

CONGENITAL TRANSMISSION OF TOXOPLASMOSIS IN DOMESTIC RABBITS

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Abstract. The results of studies on female rabbits with spontaneous toxoplasmosis confirmed that infection is transmitted from the mother to the foetus during a prolonged latent phase of toxoplasmosis (11.5% of newborns of 9 litters from 3 females were infected). Although infection was not always present in every litter from the same female, and in all newborns, it could be acquired through at least three generations. Except once, we never observed either signs of disease or malformations in the young rabbits. In order to complete our investigation, we tested the possibility of congenital toxoplasmosis in a female infected orally with mature toxoplasma oocysts; it was covered 6 months p.i. and gave birth to 8 young of which 4 were damaged congenitally and died. Differences in the course of experimental infection with tissue cysts and oocysts of *T. gondii* in rabbits, and the possibility of the influence of various factors on the occurrence of spontaneous toxoplasmosis in breeds of rabbits, are discussed in the text.

According to the mode of infection, toxoplasmosis in vertebrates has been divided into two types, i.e., the so-called acquired, and congenital toxoplasmosis. Acquired infection is frequently introduced by oral routes, mostly by ingestion of raw or underdone meat from animals with spontaneous infection harbouring toxoplasma cysts in their muscles or other organs. This, apparently, is a standard mode of infection of man (Hübner et al. 1973); it occurs with carnivores and omnivores, and if cannibalism is involved. In herbivores or grain-feeding animals, however, the problem of infestation has long been in question.

The diversity of ideas postulated in studies on congenital toxoplasmosis is remarkable. The problem of persistence of infection in man, and the possibility of congenital transmission of the infective agent, has divided the research workers into several groups; one group described congenital transmission in chronic forms, others confirmed specific malformation or abortion in acute forms, and others, again, inferred that neither acute nor chronic toxoplasmosis of the mother carried any significance in correlation with abortion and serological tests, or, possibly, that primary infection with toxoplasmosis in the mother can be transmitted congenitally to the foetus only once, and that there is no danger for the subsequent pregnancies, etc. Unfortunately, it is impossible to solve these problems in experiments with animals, because the anatomy and physiology of their reproductive organs differ from those of man, and a comparison is impossible. A similar diversity of ideas exists also with congenital transmission of toxoplasmosis in animals with experimental infection, because the results differ from species to species.

The rabbit, a commonly bred domestic animal, ranges among mammals as a species with the highest rate of infestation. This has been confirmed in surveys on serologically detected antibody against spontaneous toxoplasmosis. The percentage of positivity depends on the seroreaction used; in Czechoslovakia, e. g., Kunštýr et al. (1970) demonstrated by means of the CFT that an average of 63 % of rabbits from different breeding farms was positive. Our findings obtained from 1954—1968 by means of the SFT showed that, of the 874 rabbits from small-scale breeders, 89 % were positive. Although the percentage of positive findings was extremely high, it did not infer that all seropositive rabbits were toxoplasma carriers. By means of the microprecipitation reaction in agar gel (MPA) as the indicator of nonsterile immunity, we found a positivity of 20 % in 190 rabbits from different breeders. In spite of that, rabbits are a potentially dangerous source of infection for man, because they are bred and consumed in large numbers, especially in the country; in addition, other domestic animals and also wild animals are infected by ingesting rabbit offals and other raw meat remnants which are generally thrown on the dung heap.

What are the factors responsible for the high rate of infestation in rabbits? It appears that one of the major routes of infection is ingestion of mature toxoplasma oocysts in fodder polluted by cat faeces. The possibility of acquiring infection by contact seems of little importance; cannibalism is almost nonexistent in rabbits although it may happen that the males attack each other and bite off testes or muscles, and the females devour the foetal membranes after parturition or, sometimes, the newborns. Transmission with the mother milk may be possible during the acute stage of toxoplasmosis, but it is rare in occurrence. The last possibility is congenital transmission. Several authors (Wiktor 1950; Lalla et al. 1967; Hartcourt 1967) described epizootologies with massive mortality of young and adult rabbits, and suggested the possibility of uterine transmission, without excluding the possibility of a transmission of toxoplasma organisms with the mother milk (Hartcourt 1967; Berengo et al. 1969). The major purpose of all laboratory experiments was the assessment of the phase during which toxoplasmosis is transmissible to the progeny (Roth et al. 1957). A recent remarkable study is that by Janitschke et al. (1970) who infected female rabbits with cystogenic strains of *T. gondii* at certain intervals before copulation and during gestation; the foeti were isolated by Caesarian section and positivity assessed in culture. The authors obtained conclusive evidence of the fact that intrauterine transmission of toxoplasma organisms is possible during pregnancy with an active infection only. At the latent phase, no positive finding of toxoplasma was obtained from the foeti of females inoculated 267—35 days before copulation.

