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EXPERIMENTAL FELINE TOXOPLASMOSIS

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SUMMARY

Experimental infection by *Toxoplasma gondii* was studied in 38 kittens of 13 litters, aged 3 to 22 weeks when inoculated parenterally or *per os*, with trophozoites or cysts of 3 strains of the parasite. All strains and both stages proved virulent to kittens and capable of producing oocysts and general lethal infections. Parenteral inoculations resulted in symptomatic general disease within the first week, followed by death or rapid clinical recovery within the next 2 weeks. Recovery was associated with precocious high titers in the dye test. Feces of parenterally inoculated kittens were not infective to mice. Infections *per os* were mostly asymptomatic during the period of oocyst passing. This period was usually from the 4-5th to the 10-12th days, but for one kitten it extended through 27 days. Maternal antibodies did not prevent production of oocysts. Serological response came later and with lower titers than in parenterally inoculated animals and deaths, occurring at various intervals after the feces had become negative, were about half as frequent as in the other group.

INTRODUCTION

Natural and experimental infections of the domestic cat by *Toxoplasma gondii* have been studied by several Authors, both before and after the discovery of the schizogonic and gametogonic cycles of the parasite in the intestinal epithelium of these animals.

In this paper we describe a study in sibling kittens infected with different phases of the parasite and through different routes, including serological testing of the mother cats and prolonged follow-up of the kittens.

MATERIALS AND METHODS

The study includes 37 kittens of 12 litters, plus a stray specimen about 3 months old. Except in 3 cases, the litters were maintained with their mothers throughout the experiments.

The strains of toxoplasma were: "N", isolated by NOBREGA et al. [12] in 1952 and "R-58" isolated from a domestic rabbit in March 1969, both killing mice in few days; and "AS-28" isolated from a wild brown mouse (*Mus musculus*) in November 1969, which tends to produce chronic infections in albino mice [1].

Parenteral inoculations in the kittens were made with the peritoneal exudate of mice acutely infected with strains N or R-58. For the *per os* infections with these 2 strains acutely infected mice were fed to the kittens. Thus, in all infections with these 2 strains the infective form of the parasite was the trophozoite. Strain AS-28 has been used only for *per os* inoculations, the brains of 2-3 mice positive for cysts being fed to the kittens through a gastric tube.

The mother cats and their offspring had feces examined and dye tests (DT) made

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before inoculation of the kittens. For the latter, weight and temperature were recorded before inoculation and daily thereafter during the first weeks. Fecal examinations and DTs were made periodically throughout the observations. For the latter, undiluted serum and 4-fold dilutions up to 1:4096 and 2-fold dilutions thereafter, were used.

Fecal specimens were treated according to Willis' method, floating eggs or cysts of parasites being collected for direct examination and kept in 2.5% potassium dichromate. After various periods the material was washed and inoculated in mice through a gastric tube.

To a number of kittens and at various intervals after inoculation, pilocarpine was administered intravenously to provoke salivation. Samples of salive were injected in the peritoneum of mice.

Some kittens died or were killed, others were maintained alive for studies on resistance to reinfection. Death was sometimes accidental. At necropsy smears were made of any enlarged lymph node and of brains, heart, lungs, kidneys, liver, spleen and small intestine scrapings. Portions of these tissues were injected in lots of 4-5 mice, the follow-up of these being made according to a scheme already described [10].

RESULTS

Infection among the inoculated kittens. Of the whole group only one kitten (8-VI, Table II), which had eaten an acutely infected mouse, did not become infected.

All animals inoculated parenterally (2 by the subcutaneous, the others by the intraperitoneal route) presented symptoms of the acute disease within the first week, fever, loss of appetite and of weight being the most frequent clinical signs. For the surviving animals the weight curve was stationary or, more often, fell rapidly during the first week and went up as soon as they started recovering, at the same time that titers in the DT began to go down. Diarrhoea, dyspnoea, cough, coryza and conjunctivitis occurred in some kittens and enlargement of lymph nodes in most. Death due to toxoplasmosis occurred in 33.3%.

