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Dear Doctor Sabin:

Thank you for your careful reading of our manuscript and the excellent suggestions which you made for its improvement. I have made the changes indicated in your letter and have forwarded the manuscript to Doctor Goldforb. In keeping with the length of papers usually published in the Proceedings of the Society for Experimental Biology and Medicine we have made the introduction, literature references and discussion as short as possible.

Best wishes for a happy new year!

Sincerely yours,

Clarence R. Cole, D.V.M.
TOXOPLASMOsis

II. INTRA-UTERINE INFECTION IN DOGS, PREMATURE BIRTH AND PRESENCE OF ORGANISMS IN MILK

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Toxoplasmosis is known to be a congenital disease in the human infant. The disease has been studied in mice by Eichenwald described in utero infection and milk transmission. Our previous study of two epizootics in dogs in which pups were stillborn, born prematurely or died shortly after birth with proven toxoplasmosis 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 selected for this study. After an observation period of 3 weeks during which time they were treated for internal and external parasites and immunized against distemper, they were placed in an isolation room. A ration

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This investigation was supported in part by a research grant E-319 from the National Institutes of Health, Public Health Service.
consisting of commercially prepared cereal dog food supplemented by horse meat in a ratio of 3:1 was fed. 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 estrus cycle.

Sterile caps, masks, gowns, gloves and boots were worn by all who entered the room. Upon leaving the room all apparel, except boots which were scrubbed in 5 percent cresol solution, was placed in a cloth bag and autoclaved at 15 pounds pressure for 20 minutes. The room floor and cages were scrubbed daily with 5 percent cresol solution.

The inoculum consisted of toxoplasma-infected mouse 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 gm of tissue in 5 ml of isotonic saline. Of the 5 bitches inoculated 1 received toxoplasma of human origin, 3 the parasite of canine origin and 1 toxoplasma isolated from swine.\(^3\) At parturition 2 pups and their respective placentae from each of 4 bitches were collected in sterile

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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 percent alcohol and allowed to
dry by evaporation. A sterile breast pump was then employed to
withdraw milk samples. Doses of 1 ml were inoculated intrain-
peritoneally into each of 4 white Swiss mice. The pups aseptic-
ically collected at birth were necropsied and portions of the
brain, thoracic and abdominal exudate, heart, lungs, liver,
spoon, kidneys and lymph nodes were obtained in a sterile
manner. Each tissue was separately emulsified in isotonic
saline using 1 part tissue to 5 parts saline. One to 3 ml of
each organ emulsion was inoculated intrainperitoneally 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 gesta-
tion. The signs observed in the inoculated bitches, incidence
of in utero transfer, dissemination of toxoplasma through the
milk, as well as the signs and gross lesions in the pups are
described.

After an incubation period of 3 to 5 days all except 1
bitch manifested visible signs of disease. The bitch (No. 227)
which received toxoplasma of human origin developed a
TABLE I

PREMATURE BIRTH CAUSED BY TOXOPLASMA

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Day of Gestation When Inoculated</th>
<th>Inoculum</th>
<th>Toxoplasma Origin</th>
<th>Duration of Gestation*</th>
</tr>
</thead>
<tbody>
<tr>
<td>227</td>
<td>52</td>
<td>2.25 ml perit. exud. I.V. 10 ml pooled brain, liver, spleen and lung emulsion subcutaneously</td>
<td>Human</td>
<td>58 days</td>
</tr>
<tr>
<td>877</td>
<td>51</td>
<td>1 ml perit. exud. I.V.</td>
<td>Canine</td>
<td>56 days</td>
</tr>
<tr>
<td>607</td>
<td>50</td>
<td>1.25 ml perit. exud. I.P. 1.25 ml perit. exud. orally</td>
<td>Canine</td>
<td>54 days</td>
</tr>
<tr>
<td>638</td>
<td>30</td>
<td>1 ml perit. exud. I.V. 1 ml perit. exud. I.P.</td>
<td>Canine</td>
<td>35 days (aborted and died)</td>
</tr>
<tr>
<td>24</td>
<td>51</td>
<td>2 ml perit. exud. I.V.</td>
<td>Porcine</td>
<td>56 days</td>
</tr>
</tbody>
</table>

I.V. - intravenously
I.P. - intraperitoneally
Perit. Exud. - Toxoplasma infected mouse peritoneal exudate

