation of the invertebrate epimastigote stage in the lumen of the scent glands (anal glands) (Deane et al. 1984). The mechanisms underlying this phenomenon are not fully understood although a correlation between high parasitemia and invasion of the scent glands by the parasite has been described (Jansen et al. 1991).

Natural infections tend to be stable and very common, sometimes reaching very high rates rates as to 100% (Rodrigues & Mello 1942, Guimarães & Jansen 1943, McKeever & Gorman 1958, Deane et al. personal communication), while infected scent glands seem to be uncommon in naturally infected animals (Naiff et al. 1987, Steindel et al. 1988, Fernandes et al. 1989).

Some aspects of the maintenance of the silvatic life cycle of *T. cruzi* still require clarification: in a study carried out on Jaguanum Island (Ramal de Mangaratiba, Rio de Janeiro), 90% of opossums were found to be infected with *T. cruzi,* no scent gland infection was observed, rodents were scarce and only rare specimens of *Microtriatoma borbai* were found (Sibajev & Deane 1988).

Until quite recently marsupials were considered as "lower" mammals, an evolutionary link between oviparous mammals and viviparous placentals. However, it is now known that marsupials and placentals are closely related, and that the principal difference between tham is in reprodutive strategy. After a short gestation time, (12-13 days for *D. marsupialis*) marsupial young are born in an embryonic state, weighing only 0.01 to 0.05% of the maternal body weight, in contrast to placental young which weigh 2.3 to 3.0%.

Marsupials are born without antibodies, these are acquired in the first few hours after the commencement of suckling (Hindes & Mizell 1976).

In general, a marsupial can be considered as an immature placental that depends for its de-

Supported by CNPq.
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Received 29 April 1993
Accepted 12 January 1994

velopment on the incubation conditions of the marsupial pouch and on breastfeeding.

Marsupials are amniotic, with a well developed yolk sac. During development, a kind of chorionic-vitelline "placenta", covered by an avascular and cornified membrane, is formed. The degree of interaction of a marsupial embryo with maternal tissues is far less than that of a placental embryo; however, there is a close relationship between the mother and the young during the long lactation period. During the first 55 days, young opossums remain permanently connected to a breast nipple that extends to their stomach. Thereafter, the young opossums, which at this stage have differentiated mouths, begin to release the nipple for short periods. Lactation continues until about 100 days, with increasing independence of the young.

The long period of close association between mother and offsprings could perhaps explain the high rate of natural infection found in opossums. For this reason we decided to evaluate the importance of neonatal *T. cruzi* transmission in *D. marsupialis,* as well as to examine the role of maternal antibodies transmitted to pouch young during lactation on the course of experimental infection.

MATERIALS AND METHODS

Animals – Five adult female opossums and their litters a total of 24 specimens were used in the study. One of the adult females, reared in captivity from birth, was experimentally infected with metacyclic forms obtained from scent glands when her litter was ten days old. The others, caught in the wild, were naturally infected, as detected by routine tests previously described (Deane & Jansen 1990).

During the period of observation the adult females presented subpatent infections, detectable by hemocultures, with no parasitism of the scent glands. Total serum Ig titers, determined by an indirect immunofluorescent test (IFAT) described elsewhere (Jansen et al. 1985) remained high.

Evaluation of the influence of antibodies of maternal origin on the course of experimental infection – In order to evaluate the effect of antibodies of maternal origin on the course of infection in young opossums, a litter of five animals from an experimentally infected female were infected with metacyclic forms of *T. cruzi* from LIT medium, soon after weaning. A control litter from a laboratory reared female was experimentally infected with the same inoculum.

The follow-up of young included examination of at least two hemocultures in NNN medium with a LIT overlay every 15th day during two months, xenodiagnosis with 30 *Rhodnius neglectus* nymphs, microscopical examination of scent glands contents obtained by manual squeezing, and IFAT for Ig, IgM and IgG anti-*T. cruzi* antibodies.

