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TOXOPLASMOSIS IN RABBITS CONFIRMED BY STRAIN ISOLATION: A POTENTIAL RISK OF INFECTION AMONG AGRICULTURAL WORKERS

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Abstract: The presence of anti-*Toxoplasma gondii* IgG antibodies in a high titer of 1:8,000 was found in 2 out of 9 examined rabbits (22.2%), kept on a farm where cases of human toxoplasmosis were noted. A virulent *Toxoplasma gondii* strain was isolated in mice from the brain of a seropositive rabbit that showed clinical signs of disease: apathy, weight loss, skin lesions. The presence of *T. gondii* DNA in the peritoneal exudate of mice inoculated with the brain suspension of the dissected rabbit was confirmed by the positive results of PCR test. No toxoplasmas were found in mice inoculated with suspensions of other organs of the examined rabbit (liver, spleen, kidneys, lungs, heart, skeletal muscles). In the sections of the rabbit's brain the typical cysts of *Toxoplasma gondii* were found, filled with bradyzoites. On the basis of strain isolation and microscopic findings, toxoplasmosis of the central nervous system was diagnosed in the rabbit. The results of this study suggest that rabbits should be considered as a potential source of *Toxoplasma* infection among agricultural workers.

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INTRODUCTION

Toxoplasmosis in domestic rabbit (*Oryctolagus cuniculus* L.) was first described by Splendore in Brazil as early as in 1908 [18] and since then clinical cases of this disease among rabbits have been reported by many authors in various countries [2, 6, 13, 14, 16]. Serological response of rabbits to *Toxoplasma* antigen is variable, from 2.4–8.3% in China [15, 22] to 53% in Germany [21] and 48.4–57.9% in the Czech Republic [17].

The role of domestic rabbit in epidemiology of toxoplasmosis in humans has not been established in detail, but is probably important. Although some authors treat this role marginally [5], others place the rabbit among the animal species posing a major source of infection for man [3, 8, 12, 17]. Ishikawa *et al.* [9] described the case of cervical toxoplasmosis transmitted from rabbit to man. Beverley *et al.* [3] found very high titers of anti-*Toxoplasma* antibodies in rabbit hunters. Nevertheless, there is a lack of controlled epidemiological studies on the degree of a correlation between the prevalence of toxoplasmosis in rabbits and in humans having contacts with these animals [10].

Continuing a study of the possible animal sources of infection on an endemic area of toxoplamosis in Chełm district (eastern Poland) [19], we also examined a rabbit flock on a farm where family-environmental toxoplasmosis was diagnosed among farm inhabitants. In the course of

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this examination we found a case of clinical toxoplasmosis in rabbit confirmed by isolation of strain, as described below in detail.

MATERIALS AND METHODS

Examined rabbits. Blood serum samples from 9 mixed breed rabbits of both sexes (5 males and 4 females) aged 1–3 years were examined for the presence of anti-*Toxoplasma* antibodies. The rabbits were housed in a shed and fed with vegetables and grass grown on the farm located in Sobibór area of Chełm district (eastern Poland). Positive serological response to *Toxoplasma* antigen was found in 3 out of 4 farm inhabitants. All seropositive persons experienced during the past 2 years cervical symptoms corresponding to lymphonodular form of toxoplasmosis. Two out of 3 cats kept on the farm showed positive serological reactions with *T. gondii* antigen.

Serological examination. Rabbits' sera were examined by the direct agglutination test with modifications suggested by Desmonts and Remington to detect antibodies in IgG class [4]. Sera were treated with 2-mercaptoethanol to reduce IgM antibodies and then incubated with formalin-treated *Toxoplasma* antigen, prepared in the Department of Occupational Biohazards of the Institute of Agricultural Medicine, as described earlier [19]. Antigen was added to examined sera and the test was incubated for 24 hrs at 37ºC. Agglutination of parasites took place if the serum contained antibodies.