Among the 22 kittens infected *per os* the acute phase was clearly symptomatic in some and not in others and, when present, the symptoms occurred later than in parenterally inoculated animals. The mortality rate was 18.2%.

Finding of toxoplasma in living kittens. From each of 18 kittens several samples of saliva were obtained. For 3 animals results of inoculation were positive: in one case, infected *per os*, only the saliva collected on the 7th day was positive; in another, inoculated parenterally, the 7th day sample was negative, but those from the 9th, 14th and 20th days were infective; from the 3rd kitten, also parenterally inoculated, all 5 samples taken between the 5th and 26th days were positive.

Pools of fecal material repeatedly collected from parenterally inoculated kittens (all infected with *Toxocara*) were not infective for mice. On the contrary, similar material from kittens infected *per os* was highly infective (*). All samples tested gave positive results in mice, except those passed by the 3 siblings of litter 8 (Table II).

The 3 strains of toxoplasma and both the trophozoites and cysts produced oocysts when inoculated *per os* in kittens. Out of 19 animals, 15 passed oocysts after one infective feeding, 2 after a 2nd feeding given one week later; for the 2 siblings of litter 10 fecal examinations were always negative. The earliest day on which oocysts were seen was the 4th after inoculation and the longest period of positivity was 27 days. Marked differences were noticed in the daily and total yield per kitten.

Serology and mortality. Of the 37 infected kittens, 31 had negative DTs, even in undiluted serum, before inoculation and on the 3rd and, or, 5th day thereafter. In 27 (87.1%) of these animals the test became

(*) 4 kittens inoculated *per os* were studied before we knew of the findings on the fecal forms of toxoplasma and their significance [4, 6, 8, 13, 15, 16]. Feces of those kittens were not inoculated in mice and, although they were regularly examined, we now realize that the small toxoplasma oocysts were probably misidentified at the time.

TABLE I

Parenteral inoculation of trophozoites of *Toxoplasma gondii* (strains N or R-58) in 15 kittens of 6 litters. Maternal serology and serology, mortality and post-mortem isolation of parasite among the kittens. All kittens had negative DTs before inoculation.

Litter	Mother's DT	Kitten no.	Age at inoculation, in weeks	DTs after inoculation, max. titer, day		Mortality		Last DT *	Post-mortem isolation of parasite
						Died, on day	Killed, on day		
1	—	1-I	10	**			145	1:16	—
2	1:16	5-I	14	1:16384	15		150	1:64	—
		5-II	22	1:256	20		89	1:256	—
		5-III	22	—		12		—	...
		5-IV	22	—		15		—	...
3	undil.	6-I	10	1:16384	10		113	—	—
		6-II	10	1:4096	15		109	1:64	—
4	1:64	8-I	12	1:4096	10		16	1:1024	+
		8-II	12	1:4096	10		92	1:64	—
		8-III	12	1:4096	10		95	1:16	—
		8-IV	12	1:8196	10		16	1:8196	+
5	—	10-1	10	1:1024	20	24		1:256	+
		10-II	10	1:4	10	20		1:4	+
		10-III	10	—		8		—	+
s/n	...	12	12	1:1024	10		103	—	—

— = Negative + = Positive ... Note examined

* = at death or shortly before

** = The first test made on 35th day, positive 1:256

positive, 7 days being the shortest time required for conversion to take place.

Parenteral inoculations produced more precocious responses and higher titers during the acute phase than did the infections *per os* (Graphs 1 and 2). On the other hand relatively high titers persisted for longer in kittens of the latter group. At the end of the 2nd month most animals inoculated by a parenteral route had titers of 1:64 or 1:16, and among 8 specimens that lived for 3 months or more the test became negative in 2; both had had several positive results and, in one of them, the titer had reached 1:16384 on the 10th day (kitten 16-I, Table I).