Titration of Ig levels in milk of lactating females was done trough IFAT as already described for serum in this model host (Jansen et al. 1985).

The fluorescein isothiocyanate conjugate of goat anti-opossum heavy chains m and g was kindly supplied by Dr Maria Helena da Silva, Instituto de Microbiologia, UFRJ.

The antigens for all IFATS were prepared with the F strain epimastigotes from cultures, as previously described (Jansen et al. 1985).

The parasite strain used was G-49 (L.I), isolated from a naturally infected opossum captured in Miguel Pereira, State of Rio de Janeiro, in August 1982. Since that time, the strain has been maintained in our laboratory by means of cyclical passages through opossum-triatomid-LIT medium.

Purified LIT metacyclic forms for the inoculations were obtained by passage through of a nylon-wool column (Pinho et al. 1991). Two hundred metacyclic forms/g body weight were injected subcutaneously into each animal.

Adult animals were individually caged and fed on dog food, eggs and fresh fruits. Pouch young were maintained in the same way, after weaning, at about 120 days.

RESULTS

Neonatal transmission of *T. cruzi* was not observed to occur in the animals comprising the present study. All hemocultures and xenodiagnoses were negative, and no invasion of scent glands by the parasite took place.

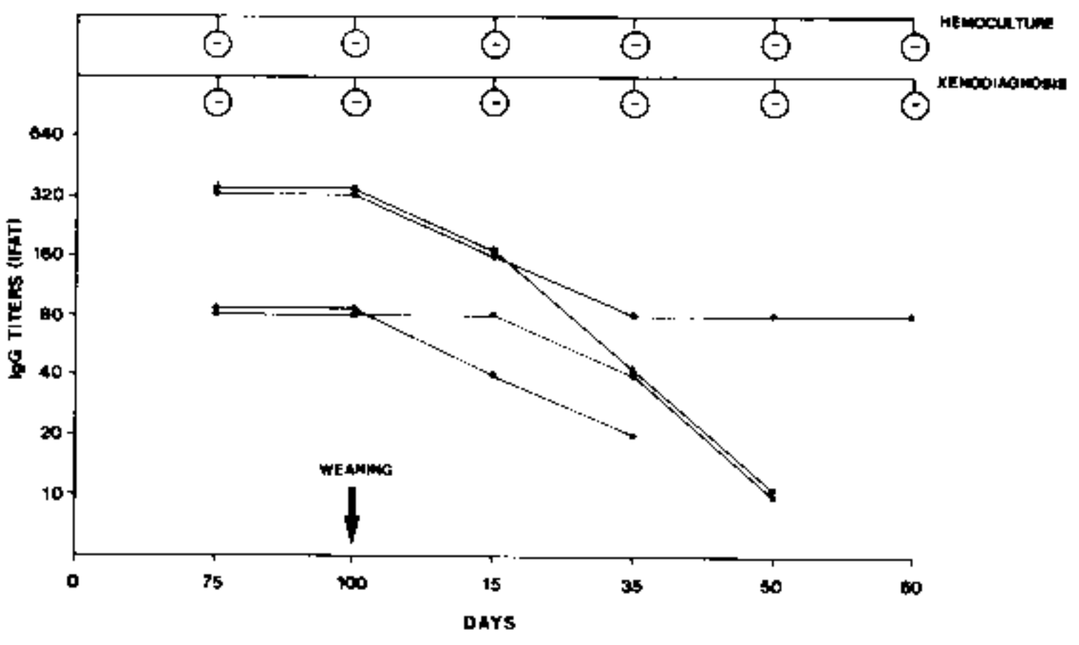

Fig. 1: serological and parasitological follow up in a litter of newborn opossums, from a naturally *Trypanosoma cruzi* infected female, before and after weaning. Each point represents the individual values for an animal. IgG antibodies were detected by an indirect immunofluorescence test.