*Normal gestation period for dogs is 61 - 63 days
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 1 hour after birth and 2 were allowed to live with their mother. After close observation for 4 days 1 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 6 apparently healthy pups. Even though the pups appeared healthy at birth all 6 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 66 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 1 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 percent of the pups born of 5 mothers inoculated parenterally with toxoplasma. Eichenwald¹ found infection in 52 percent 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
<table>
<thead>
<tr>
<th>Bitch Case No.</th>
<th>No. of Pups in Litter</th>
<th>No. of Pups Teto Isolated</th>
<th>Milk</th>
<th>Ovary of Bitch Still living</th>
<th>Fetal Membranes and Allantoic Fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>227</td>
<td>6</td>
<td>4</td>
<td>Positive</td>
<td>Still living</td>
<td>Not examined</td>
</tr>
<tr>
<td>577</td>
<td>5</td>
<td>5</td>
<td>Positive</td>
<td>Still living</td>
<td>Not examined</td>
</tr>
<tr>
<td>607</td>
<td>8</td>
<td>7</td>
<td>Positive</td>
<td>Still living</td>
<td>Negative</td>
</tr>
<tr>
<td>638</td>
<td>6</td>
<td>5</td>
<td>Not examined</td>
<td>Bitch died</td>
<td>Positive</td>
</tr>
<tr>
<td>24</td>
<td>3</td>
<td>2</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
</tr>
</tbody>
</table>
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.

**Clinical signs in pups:**

All pups born of the 5 bitches, other than those sacrificed or dead at birth, died after a course less than 2.5 months. Signs observed in the pups ranging from 1 week to 2.5 months of age were similar in nature. These signs included anorexia, depression, fever up to 104°, diarrhea, serous to mucoid ocular and nasal discharge, distended abdomen and respiratory distress. The younger pups often exhibited extreme tonic muscular spasms, opisthotonus with rigidity of spine and limbs, orthotonus, whining and tenesmus. Terminally, the body temperature dropped to a subnormal level.

**Autopsy findings:**

Prominent autopsy findings consisted of hepatosplenomegaly, gray foetid throughout the liver, focal myocarditis, nephritis, lymphadenitis and pneumonia. Microscopically, toxoplasma were seen in lesions of the brain, heart, lungs, spleen, liver, kidney, stomach, intestine and pancreas.

The finding of toxoplasma in lesions of pups at the time of birth indicates not only passage of the organism from
the infected mother to her unborn pups but also demonstrates in utero infection.

**SUMMARY:**

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 with experimentally induced toxoplasmosis. Ten of the 28 pups were stillborn and 3 were unborn.

*In utero* transmission of toxoplasmosis was demonstrated by isolation of toxoplasma from each of 6 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.

A mammary gland emulsion from one bitch and samples of milk collected aseptically from 4 lactating infected bitches each revealed toxoplasma. Thus dissemination of the agent via the milk of infected animals suggests further studies on actual milk transmission of the disease.

The interval between inoculation of the 5 pregnant mothers and premature birth of the pups was 4 to 6 days. The gestation period terminated in 35 to 58 days. In no instance did it reach the normal average duration of pregnancy of 62 days for the canine species.
### TABLE III

**COURSE OF DISEASE IN PUPS FROM TOXOPLASMA-INFECTED MOTHERS**

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>227</td>
<td>-</td>
<td>2</td>
<td>2</td>
<td>6, 9</td>
<td>6</td>
</tr>
<tr>
<td>877</td>
<td>-</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>607</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>12, 12, 13, 14</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-</td>
<td>15, 63, 68, 72</td>
<td></td>
</tr>
<tr>
<td>636</td>
<td>3</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>24</td>
<td>-</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
</tbody>
</table>

*Days of age attained by each surviving pup.*
Eight premature pups born of a totally asymptomatic mother succumbed to toxoplasmosis between 12 and 72 days of age. Mortality of 100 percent occurred in the pups (except for those sacrificed at birth); the course in no instance exceeded 2.5 months.

Toxoplasma was isolated from the ovaries of 2 infected bitches. This suggested a study now in progress on the significance of toxoplasma infection in unexplained infertility. Clinical signs in congenitally infected pups included anorexia, depression, fever up to 103°F, diarrhea, ocular and nasal discharge, tonic muscular spasms, opisthotonus, orthotonus, whining and tachycardus.

Prominent autopsy findings in pups infected in utero consisted of hepatosplenomegaly, gray foci in the liver, focal myocarditis, nephritis and pneumonia. Microscopically, toxoplasma were seen in lesions of the brain, heart, lungs, spleen, liver, kidneys, stomach, intestines and pancreas.