Infections *per os* in previously negative kittens usually produced positive tests in serum dilutions of 1:1024 or less. The period within which the maximum titer was reached was quite variable, from 2 to more than 6 weeks after ingestion of the parasite. One exception was kitten 8-V that had a titer of 1:4096 on the 15th day (Table II).

Six specimens had DTs at low titers before inoculation *per os* (Table II); there was no change in one (kitten 15-II), 2 became po-

TABLE II

Inoculation *per os* of trophozoites (strains N and R-58) and cysts (strain AS-28) of *Toxoplasma gondii* in 23 kittens of 8 litters. Maternal serology and serology, oocyst production, mortality and post-mortem isolation of parasite among the kittens.

Litter	Mother's SFT	Kitten no.	Age at inoculation, in weeks	Phase of parasite	Dye Tests			Oocysts passed		Mortality		Post-mortem isolation of parasite
					Before inoculation	After inoculation Max. titer,	day	Period, on days	Infectivity	Died, on day	Killed, on day	
4	1:64	8-V	12	Trophoz.	—	1:4096	15	(x)			18	+
		8-VI	12	Trophoz.	—	—		(x)			93	—
6	1:64	11-I	12	Trophoz.	—	undil.	12	(x)			24	+
		11-II	12	Trophoz.	—	1:1024	35	(x)			130	—
7	—	13-I	3	Cysts	—	1:4	7	4-7	+		7	+
		13-II	3	Cysts	—	1:1024	13	5-13	+	13 accid.		+
		13-III	3	Cysts	—	1:1024	15	4-12	+	18 accid.		+
		13-IV	3	Cysts	—	1:1024	15	4-30				R
8	1:64	14-I	10	Trophoz.	—	1:4	7	5-7	—		15	—
		14-II	10	Trophoz.	—	1:4	7	5-12	—		18	—
		14-III	10	Trophoz.	—	1:4	7	7	—		25	—
9	1:1024	15-I	8	Cysts	—	—		5-7	...	26		...
		15-II	8	Cysts	1:2	1:2		5-12	...	26		...
		15-III	8	Cysts	undil.	1:4	19	7	...	28		...
10	...	16-I	12	Cysts	—	1:1024	24	—				R
		16-II	12	Cysts	—	1:256	15	—				R
11*	1:64	17-I	10	Cysts	—	1:64	21	10-11	+	26		+
		17-III	10	Cysts	—	1:256	44	10-11	+			R
		17-IV	10	Cysts	—	1:256	44	5-11	+			R
		17-V	10	Cysts	1:16	1:64	4	5	+	48 accid.		...
12*	...	18-I	6	Cysts	1:16	1:4096	48	4-10	+			R
		18-II	6	Cysts	1:16	1:1024	34	4-10	+			R
		18-III	6	Cysts	1:16	1:8196	34	4-14	+			R