In our study we used animals with spontaneous infection only, because results obtained from animals with artificial infection may be different from those of animals with spontaneous infection, particularly if using strains kept for a long time in the laboratory.

MATERIAL AND METHODS

Rabbit no. I — Random serological examination of a small breed of rabbits disclosed infection in this female (MPA-positive), aged approximately 8—9 months. The animal was taken to the Institute and kept in a wooden eot in an avery under conditions similar to those in small-scale rabbit breeds in the country. The female was covered 4 times within 15 months by MPA-negative males.

Rabbit no. II — The second MPA-positive female, taken from the first litter of rabbit no. I; also this female was covered 4 times within 22 months by MPA-negative males.

Rabbit no. III — A female offspring from the 4th litter of rabbit no. II; until the present, this female was covered only once by an MPA-positive male. Living conditions were identical for all three experimental animals.

The control group consisted of 8 MPA-negative females; four of these (no. 1—4) were obtained from breeders; two (no. 5 and 6) were the offsprings of these females; two (no. 7 and 8) were offsprings of rabbit no. I (from negative litters). Breeding conditions were similar to those of the MPA-positive females. The 8 control females were covered 13 times, i.e., 10 times by MPA-negative males, 3 times by MPA-positive males.

All female rabbits were tested serologically by means of the MPA and SFT, at least half a year before the first copulation. MPA-positivity was continuous in the positive females, SFT titres ranged from 1 : 256 to 1 : 16,000. The MPA was negative in all females of the control group, SFT titres ranged from 1 : 4 to 1 : 64. (SFT-negative rabbits are extremely rare in occurrence; the results of long-term examinations of antibody level in the individual animals disclosed titres ranging from 1 : 4 to 1 : 64.) Sometimes, examination results were negative, while in the subsequent examination of the same animal, the values assessed were already within the given range of titres or vice versa. It seemed that several of these titres were unspecific as found also by Jacobs (1965) in cows. In 81 % of the 874 rabbits examined we found titres from 1 : 4 to 1 : 64. Also Záštěra (1963) found in immune sera during saturation of specific antibodies with toxoplasma antigen that, in man, antibodies can be saturated up to an SFT-negativity, while in animals (several rabbits with spontaneous infection) titres remained positive (1 : 16).

Blood for serological examination was obtained by cardiac puncture the day before copulation and again on day 21, 45, 90 and 180 after parturition (together with the blood of the young).

None of the parents was a descendant of a racially pure line.

All animals, i.e., females and young (except the females kept for reproduction and studies on the young) were killed 6 months after the onset of the experiment; their brain was injected to white mice (for techniques see: Hübner et al. 1969).

The two methods used simultaneously for the serological examination of the animals were the MPA (Hübner et al. 1970—with attached corrigenda) and the SFT (Hübner et al. 1974).

RESULTS

A. MPA-positive females (Table 1)

Female no. I gave birth to 30 young (four litters). Toxoplasma organisms were found in two young from the first litter (disclosed by both serological tests and isolation experiments). In the remaining young, the results of isolation experiments and the MPA were negative, SFT titres ranged from 1 : 4 to 1 : 16.

Female no. II delivered 19 young in four litters; of these toxoplasmosis was disclosed with serological tests in one animal of the second litter and in two animals of the fourth litter. The strain was isolated from one young of the second litter, and from one of the two animals from the fourth litter; the second animal of this litter was the female rabbit no. III which is still alive. In the remaining animals, the results of the MPA and of isolation experiments were negative, the titres of the SFT ranged from 1 : 4 to 1 : 64. Rabbit no. III had produced as yet one litter only with three young, of which two were negative in both MPA and culture, with an SFT titre of 1:14 — 1:16; the third

Table 1. MPA-positive females

Female no. I	Female no. II	Female no. III
4 litters	4 litters	1 litter*)-3 young
7 young-2 positive 9 young-negative 11 young-negative 3 young-negative	4 young-negative 5 young-1 positive 5 young-negative 5 young-2 positive	1 positive
Total 30 young-2 positive	19 young-3 positive	3 young-1 positive

*) = the female giving birth to this litter was covered by a MPA-positive male

young showed signs of CNS affections, its MPA was positive. This young will be used in a further test.

B. MPA-negative females (Table 2). The 8 females of this group produced a total of 80 young in 13 litters. Females and young were negative in both MPA and culture, the SFT titres ranged from 1:4 to 1:64.