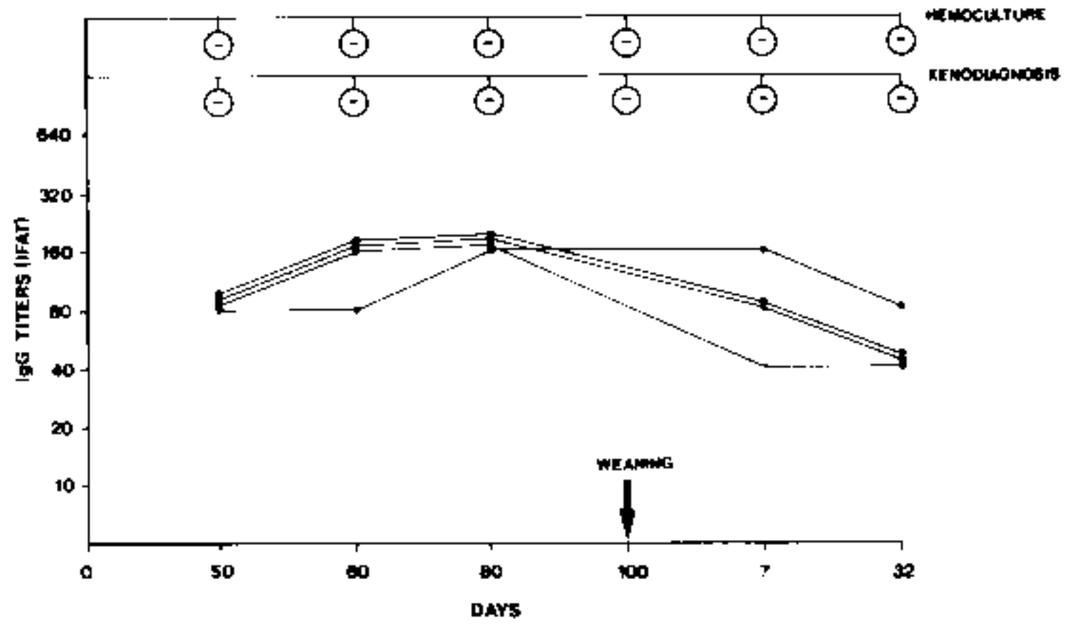

Fig. 2: as in Fig. 1; another example.

