

Seroprevalence of *Encephalitozoon cuniculi* in Pet Rabbits in Korea

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Abstract: *Encephalitozoon cuniculi* is a microsporidian parasite commonly found in rabbits that can infect humans, causing encephalitozoonosis. The prevalence of encephalitozoonosis is not well documented, even when many clinics suspect pet rabbits as being highly infected. This study investigated the seropositivity of *E. cuniculi* using ELISA. The examination of 186 rabbits using ELISA showed that 22.6% (42/186) were seropositive against *E. cuniculi*. In analysis with healthy status, all 42 seropositive sera were collected from clinically normal rabbits. Moreover, the gender and age of pet rabbits did not have any significant effect on *E. cuniculi* infection. To the best of our knowledge, this is the first report to describe the seroprevalence of *E. cuniculi* in pet rabbits and suggests that pet rabbits could act as an important reservoir of encephalitozoonosis for both pet animals and humans in Korea.

Key words: *Encephalitozoon cuniculi*, pet rabbit, seroprevalence

The intracellular microsporidium *Encephalitozoon cuniculi* is a single-celled, spore-forming, obligate intracellular parasite that infects a wide range of vertebrate animals, including rabbits, mice, dogs, cats, goats, pigs, and horses [1-3]. *E. cuniculi* is also a zoonotic and opportunistic pathogen in human patients with acquired immunodeficiency syndrome (AIDS) or in immunocompromised patients [2].

The rabbit is known as a main host for *E. cuniculi*, and can be infected through transplacental transmission, inhalation of spores, or ingestion of food contaminated with infected urine [4,5]. Although many cases of rabbits infected with *E. cuniculi* do not show any symptoms, some rabbits suffering from encephalitozoonosis display various clinical signs, such as renal failure, eye lesions, neurological signs, and sudden death [6,7]. The in vivo diagnosis of this disease is difficult, because many animals are subclinically infected. Several diagnostic tools, including neurological and ophthalmological examinations, serological test, microscopic spore detection, and PCR are used for detection of *E. cuniculi* infection in humans and animals

[5]. In living animals, the serological detection of antibodies, such as ELISA and indirect fluorescent antibody technique (IFAT), is the most important diagnostic method for diagnosis of *E. cuniculi* infection [5]. Serological surveys showing high seroprevalence rates (37-68%) around the world suggest that *E. cuniculi* infection is ubiquitous in rabbits [8]. However, the information on the prevalence of *E. cuniculi* in rabbits is not available in Korea. Therefore, this study evaluated the prevalence of *E. cuniculi* antibodies in pet rabbits in Korea.

The study material was collected from local veterinary hospitals (Daejeon city; n = 11, Gyeongbuk province; n = 100, Chungnam province; n = 75) in Korea. Serum samples from Chinchilla (n = 100), New Zealand white (n = 30), Rex (n = 18), Lionhead (n = 8), Dutch (n = 2), Dwarf (n = 1), and cross-breed rabbits (n = 27) were collected from January 2011 to February 2013. For each sampled animal, sex, age, and health status (symptomatic/asymptomatic) were recorded. With respect to sex, there were 74 male and 112 female rabbits used in the study. These animals were classified into 3 age groups: young (<4 months old), adults (4-12 months old) and old (>12 months old), and the sample number of each group was 48, 88, and 50, respectively. Out of 186 samples, 163 were taken from rabbits that showed no clinical signs, and 23 were collected from rabbits showing anorexia, uveitis, head-tilt, cachexia, renal failure, and hepatic failure.

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Serological examination was carried out using ELISA (Medicago, Uppsala, Sweden) according to the manufacturer's instructions. The serological test revealed that 42 out of 186 (22.6%) sera were positive for *E. cuniculi* antibodies. These samples were collected from rabbits that originated from Gyeongbuk and Chungnam provinces and showed 13.0% (13 out of 100) and 38.6% (29 out of 75) seropositivity, respectively, resulting that the seropositive rate of Chungnam was significantly higher than that of Gyeongbuk province (Pearson's chi-square test) (Table 1).

Comparison of the infection rate by sex showed that 17/74 (22.9%) in male and 25/112 (22.3%) in female were seropositive, and all seropositive samples were collected from clinically normal rabbits (Table 2). Furthermore, comparison of the infection rate by age showed that 12/48 (25.0%) in young, 15/88 (17.0%) in adult and 15/50 (30.0%) in old groups were seropositive.

From statistical analysis using Statistical Package for the Social Sciences (SPSS, IBM, USA), rabbit gender ($\chi^2=0.691$, $P=0.406$), age ($\chi^2=0.549$, $P=0.76$), and health status ($\chi^2=0.319$, $P=0.116$) did not have any significant effect on *E. cuniculi* infection, as reported by other studies [9-12], which is in contrast to the reports presented by Dipineto et al. [9], Santaniello et al. [11] and Tee et al. [12].