The average number of newborns per litter was approximately the same in both groups, i.e., 5.77 for group A, 6.15 for group B. The percentile occurrence of toxoplasmosis in the young of group A was 11.5 % in all litters.

Table 2. Control females MPA-negative

	No. of litters	No. of young per litter	Results of MPA and isolation experiments
Female no. 1	2	6 + 9	negative
Female no. 2	2	6 + 8	negative
Female no. 3	2	7 + 7*)	negative
Female no. 4	1	10	negative
Female no. 5	1	5	negative
Female no. 6	1	4*)	negative
Female no. 7	3	6 + 6 + 4*)	negative
Female no. 8	1	2	negative
Total:	13	80	negative

*) — the female giving birth to this litter was covered by a MPA-positive male

DISCUSSION

In spite of our limited experimental material, it is clearly indicated by our results that congenital infection is possible in females with a prolonged latency period of toxoplasmosis. Infection is not transmitted to every litter brought forth by one female, and not to all newborns, but the possibility of congenital infection persists for at least three generations. No marked symptoms of infection were observed in the positive females and their offsprings, except for one newborn with signs of damage apparently of congenital origin. Abortion did not occur in females of these litters, which were kept for breeding.

It may be suggested that infection of the young had not been acquired by congenital transmission, but by the transport of toxoplasma organisms with the mother milk, or by contact with the infected mother, or even by ingesting food contaminated with toxoplasma oocysts. Release of toxoplasma organisms with the mother milk may occur only with an infection at its initial or acute stage, when free toxoplasma organisms circulate in the body. Blood from female no. 1 taken three times during gestation and three times during the first fortnight after parturition was inoculated into mice, but parasitaemia could not be demonstrated. This excluded also the possibility of acquiring infection by contact (faeces, urine, saliva). The possibility of the release of toxoplasma organisms with the urine and excrements of rabbits during the acute stage of infection with *T. gondii* was tested by one of the present authors, but only standardly negative results were obtained. We also excluded the possibility of infection by food (fresh grass) contaminated with toxoplasma oocysts, because all animals, including those of control group B, had been fed with food from the same source and, yet, none of the 88 animals of group

B had acquired infection; also the results of the MPA and cultivation were negative. These findings support our assumption that transmission of toxoplasmosis to the young of group A was intrauterine and, therefore, this mode of transmission should be considered in the epizootiology of toxoplasmosis in rabbit, and also in the practice, when

