Susceptibility of Common Voles to Experimental Toxoplasmosis

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ABSTRACT: Common voles (*Microtus arvalis*) in groups of nine to 10 animals were inoculated per os with a dose of 1, 10, 1×10^2, 1×10^3, and 1×10^4 oocysts of the K1 strain of *Toxoplasma gondii.* All the common voles inoculated with 1 to 1×10^3 oocysts remained subclinical and survived. Three of the 10 voles inoculated with 1×10^4 oocysts died between days 7 and 12 post inoculation (p.i.). Antibodies were demonstrated in all the infected voles killed on day 60 p.i. The highest antibody titres in voles detected by the dye test (DT) and latex agglutination test (LAT) were 1,024 and 1,280, respectively.

Key words: Experimental infections, pathology, rodents, serology, *Toxoplasma gondii.*

Toxoplasma gondii parasitizes birds and mammals worldwide (Dubey and Beattie, 1988). The common vole (*Microtus arvalis*), a small rodent, is one of the most abundant mammalian species in agrocenoses of central Europe (Zejda, 1996). Little attention has been given to the host-parasite interaction between *T. gondii* and the common vole. With the complement-fixation test, antibodies to *T. gondii* were found in 6% of common voles in Slovakia (Čatár, 1972). In the Czech Republic, *T. gondii* recently has been isolated in 1% of common voles (Hejlíček et al., 1997). Clinical toxoplasmosis has not been described among free-ranging common voles. Starzyk et al. (1970) experimentally (intraperitoneally, subcutaneously and intranasally) infected common voles with *T. gondii* tachyzoites of a "virulent" GM strain and found that common voles were more resistant to parenteral infection than laboratory mice. However, "avirulent" *T. gondii* strains seem to be more frequent (Literák et al., 1998), in wild rodents and the main *T. gondii* transmission route is ingestion of animal tissues containing tissue cysts, or ingestion of food or water contaminated with oocysts from cat feces (Dubey and Beattie, 1988). The aim of the present

study was to determine the degree of susceptibility of common voles to experimental oral infection with *T. gondii* oocysts.

The common voles used in experimental infections came from a breeding station (Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Brno, Czech Republic). They were descendants of the sixth to the eighth generations of voles bred in captivity. Voles were placed in groups in facilities for laboratory rodents. They were fed a commercial complete feed for laboratory mice, and given drinking water ad libitum. Adult voles were inoculated orally with *T. gondii* oocyst suspension (0.5 ml inoculum) in phosphate buffered saline (PBS, 7.2 pH) using a gastric tube and diethyl ether as an anaesthetic. Forty nine adult voles were divided into five groups of 10, 10, 10, 9, and 10 and were inoculated with a dose of 1, 10, 1×10^2, 1×10^3, and 1×10^4 oocysts, respectively. Another 20 uninfected voles randomly selected from the same breeding station were killed and serologically tested for antibodies to *T. gondii.* After the inoculation, animals were inspected daily for signs of the disease. The animals that survived acute toxoplasmosis were killed on day 60 post inoculation (p.i.).

The K1 strain of *T. gondii* used in the experimental infection was isolated in 1995 from a latently infected dog and, based on a genotype characteristics, classified as a strain from the clonal line of the so-called avirulent strains (Literák et al., 1998). The method used to obtain oocysts has been described elsewhere (Dubey and Beattie, 1988). Briefly, oocysts were obtained from the feces of experimentally inoculated cat, sporulated in 2% sulphuric acid and stored for 3 mo at 4 C before they were used in the experiment. Before the

voles and mice were inoculated, the oocyst suspension had been neutralized with 3.3% NaOH.

The Sabin Feldman dye test (DT) and the latex agglutination test (LAT) were used to examine the serum of the voles. The DT was performed in a modification described earlier (Literák and Hejlíček, 1993); the sera were diluted in a twofold series starting from the basic dilution of 1: 4, with titres ≥4 being considered positive. The LAT (Sanofi Diagnostics, Pasteur, France) was made according to the manufacturer's instructions. The testing sera were diluted in a twofold series starting from the basic dilution of 1:10, with titres ≥20 being considered positive. The peritoneal exudate of voles that died of acute toxoplasmosis was examined microscopically for the presence of *T. gondii* tachyzoites (Dubey and Beattie, 1988).

The voles that died or that were killed at the end of the experiment were necropsied. Portions of the brain, cerebellum, heart, skeletal muscles, liver, lung, spleen, kidneys, small and large intestines, and gonads were fixed in 10% neutral buffered formalin. Paraffin-embedded sections were cut at 5 μm thickness, stained with hematoxilin and eosin, and examined microscopically.