Although worldwide surveys have shown high rates (43-100%) of *E. cuniculi* infection in rabbits with neurological signs, vestibular disease or ocular lesions, asymptomatic rabbits also have shown high rates (37-68%) of infection in various countries, including UK, Austria, Italy, and Japan [5,8,9]. In the current study, the total number of symptomatic rabbits was 23 which included 3 (neurological signs), 11 (anorexia), 3 (ocular lesion), 2 (renal failure), and 4 rabbits (others), whereas 42/163 (25.8%) of asymptomatic rabbits were positive. This seropositive rate of asymptomatic rabbits (25.8%) in the current study was higher than those reported in Nigeria (16.5%) but lower than in UK (36.8%), Japan (57.9%), and Italy (68.1%) [5,13]. Reports from different countries have shown different incidences

Table 1. Prevalence of seropositive rabbits and statistical analysis among different locations in Korea

Province	No. of tested	No. of positive	% Positive	P^a
Gyeongbuk	100	13	13.0	0.000
Chungnam	75	29	38.6	
Daejeon	11	0	0	
Total	186	42	22.6	

^aanalyzed by Pearson's chi-square test for independence.

es suggesting that the prevalence may vary strongly in the same country from one locality to another due to certain ecological factors and breeding system [14]. In the current study, samples were collected from 3 areas including Daejeon city, Gyeongbuk, and Chungnam province in Korea, and the results of the seropositive rate showed different in each area. This result suggests that certain ecological factors and breeding system affect the *E. cuniculi* prevalence in different areas. In addition, the high percentage of seropositive healthy rabbits suggests that healthy rabbits may be considered as a potential zoonotic risk.

The distribution of seropositive samples by age in asymptomatic rabbit population in this study were 12/48 (25.0%) from young rabbit group, 15/88 (17.0%) from adult rabbit group, and 15/50 (30.0%) from old group. This finding showed that rabbit's age had no significant effect on *E. cuniculi* infection, which is in agreement with the study performed on domestic rabbits [10]. On the other hand, a previous study conducted on pet rabbits showed that rabbit's age, primarily adult rabbits aged older than 4 months, exhibited significant effect for *E. cuniculi* infection [9]. In immunological aspect of *E. cuniculi* infection, maternal antibodies passed to the offspring are present until 4 weeks of age, and from 4 to 8 weeks of age, the animals are seronegative [15]. Although not significant, this may explain why in the current study several asymptomatic rabbits from young age group had antibodies for *E. cuniculi*. Furthermore, this study also showed that rabbit gender has no significant effect on *E. cuniculi* infection, as reported by other studies [9,10].

Since in vivo diagnosis is difficult, serology remains the most important diagnostic tool for the diagnosis of *E. cuniculi* infection in living animals [5,7]. Indirect fluorescent antibody

Table 2. ELISA results and statistical analysis according to the sex, age, and health status of the rabbits

Rabbit data	No. tested	No. of positive	% Positive	95% CI (%)	P^a
Sex					
Male	74	17	22.9	0.6-16.8	0.416
Female	112	25	22.3	5.4-22.2	
Age					
Young (<4 months old)	48	12	25.0	0-22.2	0.760
Adults (4-12 months old)	88	15	17.0	5.1-21.3	
Old (>12 months old)	50	15	30.0	0-19.3	
Health status					
Symptomatic	23	0	0	0	0.116
Asymptomatic	163	42	25.8	7.4-22.2	
Total	186	42	22.6	5.7-17.7	

^aanalyzed by Pearson's chi-square test for independence.

technique (IFAT), carbon immunoassay (CIA), and ELISA are the most common serological tests for *E. cuniculi* infection in rabbits [7,9,16]. In comparison of the sensitivity between CIA and ELISA, the serological results seem almost the same [9,16].

E. cuniculi is a potential zoonotic and opportunistic pathogen which may pose potential risk for *E. cuniculi* infection to pet rabbit owners [2]. The main host for *E. cuniculi* is the rabbit, and the infected rabbits excrete the spores of *E. cuniculi* intermittently. The infection is transmitted horizontally to other hosts, including humans by direct contact or through environmental contamination [5]. In fact, *E. cuniculi* infections as determined by immunological and/or molecular methods reported in several patients with HIV, undergoing organ transplantation, and with idiopathic CD4⁺ T lymphocytopenia from Europe and the USA proved the infectivity of *E. cuniculi* to immunodeficient individuals [17]. In addition, the identification of a human isolate of *E. cuniculi* type I from Italy has been reported [16].

In conclusion, the findings of the present survey, the first examined in Korea, showed that *E. cuniculi* is present and spread in pet rabbits in Korea. Based on zoonotic potential and public health concerns, further studies aimed at studying the transmission dynamics of this zoonotic parasite are required. We advise serological checks for *E. cuniculi* infection both in symptomatic and asymptomatic pet rabbits.

CONFLICT OF INTEREST

We have no conflict of interest related to this study.

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