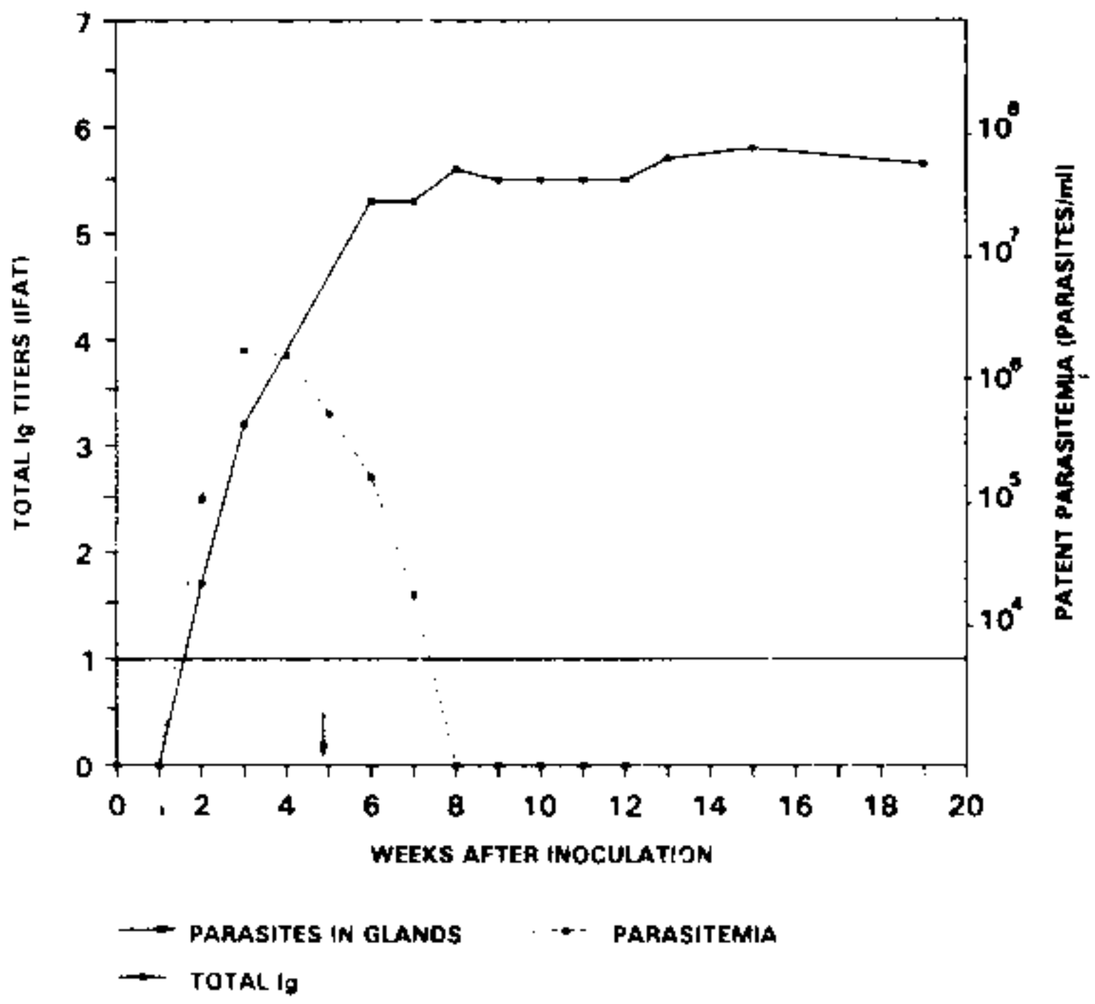

Fig. 3: Ig antibodies and patent parasitemia after experimental infection with *Trypanosoma cruzi*, in a litter of seven opossums from an uninfected, laboratory reared *Didelphis marsupialis* female. Each point represents the mean values for the litter and the curves were plotted as log 2 of the dilution titers. The horizontal line in paralel with the abscissa, corresponds to the 1:20 dilution, above what the reaction was considered positive.