— = Negative + = Positive ... = Not examined R = Kept alive for reinoculation

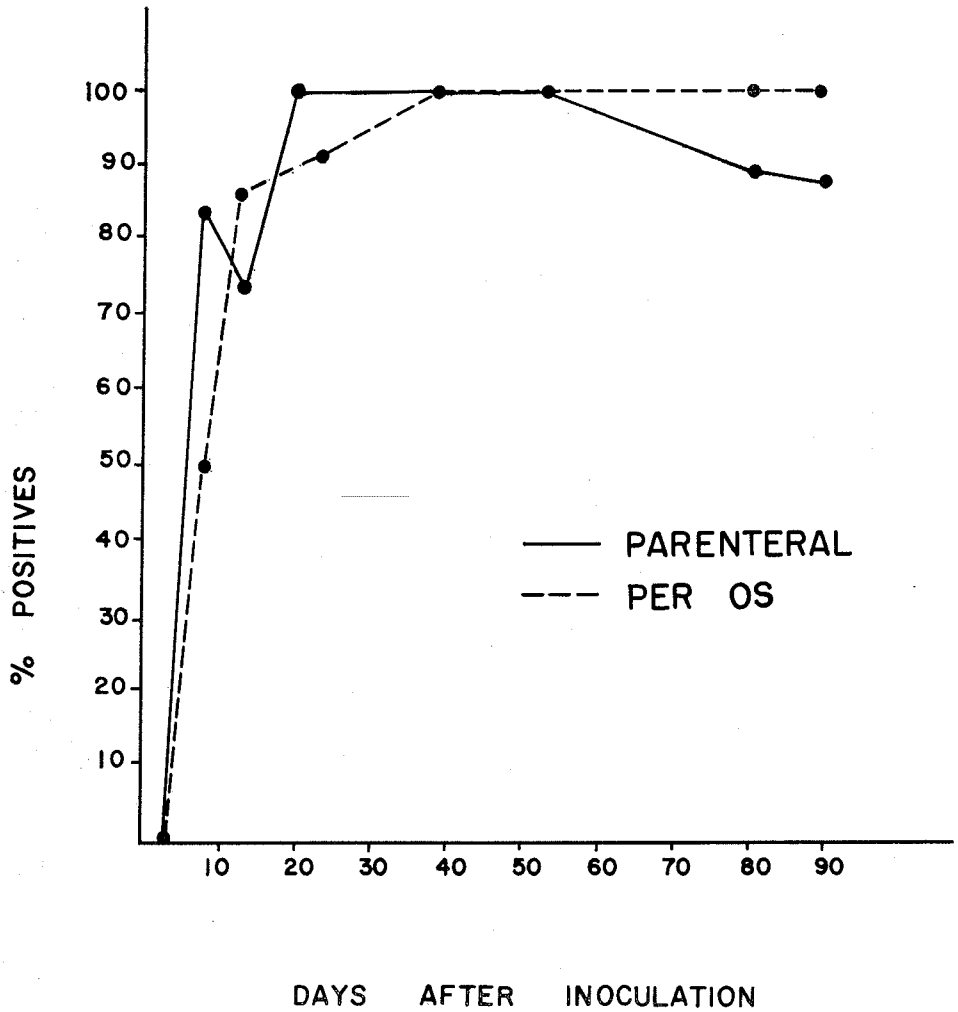
* These 2 litters received double inocula, in one doses for litter 12, in 2 doses at one week interval for litter 11

(x) About these kittens see footnote on page 132

sitive up to the next serum dilution (kittens 15-III and 17-V) and a marked increase occurred in the 4 siblings of litter 12 that started with a titer of 1:16 and received exceptionally heavy inocula.

Among kittens inoculated parenterally with the very virulent trophozoites of strains N and R-58, there was a good correlation between early serological response with high titers and survival through the acute infection. Kittens showing no conversion or only weak and late responses died of acute toxoplasmosis. The one exception (kitten 5-II, Table I), started with fluctuating low titers and was severely ill but managed to survive with a positive DT at 1:256 from the 20th day on.

Among kittens inoculated *per os* (Table II) there were 4 deaths attributed to toxoplasmosis, corresponding to 4 specimens in which serological response was weak or absent, while no deaths due to the disease occurred among those responding with relatively high titers. On the other hand, in kitten 17-V



Graph 1 — Positivity of the dye test among previously negative kittens inoculated parenterally or *per os* with *Toxoplasma gondii*. Fatal cases of acute toxoplasmosis without serological conversion are responsible for the downward curve between the 10th and 20th days. Among the parenterally inoculated kittens, 2 were negative at the end of the period of observations.

the titer of 1:16 prior to inoculation increased only to the next dilution, maintaining this level up to the 48th day, when the animal died accidentally following a heart puncture.

Serology and oocyst production. Completion of the gametogonic cycle occurred independently of maternal serology and of the positivity of the DT in the kittens themselves prior to inoculation (Table II).

One kitten (13-IV) continued to pass oocysts for 2 weeks after a maximum titer of 1:1024 had been reached, but in 5 other specimens the highest titers were attained long after the feces had become negative.