Isolation of strain. An attempt was made to isolate a *Toxoplasma gondii* strain from 1 rabbit showing strong serological response and clinical signs of infection. This was a 1-year old mixed breed male. The rabbit was killed by intraperitoneal injection of sodium pentabarbiton and dissected. Five gram portions of liver, spleen, kidneys, lungs, heart, skeletal muscles and brain were excised and each blended in 5 volumes of 0.9% NaCl in a blender for disruption of tissue. Then, the homogenate of brain was inoculated intraperitoneally into 4 female Swiss mice in the volume of 1 ml per mouse, while the homogenates of other organs were digested in pepsin according to recommendations by Dubey and Beattie [5]. To 30 ml of each homogenate were added: 25 ml of digestion fluid (pepsin with biological activity of 1:10000, 2.6 g; NaCl, 5.0 g; HCl, 7.0 ml; and distilled water to make 500 ml of solution). The homogenates were incubated at 37ºC for 90 min in a shaker, then filtered through gauze and centrifuged at 3,000 rev./min for 10 min. The supernatants were poured off while sediments resuspended in 5 ml of 0.9% NaCl. The suspensions were centrifuged as above and sediments were resuspended in antibiotic saline solution, each in 5 ml of 0.9% NaCl containing 1,000 penicillin units and 100 mg streptomycin per 1 ml. Each of the antibiotic saline solutions of digested homogenates of rabbit organs was inoculated intraperitoneally into 4 female Swiss mice, in the volume of 1 ml per mouse.

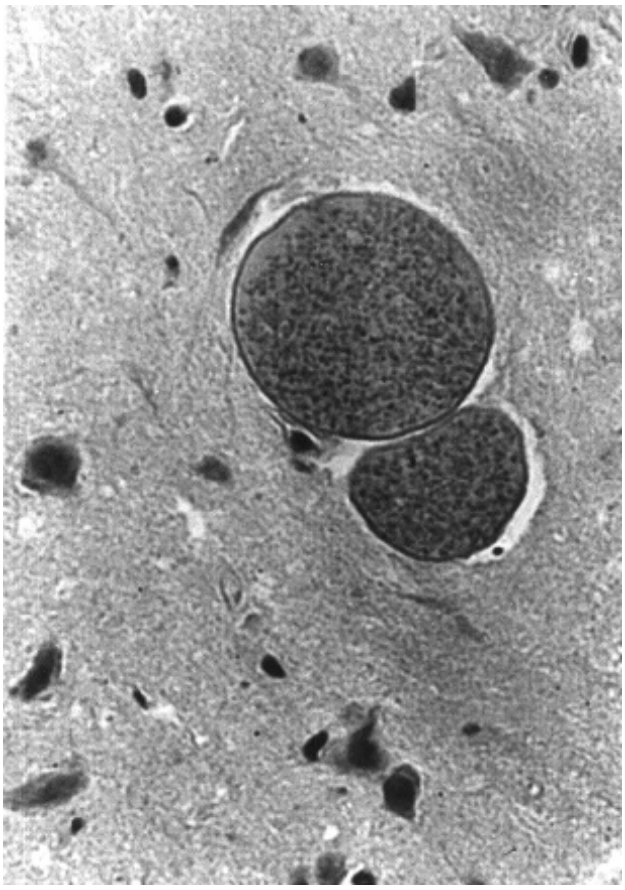

Figure 1. Two cysts of *Toxoplasma gondii* in the brain section of spontaneously infected rabbit. H+E, × 670.

After 5 days post inoculation, randomly selected mice of each group (one mouse per group) were dissected and the smear preparations from peritoneal exudate, liver, lungs and brain were stained with Giemsa and checked for the presence of *Toxoplasma gondii* tachyzoites. Besides, the mice were observed each day for appearance of symptoms. Symptomatic mice were dissected and checked for the presence of *T. gondii*. After 6 weeks post inoculation, the remaining mice were dissected and the smear preparations from brain suspension in 0.9% NaCl were stained with Giemsa and checked for the presence of *Toxoplasma gondii* cysts containing bradyzoites. The mixed suspensions of brain, liver, spleen, heart and lungs from the dissected mice were inoculated into the next group of mice using the technique as above. The mice of this passage were checked after 5 days and 6 weeks for the presence of *T. gondii*.

Histological examinations. The portions of brain, liver, spleen, lung, heart and kidney excised from the rabbit dissected for strain isolation and from mice inoculated with rabbit's organ were examined with histological techniques. All organs were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned 6 µm thick, and stained with hematoxylin and eosin (H+E).

