TABLE I. Cholesterol Esterase Activity in Serum of 3 Dogs Before and After Pancreatectomy. Enzyme digests were prepared as previously described(2). Substrate for esterification, cholesterol, 25 mg, oleic acid, 54.8 mg. pH of digests, 6.2. Bile salt solution, 1 cc 10% sodium taurocholate. Enzyme, 2 cc of dog serum.

No. of dog	Days after operation	Raw pancreas in diet	Esterification* 24 hr, %
1	Pre	—	39.1
	14	—	.9
	16	—	.7
	20	—	.0
	24	+	1.0
	28	+	1.0
2	Pre	—	17.2
	14	—	.7
	16	—	.0
	20	—	.5
	24	+	.3
	28	+	2.0
3	Pre	—	
	14	+	40.0
	16	+	1.0
	20	—	.8
	24	+	2.0
	28	+	.5

* The values obtained after the operation can be attributed to the limit of error of the method(2).

terol esterase in serum. Upon the removal of the pancreas, no activity could be detected. The feeding of raw pancreas did not produce any change, possibly because gastric acidity inactivated the enzyme(8).

Summary. Pancreas appears to be the sole source of dog serum cholesterol esterase.

1. Yamamoto, R. S., Goldstein, N. P., and Treadwell, C. R., *J. Biol. Chem.*, 1949, v180, 615.

2. Swell, L., and Treadwell, C. R., *J. Biol. Chem.*, 1950, v182, 479.

3. Swell, L., and Treadwell, C. R., *J. Biol. Chem.*, 1950, v185, 349.

4. Swell, L., Byron, J. E., and Treadwell, C. R., *J. Biol. Chem.*, 1950, v186, 543.

5. Sperry, W. M., *J. Biol. Chem.*, 1935, v111, 467.

6. Sperry, W. M., and Stoyanoff, V. A., 1937, v121, 101.

7. Markowitz, J., *Experimental Surgery*, 2nd Ed., Baltimore, Williams and Wilkins, 1949.

8. Swell, L., Cassidy, J. W., and Treadwell, C. R., *Fed. Proc.*, 1951, v10, 256.

Received December 16, 1952. P.S.E.B.M., 1953, v82.

Toxoplasmosis. II. Intra-Uterine Infection in Dogs, Premature Birth and Presence of Organisms in Milk.* (20065)

D. M. CHAMBERLAIN, F. L. DOCTON, AND C. R. COLE.
(Introduced by Albert B. Sabin.)

From the Department of Veterinary Pathology, College of Veterinary Medicine, The Ohio State University, Columbus.

Toxoplasmosis is known to be a congenital disease in the human infant. The disease has been studied in mice by Eichenwald(1), who described *in utero* infection and milk transmission. Our previous study of 2 toxoplasmosis epizootics in dogs in which pups were stillborn, born prematurely or died shortly after birth prompted this investigation. This report describes experimental congenital toxoplasmosis in dogs associated with premature birth and the presence of toxoplasma in the milk.

Materials and methods. Five healthy virgin bitches 1.5 to 2 years of age and a 3-year-old male dog were isolated for 3 weeks during which time they were treated for internal and external parasites and immunized against distemper. Diet consisted of commercially prepared cereal dog food supplemented by horse meat in a ratio of 3:1. Two preinoculation serological studies conducted prior to estrus according to the method of Sabin and Feldman(2) revealed no toxoplasma antibodies. Toxoplasma organisms of canine and human origin were used in the serological studies. The male and female were allowed to copulate daily throughout the course of the estrous cycle. Sterile caps, masks, gowns, gloves and boots were worn by all who entered the room.

* This investigation was supported in part by a research grant from the National Institutes of Health, Public Health Service.

TABLE I. Premature Birth Caused by Toxoplasma.

Dog No.	Day of gestation when inoculated	Inoculum	Toxoplasma origin	Duration of gestation,* days
227	52	2.25 ml perit. exud. I.V. 10 ml pooled brain, liver, spleen and lung emulsion subcut.	Human	58
877	51	1 ml perit. exud. I.V.	Canine	56
607	50	1.25 " " " I.P. 1.25 " " " orally	"	54
638	30	1 " " " I.V. 1 " " " I.P.	"	35
24	51	2 " " " I.V.	Porcine	56

* Normal gestation period for dogs is 61-63 days.
I.V. = intravenous; I.P. = intraperitoneal; Perit. exud. = toxoplasma infected mouse peritoneal exudate.

The room floor and cages were scrubbed daily with 5% cresol solution. The inocula consisted of peritoneal exudate and/or a pooled emulsion of brain, heart, lungs, liver, spleen, and kidneys from toxoplasma infected mice. All emulsions were prepared using 1 g of tissue in 5 ml of isotonic saline. Of the 5 bitches inoculated one received toxoplasma of human origin, 3 the parasite of canine origin, and one organisms isolated from swine(3). At parturition 2 pups and their respective placentae from each of 4 bitches were collected in sterile containers while the surviving litter mates were allowed to nurse their mothers. The mammary glands of the bitches were washed with soap and water, dried, bathed in 70% alcohol and milk samples withdrawn. Doses of 1 ml were inoculated intraperitoneally into each of 4 white Swiss mice. The pups aseptically collected at birth were necropsied and portions of the brain, thoracic and abdominal exudate, heart, lungs, liver, spleen, kidneys, and lymph nodes were obtained in a sterile manner. Each tissue was separately emulsified in isotonic saline using one part tissue to 5 parts saline. One to 3 ml of each organ emulsion was inoculated intraperitoneally into each of 3 mice. A total of at least 24 mice were used per newborn animal. The placentae were treated in a similar manner.