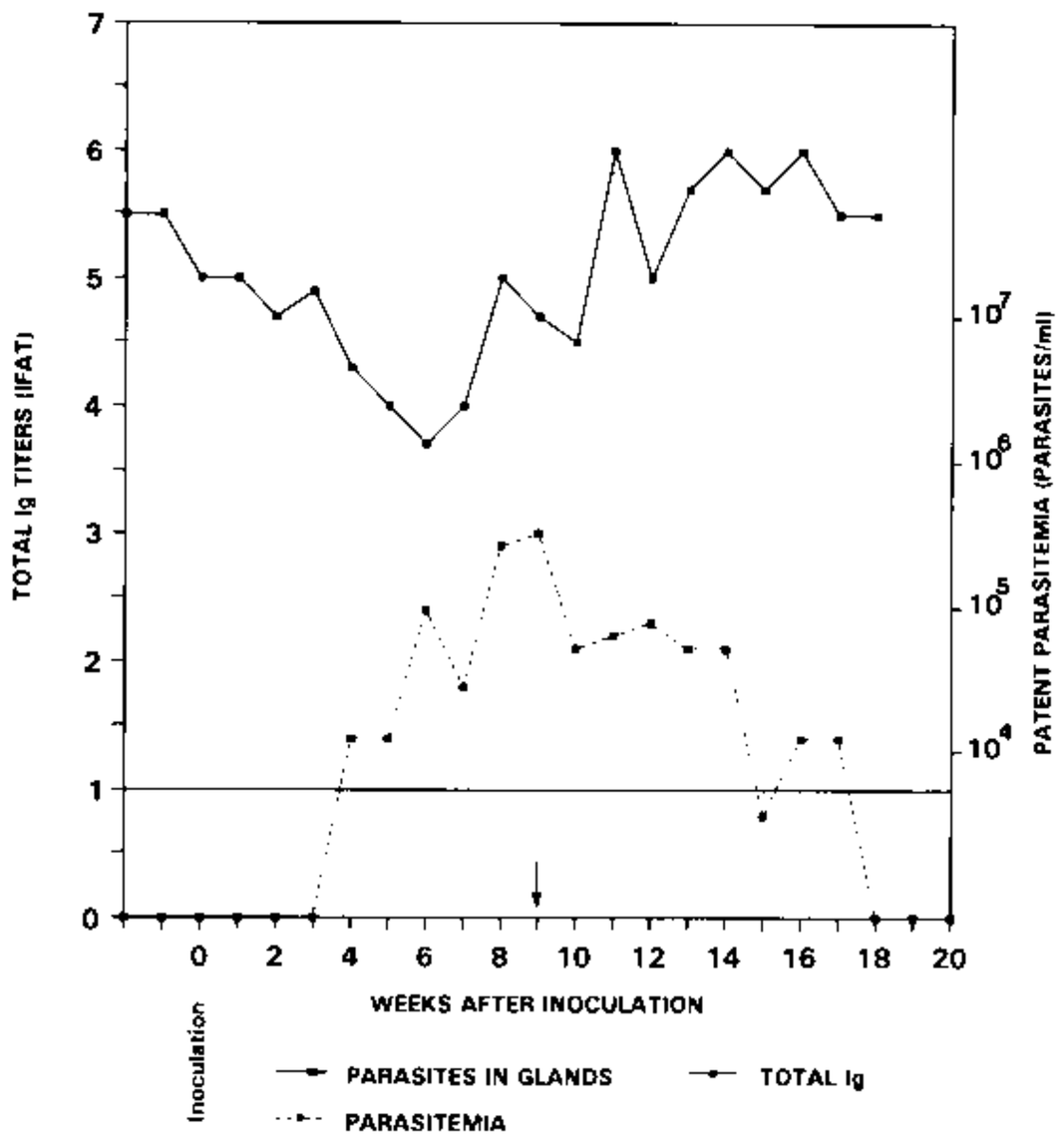

Fig. 4: Ig antibodies and patent parasitemia after experimental infection with *Trypanosoma cruzi*, in a litter of five opossums from a *Didelphis marsupialis* female, experimentally infected with *Trypanosoma cruzi*. Other data as in legend to Figs 1,3.

High levels of anti *T. cruzi* IgG antibodies, reaching sometimes 1:160, were observed in the serum of pouch young and in maternal milk, but the same samples were always negative for IgM. Titres of maternal *T. cruzi* antibodies begin to decline in young opossum sera from the 30th day after weaning. Figs 1 and 2 show the follow up of two of the litters studied.

Maternal anti *T. cruzi* antibodies transmitted during lactation conferred a partial protection on pouch young. Inoculated pouch young from infected mothers displayed a double prepatency period in relation to the control group; in addi-

TABLE

Trypanosoma cruzi infection in the opossum *Didelphis marsupialis*: parasitological and serological data on experimentally infected newly-weaned infants from normal and infected females. Inocula: 200 metacyclic forms/body weight subcutaneously

	Parasitemia				Parasites in scent glands		Total Ig
	Prepatent period (days)	Parasites at peak/ml	Peak time (days)	$\bar{X}$ duration (weeks)	Animals (+)/Total	Time x (weeks)	Titre at peak
7 newly-weaned infants (120 days) normal female	13.5	2.9×10^6	27	7	7/7	4.6	1:1280
5 newly-weaned infants (120 days) infected female	28.4	6.5×10^5	50	14	4/5	9	1:640

tion peak levels of circulating parasites were 4,5 fold lower. The onset of patent parasitemia was coincident with the drop of Ig anti *T. cruzi* antibodies of maternal origin and the drop in the number of circulating parasites, with the rise of specific Ig antibodies from offsprings. The patency of parasitemia however was twice as long in offsprings from infected mother than in the control group (Table).