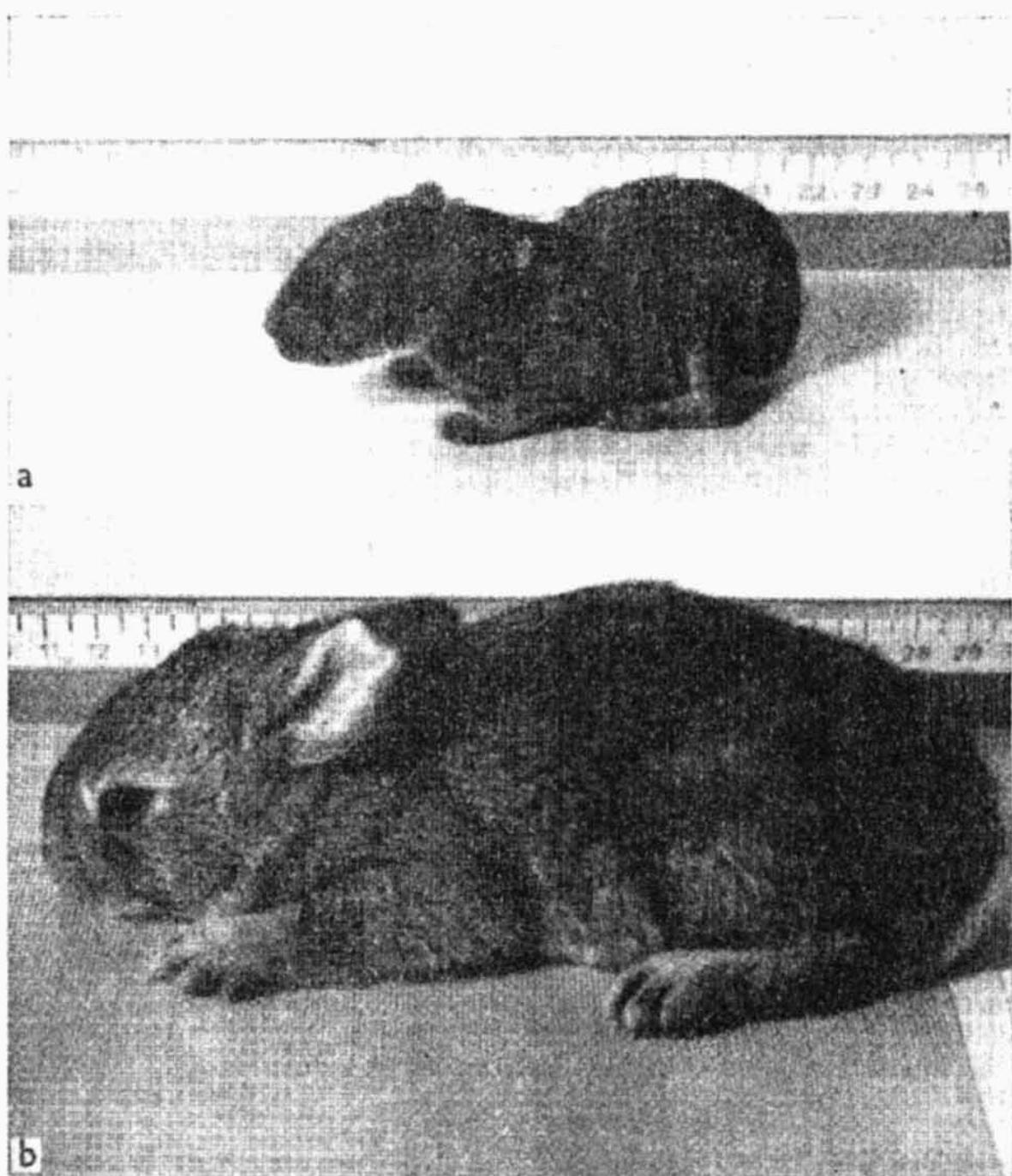


Fig. 1. A 15 day-old young with congenital damage.
Fig. 2. A healthy, 15 day-old young from the same litter.

small-scale breeders exchange female rabbits for breeding purposes. In the latter case, approximately 50 % of rabbit infestation with toxoplasmosis (MPA test) may have been acquired by congenital transmission.

Since there was no explanation of the mode of infection of the other 50 % of MPA-positive rabbits, we fed three young female rabbits from our MPA-negative breeds with grass contaminated with mature toxoplasma oocysts from the faeces of experimentally

infected cats. Infection with a small dose analogous to conditions in nature was manifested by signs of malacia, diarrhea and considerable loss of weight in all animals; high SFT titres and a positive MPA indicated the presence of acute toxoplasmosis. One of the animals died shortly afterwards. The course of infection with oocysts was very different from experimental infection with tissue cysts administered orally; in the latter case, the presence of infection was not signalled by signs of disease. One of the females was covered 6 months after infection and gave birth to 8 young; five of these developed normally; three were much smaller at birth and looked "exsiccated"; two died within 11 days after birth. Isolation experiment disclosed toxoplasma organisms in one of them, but during the second isolation experiment the mice died of concomitant bacterial infection. The third newborn photographed at the age of 15 days together with the second, normal newborn (Fig. 1), died two days later and was liquidated during our absence. In one of the 5 normally developing baby-rabbits, a cataract on both eyes was observed at the age of 5 weeks; it continued to grow worse, the animal became cachectic and died at the age of 11 weeks. Material from this animal was not used in isolation experiments on mice. SFT titres of the four remaining newborns ranged from 1 : 4 to 1 : 16, MPA was negative. In none of them did we observe signs of damage. This experiment indicated that several of the newborns had acquired toxoplasmosis by congenital transmission, with a lethal outcome. Hypothetically, this may offer an explanation of outbreaks and epizooties in rabbits, and the numerous cases of mass mortality of newborn rabbits recorded by many small-scale breeders, if we excluded the effect of facultative, pathogenic rabbit coccidia of the genus *Eimeria*, or that of other factors.

The course of infection acquired from toxoplasma oocysts appears to be more severe than that of infection caused by tissue cysts; also congenital infection may damage considerably the foetus and, subsequently, the newborn.

As regards the circulation of toxoplasmosis among animals in the field, it seems very probable that, in a certain percentage of cases, infection may be caused by the ingestion of toxoplasma oocysts, particularly among herbivores. This suggests that in the rabbit, this mode of infection may supplement congenital transmission, or vice versa. The problem of the likelihood of oocyst infection in herbivores, i.e., how many times the cat is capable of releasing toxoplasma oocysts during its span of life, and the percentage of cats acting as a potential source of toxoplasmosis, are the subject of another communication (Hübner et al. 1974, in press).