Results. Table I summarizes the stage of gestation when inoculated, type of inoculum, origin of toxoplasma, and duration of gestation.

Clinical signs in bitches. After an incubation period of 3 to 5 days all except one bitch manifested visible signs of disease. The bitch (No. 227) which received toxoplasma of human origin developed a sanguineous diarrhea on the third day after inoculation. This was immediately followed by a mucoid ocular discharge, serous nasal discharge, progressive sensitivity to lumbar palpation, extreme depression, anorexia and anemia. Although parturition extended over a period of 6 hours leaving the mother extremely weak, she gave birth to 6 live pups without assistance. Two of the newborn were collected aseptically and sacrificed as they emerged from the birth canal, 2 died within one hour after birth and 2 were allowed to live with their mother. After close observation for 4 days one of the 2 remaining pups became depressed, inactive, and remained separated from its mother and litter mate for long periods of time. These signs as well as distended abdomen, anorexia and periodic tenesmus were manifested until death on the sixth day. The bitch had ample milk and showed concern over her young at all times. The sixth or remaining pup showed similar signs on the seventh day and died at 9 days of age. Toxoplasma was isolated from 2 pups collected at the time of birth, from 2 pups living 6 and 9 days respectively, and from the mother's milk.

One of the 3 bitches (No. 607) inoculated with toxoplasma of canine origin was asymptomatic and gave birth to 8 apparently healthy pups. Even though the pups appeared healthy

TABLE II. Isolations of Toxoplasma by Mouse Inoculation.

Bitch No.	No. of pups in litter	No. of pups toxo isolated	Milk	Ovary of bitch	Fetal membranes and allantoic fluid
227	6	4	Pos.	S.L.	*
877	5	5	"	"	*
607	8	7	"	"	Neg.
638	6	5	*	Pos.	Pos.
24	3	2	Pos.	"	Neg.

* Not examined.
Pos. = positive; Neg. = negative; S.L. = still living.

at birth all 8 died of toxoplasmosis within 2.5 months. Two died at 12 days of age, one at 13 days, one at 14 days, one at 15 days, one at 63 days, one at 68 days, and the final pup died when 72 days of age. Since toxoplasma was isolated from 7 of 7 pups tested by mouse inoculation and no bacterial, viral or other disease could be incriminated, toxoplasmosis was considered to be the cause of death. Milk from the mother on the day of parturition contained toxoplasma.

Two bitches (Nos. 877 and 638) showed restlessness, vomiting, sanguineous diarrhea, depression, sensitivity to lumbar palpation and cough. Mastitis developed in one animal (No. 877) after she gave birth to 5 pups. Two of the pups were stillborn, 2 were collected aseptically and sacrificed at birth, and one died when 4 days of age. Toxoplasma was isolated from all 5 pups and from the milk of the mother. The second bitch (No. 638) aborted 3 pups after 35 days gestation, developed convulsions and died. At autopsy 3 unborn pups were found in her uterus. Toxoplasma was isolated from 2 aborted pups, 3 unborn pups, fetal membranes, allantoic fluid and from the ovary of the bitch.

The fifth bitch (No. 24) studied was inoculated with toxoplasma of porcine origin. Four days later she developed extreme respiratory distress and pulmonary edema followed by premature birth of 3 pups and death on the fifth day. Toxoplasma was isolated from 2 of the premature pups examined. Milk collected at the time of parturition, as well as her ovaries and mammary glands collected at autopsy yielded toxoplasma.

A summary in Table II shows that congenital infection occurred in 82% of the pups born of 5 mothers inoculated parenterally with toxoplasma. Eichenwald(1) found infection in 52% of young mice born and nursed by mothers which were infected by ingestion of toxoplasma-infected mouse liver.

Upon isolation of the organism from these infected animals its identity was proven by morphologic similarity to the toxoplasma inoculated, and its pathogenicity for mice. In addition the reisolated organism was proven to be antigenically identical to known toxoplasma when employed as the antigen in the Sabin-Feldman test. It also was capable of producing specific toxoplasma antibody.

Summary. 1. Toxoplasma of human, canine, and porcine origin was pathogenic for pregnant bitches and caused abortion or premature parturition. The organism was isolated from 23 of 28 pups born of 5 bitches. 2. *In utero* transmission of toxoplasmosis was demonstrated by isolation of toxoplasma from each of 8 pups (born of 4 bitches) which were collected aseptically and sacrificed at the time they emerged from the birth canal. Isolation of organisms from the placenta and allantoic fluid was further evidence of *in utero* transmission. 3. A mammary gland emulsion from one bitch and samples of milk collected aseptically from 4 lactating infected bitches each revealed toxoplasma.

1. Eichenwald, H., *Am. J. Dis. Child.*, 1948, v76, 307.

2. Sabin, A. B., and Feldman, H. A., *Science*, 1948, v108, 660.

3. Farrell, R. L., Docton, F. L., Chamberlain, D. M., Cole, C. R., *Am. J. Vet. Res.*, 1952, v13, 47.

Received December 22, 1952. P.S.E.B.M., 1953, v82.