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Experimental Infection of a Bison with *Toxoplasma gondii* Oocysts

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The ingestion of meat infected with tissue cysts of *Toxoplasma gondii* is one of the two major sources of infection for humans. Encysted *T. gondii* has been found in sheep, goats, pigs, chickens, moose, and mule deer killed for human consumption in the United States (Dubey, 1977, *In Parasitic Protozoa*, Kreier (ed.), Academic Press, New York, pp. 101-237). The objective of this report was to study the distribution of *T. gondii* cysts in the tissues of an experimentally inoculated bison (*Bison bison*).

A 1-day-old bison was removed from his dam at the National Bison Range, Moiese, Montana, and housed in isolation at the Veterinary Research Laboratory (VRL), Bozeman, Montana, until necropsy. The bison was part of a study on sarcocystosis and had been fed 10⁵ sporocysts of *Sarcocystis cruzi* at 7 days of age (Dubey, 1982, *J. Am. Vet. Med. Assoc.* 181: 1272-1274). The same bison was fed 10⁴ infective oocysts of the GT-1 strain of *T. gondii* (Dubey, 1980, *Am. J. Vet. Res.* 41: 427-429) when 55 days old. The number of infective oocysts in the inoculum was determined by inoculating 10-fold dilutions of oocysts into mice, 4 wk prior to inoculating the bison (Dubey, 1980, op. cit.). Rectal temperatures of the bison were taken every day from the day of inoculation of *Toxoplasma* until 17 days postinoculation (DPI).

The bison was killed 28 days after inoculation with *T. gondii*, exsanguinated, and necropsied. Portions (50 g or whole organ) of lung, heart, spleen, diaphragm, limb muscles, tongue, kidneys, small intestines, mesenteric lymph nodes, prescapular lymph nodes, brain, spinal cord, retina and choroid from one eye, and testes

were each homogenized in an acid-pepsin solution, incubated for 90 min at 37 C to kill tachyzoites, washed by centrifugation, and suspended in an antibiotic saline solution. Washed homogenates of individual tissues were inoculated subcutaneously into mice, six mice per tissue (for procedural details see Dubey, 1980, *J. Am. Vet. Med. Assoc.* 177: 1203-1207). Six ml of blood from the jugular vein of the calf were collected in vacuum tubes containing ethylenediaminetetraacetic acid and were inoculated subcutaneously into six mice, 1 ml into each mouse. In addition, six 50-g portions of liver digested in the acid-pepsin solution were inoculated subcutaneously into each of 36 mice as described previously (Dubey, 1980, op. cit.).

In addition to mouse inoculation, portions (1 kg or whole organ) of tongue, heart, kidneys, limb muscles, diaphragm, and brain were chopped and fed to seven *Toxoplasma*-free cats over a period of 2-7 days, one cat for each tissue. The cats were 3-4 mo old and came from the *Toxoplasma*-free cat colony maintained in VRL. The cats had never eaten meat before the experiment and had no detectable serum antibody to *T. gondii* as determined by the Sabin-Feldman dye test (Sabin and Feldman, 1948, *Science* 108: 660-663). The feces of the cats were examined daily for *Toxoplasma*-like oocysts for 3 wk after ingesting the bison tissue. The identity of *Toxoplasma* oocysts was determined by mouse inoculation.

The mice were examined for *Toxoplasma* infection. Impression smears of lungs and brains of mice that died were examined microscopically after staining with Giemsa's stain. Survivors were exsanguinated 21 DPI and their serum samples were examined for antibody to *T. gondii* in the Sabin-Feldman dye test.

The cat fed bison liver shed *T. gondii* oocysts 5-15 days later. The oocysts from the feline feces were infectious to mice and the tachyzoites were maintained by mouse to mouse pas-

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sage. None of the other six cats fed other bison tissues shed *T. gondii* oocysts. None of the mice inoculated with the bison tissues became infected with *T. gondii*.

Results of this preliminary study showed that bison, like ox (Fayer and Frenkel, 1979, *J. Parasitol.* 65: 756–762), may be resistant to *Toxoplasma* infection or can eliminate *T. gondii* from most of their tissues. The persistence of *T. gondii* cysts in the liver is similar to that in

goats inoculated with the GT-1 strain of *T. gondii* (Dubey, 1980, *op. cit.*).

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***Toxoplasma gondii* Infection in Rodents and Insectivores from Montana**

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Felidae, the reservoir host of *Toxoplasma gondii*, are postulated to become infected in nature by ingesting tissues of small mammals and birds infected with the tissue cysts of *T. gondii*. Limited information is available regarding the prevalence of *T. gondii* in small mammals in the United States (Gibson and Eyles, 1957, *Am. J. Trop. Med. Hyg.* 6: 990–1000; Wallace, 1973, *Am. J. Trop. Med. Hyg.* 22: 456–464; Dubey et al., 1981, *Am. J. Vet. Res.* 42: 1007–1010). The objective of this report was to determine the prevalence of *T. gondii* in rodents and insectivores around Bozeman, Montana.

From April 1979 to February 1982 tissues were collected from 500 Richardson's ground squirrels (*Spermophilus richardsoni*), locally called gophers), 99 deer mice (*Peromyscus maniculatus*), 84 muskrats (*Ondatra zibethicus*), 52 meadow voles (*Microtus pennsylvanicus*), 27 beavers (*Castor canadensis*), six long-tailed voles (*Microtus longicaudus*), four red-backed voles (*Clethrionomys gapperi*), 13

house mice (*Mus musculus*), four Rocky Mountain jumping mice (*Zapus princeps*), three masked shrews (*Sorex cinereus*), four water shrews (*Sorex palustris*), five vagrant shrews (*Sorex vagrans*), and three yellow pine chipmunks (*Eutamias amoenus*). Animals other than gophers were trapped alive or were found dead in traps around Bozeman. Most gophers were shot in open range in June and July 1979. Animals were killed with ether and necropsied.

Samples of skeletal muscles, heart, brain, and spleen (total 5–10 g) of each animal (except beavers) were pooled and ground in a pestle with a mortar using about 5 volumes of 0.9% NaCl solution (saline). The homogenate from each animal was strained through gauze, and 1 ml (about 1/100 of pooled tissues) was mixed with 1 ml of saline containing 2,000 units of penicillin and 200 µg of streptomycin (antibiotic saline); 1 ml of the mixture was inoculated subcutaneously into each of two mice. Thus, 1,554 mice were inoculated with tissues from 777 animals, excluding beavers. The tissues of the beavers were digested in an acid-pepsin solution (Sharma and Dubey, 1981, *Am. J. Vet. Res.* 42: 128–130) before inoculation into mice. For this, samples of brain, heart, and skeletal muscle (total 50 g) were pooled for each beaver, homogenized in a blender, digested in

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