All animals of the two groups but one, from the infected mother, presented parasites in scent glands close to the parasitemic peak and this parasitism was confirmed at necropsy of most of animals of the two groups several months after (Table, Fig. 1).

DISCUSSION

Our results strongly suggest that the high natural *T. cruzi* infection rates observed for the opossum *D. marsupialis* are not due to neonatal transmission. On the contrary, infected mothers transferred to their pouch young, significant amounts of specific IgG antibodies which conferred a partial protection against experimental infections.

Congenital transmission of *T. cruzi* in placental laboratory hosts does occur, although relatively rarely (Miles 1972). Congenital transmission in humans is also considered uncommon, although in certain areas it can be an important route of infection (Bittencourt 1967, Bittencourt & Gomes 1967, Howard & Rubio 1968, Miles 1972, Bittencourt et al. 1985, 1988. Bittencourt 1992, Zaidenberg & Segovia 1993).

In *D. marsupialis* we observed no cases of congenital transmission: indeed, the short gestation time and the characteristic maternal-embryonic barrier ocurring in marsupials makes this route of contamination very improbable.

Reports of the transmission of *T. cruzi* into the milk of different placental species are scarce: though Disko and Krampitz (1971) demonstrated the presence of trypanosomes in the milk of infected mice, but not the transmission of the infection to sucklings. In humans, it was demonstrated that transmission through breast feeding rarely occurs even in the acute phase of the disease, unless there were nipple bleeding (Bittencourt et al. 1988).

In spite of the close physical contact and length of lactation in didelphids, we did not obseve any *T. cruzi* transmission during this period. Although protection conferred by specific antibodies of maternal origin ensured to the weaned young a significantly longer prepatent period and 4,5 fold lower peak levels of circulating parasites, the patent parasitemia persisted for twice as long. The longer patent period seems to be a peculiar trait of this model host, and in nature it could be one of the factors accounting for its high eficiency as a reservoir.

The invasion of scent glands of the two experimentally infected litters observed close to the time of peak parasitemia reinforces our previous observations with regard to the correlation between parasitism of this site and a high bloodstream parasite population. The single animal whose scent glands were not colonized by *T. cruzi* suggests that additional, as yet uncharacterized factors are also involved in this process.

Protection through lactation is well known in parasitic diseases.

Immune and non immune anti-parasitic agents involved in protection against entero protozoan parasites have been described in human milk (Gillin et al. 1983).

Protection against trypanosomatids of the brucei group via lactation has been described in ungulates (Stephen 1966, Whitelaw & Jordt 1985) in addition, an anti *T. b. rhodesiense* activity has been observed in colostrum of animal and human origin (Mulla et al. 1985).

Marsupials are born after a very short gestation period and with only the vital physiological functions. The immunological incompetence phase, before the maturation of the immune system takes place, occurs in the marsupial pouch. Very little is known about the immune and non immune mechanisms which protect the newborn in this condition: the transmission of maternal antibodies is probably one of the most important, although other as yet unidentified factors may also be transferred during lactation.

Although Rowlands and Dudley (1969) did not observe the transfer of antibodies during lactation of *D. virginiana,* the phenomenon was described in a later publication by Hindes and Mizell (1976). The importance of transmission of antibodies to newborn marsupials via lactation was corroborated in *Monodelphis domestica* (Samples et al. 1986), *Setonix brachyuris* (Yadav 1971), *Trichosurus vulpecula* (Yadav & Eadie 1973), and *Macropus robustus* (Deane & Cooper 1984).

Our observations indicate that, in experimentally infected pouch young, parasitemia becomes patent only after levels of antibodies of maternal origin begin to decline. This leads us to suggest that, in nature, *D. marsupialis* maternal antibodies may serve to protect pouch young during the period of maturation of intrinsic mechanisms involved in the control of *T. cruzi* infection.

ACKNOWLEDGEMENT

To Alcidineia Ivo for technical help.

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