In conclusion, we should like to draw attention to the fact that the correlation between the MPA and isolation experiments was 100 % in all cases. Obviously, the MPA technique employed standardly in veterinary medicine, may also find its way into the general practice to be used, e.g., for the selection of healthy animals for replenishing stocks, or for the detection of infected animals, particularly in populations bred for laboratory purposes.

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ВРОЖДЕННАЯ ПЕРЕДАЧА ТОКСОПЛАЗМОЗА У ДОМАШНЕГО КРОЛИКА

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Резюме. Результаты изучения спонтанного toxoplasmosis у самок домашнего кролика подтвердили, что инфекция передается матерью в плод во время затянувшейся латентной фазы toxoplasmosis (11,5 % новорожденных детеныш из 9 выводков от 3 самок оказались зараженными). Не смотря на то, что не всегда в всех детенышах в каждом из выводков от

одной и той же самки обнаружено наличие токсоплазмоза, это заболевание может быть приобретено на протяжении по крайней мере трех генераций. За исключением одного случая, мы не наблюдали никаких признаков заболевания или уродств у молодых кроликов. В завершение наших исследований мы поставили опыт по выяснению возможности врожденного токсоплазмоза у самки, зараженной через рот пологозрелыми токсоплазмовыми ооцистами; 6 месяцев после заражения самка была оплодотворена и родила 8 детеныш, из которых 4 оказались с врожденными повреждениями и погибли. В статье далее излагаются отличия, возникающие во время экспериментального заражения кроликов цистами и ооцистами *T. gondii*, и возможность влияния разных факторов на наличие спонтанного токсоплазмоза в их потомстве.

REFERENCES

BERENGO A., DE LALLA F., CAVALLINI-SAMPIERI L., BECHELLI G., CAVALLINI F., Prevalence of toxoplasmosis among domestic and wild animals in the area of Siena, Italy. A serologic and parasitologic study. Am. J. trop. Med. Hyg., 18: 391—394, 1969.

HARTCOURT R. A., Toxoplasmosis in rabbits. Vet. Rec., 81: 191—192, 1967.

HÜBNER J., UHLÍKOVÁ M., Use of the MPA method in the diagnostics of toxoplasmosis. I. J. H. E. M. I., 13: 514—520, 1969.

—, Use of the MPA method in the diagnostics of toxoplasmosis. II. J. H. E. M. I., 14: 499—506, 1970.

—, Use of the MPA method in the diagnostics of toxoplasmosis. III. J. H. E. M. I., 17: 70—84, 1973.

—, Mikromodifikace Sabin-Feldmanova dye testu pro serodiagnostiku toxoplasmosy. Čs. E. M. I., 1974 (in press).

—, Sexuální stadium prvoka *Toxoplasma gondii* a jeho role v epidemiologii a epizootiologii toxoplasmosy. J. H. E. M. I. 1974 (in press).

JACOBS L., Personal communication 1965 (in: Work K., Toxoplasmosis. Munksgaard Copenhagen, p. 17, 1971).

JANITSCHKE K., JÖRREN H. R., Untersuchungen über die Bedeutung der intrauterinen Übertragung von Toxoplasmen für die Verbreitung der Infektion bei Hauskaninchen. Z. Tropenmed. Parasit., 21: 246—251, 1970.

KUNSTÝR I., JÍRA J., PRINCOVÁ D., MÍKA J., Survey of *Toxoplasma* antibodies in domestic rabbits. Folia parasit. (Praha) 17: 277—280, 1970.

LALLA F. DE, BECHELLI G., CAVALLINI-SAMPIERI L., Osservazioni su un facolato enzootico di toxoplasmosi parassitologicamente dimostrata nei conigli dell'area di Siena. Clin. Vet. (Milano), 90: 134—148, 1967.

ROTH W., WERTHEMANN A., HESS R., Postnatale und intrauterine experimentelle Infektion des Kaninchens mit einem abgeschwächten Stamm von *Toxoplasma gondii*. Schweiz. Z. allg. Path. 20: 257—280, 1957.

UHLÍKOVÁ M., HÜBNER J., Sexual phase of *Toxoplasma gondii* development and its role in epidemiology and epizootiology of toxoplasmosis. J. Protozool. 18, Supplement, 45, 1971.

WIKTOR T. J., Toxoplasmosis animale. Sur une épidémie des lapins et des pigeons à Stanleyville (Congo Belge). Trop. Dis. Bull. 47: 889, 1950.

ZÁSTĚRA M. Personal communication, 1963

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