Polymerase chain reaction (PCR). PCR test was used for confirmation of the presence of *Toxoplasma gondii* DNA in the peritoneal exudate of 4 mice inoculated with the brain suspension of the dissected rabbit. Isolation and amplification of *T. gondii* DNA was performed using a PCR kit obtained from DNA-GDAŃSK II s.c. (Gdańsk, Poland). Detection of *T. gondii* DNA was based on amplification of gene fragment coding 65 kDa antigen protein in 2 subsequent reactions with the same pair of primers. The size of the amplified fragment was 262 base pairs.

RESULTS

The presence of anti-*Toxoplasma gondii* antibodies of IgG class was found in 2 out of 9 examined rabbits (22.2%), in a high titer of 1:8,000. One of the seropositive rabbits showed clinical signs of disease: apathy, weight loss, skin lesions. Using the procedure described above, a virulent *Toxoplasma gondii* strain was isolated in mice from the brain of this rabbit. After 13 days post inoculation, signs of slight apathy were noticed in 2 out of 4 mice originally inoculated with the brain suspension of the rabbit. The mice were dissected and the presence of rare *Toxoplasma gondii* tachyzoites was noted, in their peritoneal exudate and liver. In the course of subsequent passages by injecting the exudate to healthy mice at the constant dose of 1 ml per mouse, the proliferation of parasites occurred, shown by earlier appearance of signs of disease: at 10 days post inoculation at the second passage and at 6 days post inoculation at the third and later passages. The *T. gondii* tachyzoites were more abundant in organs and exudate of infected mice; most of them occurred intracellularly. After the third passage, the concentration of toxoplasmas in peritoneal exudate maintained at the steady level of 10^4/ml. The presence of *T. gondii* DNA in the exudate of mice inoculated with the brain suspension of the dissected rabbit was confirmed by the positive results of PCR test. No toxoplasmas were found in mice inoculated with suspensions of other organs of the examined rabbit (liver, spleen, kidneys, lungs, heart, skeletal muscles).

In the sections of the rabbit's brain the typical cysts of *Toxoplasma gondii* were found, filled with bradyzoites (Fig. 1). The cysts measured on the average 25.4–51.2 µm. Neither cysts nor other forms of *Toxoplasma gondii* were found in the other organs of the rabbit. On the basis of strain isolation and microscopic findings, toxoplasmosis of the central nervous system was diagnosed in the rabbit.

DISCUSSION

The described case of clinical toxoplasmosis in rabbit is noteworthy because of the localization of parasites only in the brain since until recently the presence of toxoplasmas and pathologic lesions were reported mostly in the spleen, liver, heart and lung of rabbits [2, 6, 7, 13]. Kapperud [11] found no *Toxoplasma* cysts in histological examination of brain tissue from 51 wild rabbits. The present work confirms the possibility of spontaneous development of *T. gondii* in rabbits' brain that was first described by Levaditi *et al.* [14] and further evidenced by the isolation of *Toxoplasma* strains by Lainson [12] and Werner [21]. Tośk-Luty [20] has demonstrated proliferation of parasites in the brains of rabbits experimentally infected with two strains of *T. gondii*.

In spite of numerous descriptions of clinical toxoplasmosis in rabbits, *Toxoplasma* strains have seldom been isolated from these animals, to the best of our knowledge in less than 10 countries. The isolations of rabbit strains of *T. gondii* have been reported from the United Kingdom [12], Germany [21], and Bulgaria [1]. This paper presents the first isolation of *Toxoplasma gondii* strain from rabbit in Poland.

A possibility that infected rabbits might be a source of toxoplasmosis stated in farm inhabitants cannot be excluded, but is uncertain, the more so as seropositive cats lived on the same farm. Thus, a probability of the transmission of disease from rabbit to human is less in our case compared to the case described by Ishikawa *et al.* [9].

A limitation of this preliminary study is the small number of examined animals which does not allow the drawing of any firm conclusions. Nevertheless, the obtained results suggest that rabbits should be considered as a potential source of *Toxoplasma* infection among agricultural workers.

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