All 20 uninoculated voles were serologically negative both in the DT and the LAT.

Three of the voles inoculated with 10^4 oocysts died on seven, seven, and 12 days p.i., respectively. No clinical signs of the disease were observed in other experimentally inoculated voles.

On day 60 p.i., the occurrence of specific antibodies was proof of a successful infection with *T. gondii* oocyst in 39 serologically tested voles (of a total of 49 experimentally infected). In six voles inoculated with one oocyst and in one vole inoculated with 10 oocysts and sacrificed on day 60 p.i., *T. gondii* infection was not demonstrated either serologically or by any of the other methods used. Three voles killed on day 60 p.i. were not bled

TABLE 1.   Serological response of common voles on day 60 p.i. of *Toxoplasma gondii* oocysts.

Number of oocysts	DT[a]	LAT[b]
1	40[c] (3)[d]	320 (4)
10	23 (4)	235 (9)
10^2	52 (7)	422 (10)
10^3	81 (6)	403 (9)
10^4	323 (6)	707 (7)

[a] Dye test.
[b] Latex agglutination test.
[c] Geometric mean of titers.
[d] Number of seropositive animals.

and serologically tested. The seroprevalence as well as titre values, however, differed according to the serological tests used for the antibody detection (Table 1). The overall seroprevalence ascertained by the DT was 74% (26 positive/35 tested). In the voles infected with a dose of 1, 10, 1×10^2, 1×10^3, and 1×10^4 oocysts, the seroprevalence levels ascertained by the DT were 75%, 50%, 78%, 75% and 100%, respectively. The highest antibody titre detected by the DT (1,024) was found in two voles infected with 1×10^3 and in one vole inoculated with 1×10^4 oocysts. In the LAT, seroprevalence was 100% (39 positive/39 tested). The highest antibody titre detected by the LAT (1,280) was found in one, three, two and four voles infected with a dose of 1, 1×10^2, 1×10^3 and 1×10^4 oocysts, respectively.

Necropsy of three voles which died between days seven and 12 p.i. had no marked gross pathological changes of their organs. In all three voles, histopathological changes were demonstrated in the liver, small intestines, mesenteric lymph nodes, lungs, spleen, and brain. The liver had a marked periportal mixed infiltration, infiltration of sinusoids, foci of necrosis and cholestasis, and *T. gondii* tachyzoite clusters were found in the liver parenchyma. Other findings included edema and hyperemia in the small intestines, edema, hyperemia and hemorrhage-necrotic inflammation in mesenteric lymph nodes, acute catarrhal bronchopneumonia in the lungs

and foci of necrosis in the spleen. The brain contained tachyzoite clusters without cellular reaction. In one vole that died, myositis was found. In one half of the voles inoculated with higher doses (1×10^3 or 1×10^4) of oocysts and sacrificed on day 60 p.i. CNS findings included submeningeal infiltrations, tiny granulomas, glia nodes, and numerous tissue cysts in the cerebrum but not in the cerebellum. In the remaining voles, only tissue cysts, both in the grey and the white matter, were found in brain.

Toxoplasmosis does not represent an immediate health risk to common voles and their mortality due to acute toxoplasmosis is low even at higher levels of infection doses of oocysts. The virulence of the K1 for laboratory mice was higher. None of 10, 8 of 10, and 8 of 10 CD1 mice infected orally by 1×10^2, 1×10^3, and 1×10^4 oocysts of K1 strain, respectively, died (Sedlák and Literák, unpubl. observations). It seems that the susceptibility to toxoplasmosis is lower in common voles compared to laboratory mice.

A higher resistance to *T. gondii* infections in the voles compared with the mice was also found in experimental intraperitoneal, subcutaneous and intranasal applications of *T. gondii* tachyzoites of the GM strain (Starzyk et al., 1970). The infection intensity was classified according to the number of tachyzoites in impression preparations of different organs, and of peritoneal exudate. In all three types of parenteral application, mice were found to have higher numbers of tachyzoites and a higher sensitivity to infection than voles.

Histological examinations of the livers of the voles that died consistently showed marked periportal infiltrations and infiltration in the sinusoids. In laboratory mice, however, necrosis of vessels and of lamina propria cells appears to be the main lesion leading to fatal enteritis in animals fed oocysts, the intestinal lesions heal rapidly if the animal recovers from the enteritis, pneumonitis is the main lesion during the second and third weeks after oocysts ingestion (Dubey et al., 1997).

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