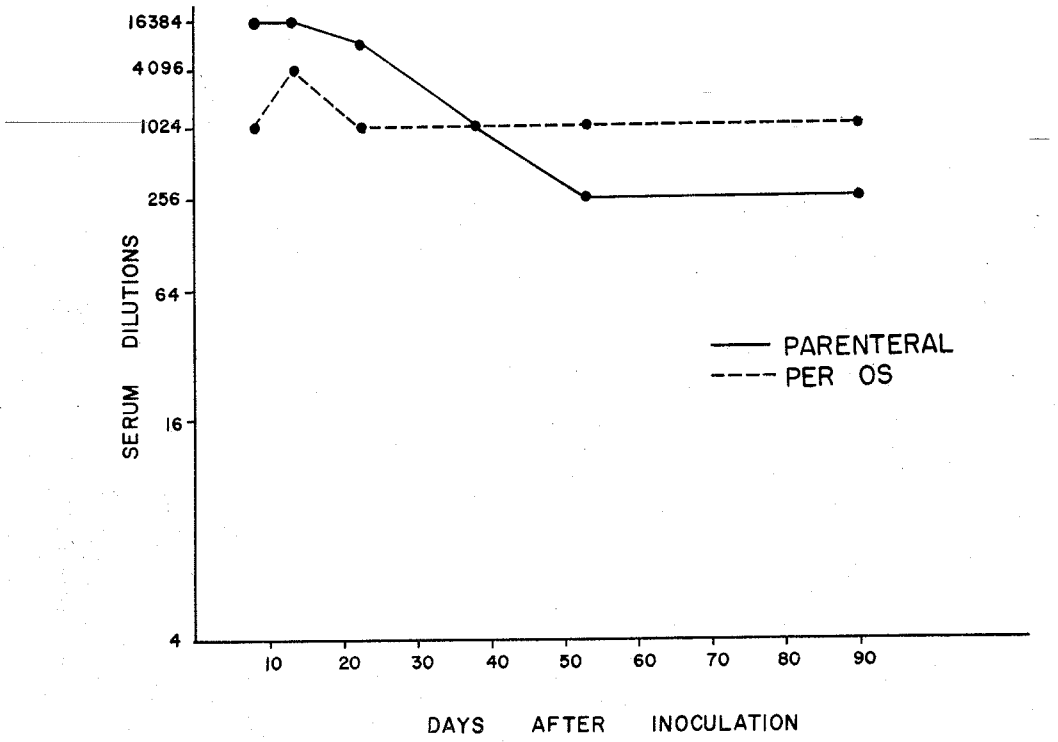
Oocysts were never found in the feces of 2 siblings (litter 10) coming from a non-examined mother. In both the DT was negative before inoculation and became positive thereafter, the titer being 1:4 and 1:256 on the 10th and 15th days, and reaching 1:1024 for one of them on the 24th day.

Post-mortem isolation of the parasite. The parasite was reisolated from the tissues of 11 of the 23 animals examined. All positive findings were among 14 kittens dead or killed within the first month of inoculation. This makes for a proportion of 78.6% isolations for this group against none for the 9 kittens killed 3 months or more after inoculation, although in most of the latter the DT was positive up to the last day of their lives.

DISCUSSION

All 3 strains of toxoplasma used and both the trophozoites and cysts proved virulent to kittens and capable of going through the intestinal cycle up to the oocyst stage and producing lethal general disease. It was the route of inoculation rather than the strain that seemed to influence the outcome of infection.

Following parenteral inoculations the course of infection was as expected for any general disease caused by a rapidly spreading organism, the acute phase being always sympto-



Graph 2 — Maximum titers in the dye test among previously negative kittens inoculated parenterally or *per os* with *Toxoplasma gondii*

matic within the first week and prompt clinical recovery or death ensuing within the next 15 days. In recovering kittens high titers in the DT were reached between the 7th and 15th days. There was no evidence of an intestinal cycle among kittens infected parenterally, their pooled feces being consistently non infective to mice.

On the other hand, the gametogonic cycle was completed in most kittens infected *per os* but symptoms of a general infection were absent or, when present, appeared 10-20 days after oocyst passing had stopped. The rising in DT titers was slow, sometimes taking more than one month (once almost 4 months) to reach their highest points. These were usually lower than in the other group.

The above observation indicate a certain degree of independence between the intestinal and extraintestinal cycles as far as disease and immunity are concerned. As suggested by DUBEY & FRENKEL [3] it seems that antibody production follows extraintestinal infection.

When the trophozoites of a virulent strain are introduced parenterally in non-immune kittens it is probable that the parasite continues to multiply by endodiogeny, rapidly invading the tissues and causing death or an effective immune response soon enough to prevent, in both cases, further spreading. This would explain why parenteral inoculations do not always lead to production of the fecal forms. As in our observations, DUBEY & FRENKEL [3] could not find any intestinal stage of the parasite in 4 kittens inoculated subcutaneously with toxoplasma cysts and dying from acute toxoplasmosis, although in JANITSCHEKE's [11] experiments similar inoculations were followed by oocyst production.

It has been suggested [2] that when toxoplasma cysts are introduced *per os* in cats, some trophozoites start the intestinal cycle while others immediately make their way to other tissues. Studying, in new born kittens, the time required for the parasite to reach various organs, DUBEY & FRENKEL [3] found great differences which they attributed to differences in the number of cysts (from 1 to 23 infected mouse brains) fed to the animals. It is possible than when smaller inocula are used for older kittens, invasion of extraintestinal territories is slow and gradual, or is held in check until a mass of trophozoites accumulates and overcomes any barrier. Gradual invasion by small numbers of parasites could explain the slow rising in titer of the DT without disease.

Another aspect worth discussing is the value ot the DT. That a negative result does not always mean absence of previous infection is demonstrated by the fact that in 2 of our parenterally inoculated kittens the test became negative in a little over 3 months. This may be related to the consistently negative results of inoculations of tissues from kittens killed 3 months or more after infection, indicating that our strains left a very scanty residual parasitism, if any. However, there are several reports on the isolation of toxoplasma from serologically negative animals [9]. That the antibodies involved in the DT are not protective antibodies has been demonstrated by HULDT [7], who found that high titers obtained through artificial immunization with dead parasites did not protect rabbits against infection. Also, experimental work by several Authors [5, 14] has indicated that in toxoplasmosis as in other infections by intracellular protozoa, protection is chiefly due to cellular immunity and circulating antibodies do not play a decisive role in resistance to reinfection.

The isolation of toxoplasma from the saliva of some kittens points out to another possible means of transmission of toxoplasmosis among cats.

RESUMO

Toxoplasmose experimental em felinos

A infecção experimental por *Toxoplasma gondii* foi estudada em 38 gatinhos de 13 ninhadas, tendo de 3 a 22 semanas quando inoculados, por via parenteral ou digestiva, com trofozoitos ou cistos de 3 cepas do parasita. As 3 cepas e as duas formas evolutivas mostraram-se virulentas e capazes de produzir o ciclo intestinal nos gatinhos.

As inoculações parenterais causaram infecção geral sintomática após uma semana, seguindo-se morte em 33,3% ou recuperação rápida acompanhada de altos títulos no teste do corante. Em dois animais que sobreviveram tendo apresentado altos títulos o teste do corante tornou-se negativo em 3-4

meses. As fezes dos animais inoculados por via parenteral não foram infetantes para camundongos.

As inoculações *per os* resultaram na produção de oocistos infetantes para camundongos. As infecções foram assintomáticas durante o período de eliminação de oocistos, o qual foi do 4.°-5.° até o 10.°-12.° dias, e em um animal estendeu-se por 27 dias. Em alguns animais ocorreram sintomas gerais em tempos variáveis após a negativação das fezes. Os títulos mais altos no teste do corante foram em geral mais baixos e atingidos bem mais tardiamente que entre os inoculados por via parenteral. A mortalidade foi 18,2%.

Amostras de saliva colhidas durante a infecção e inoculadas em camundongos deram resultados positivos em 16,6% dos gatinhos.

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