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Short communication

Seroprevalence of *Encephalitozoon cuniculi* in Humans and Rabbits in China

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Abstract

Background: *Encephalitozoon cuniculi* is a microsporidian parasite commonly found in rabbits that can infect humans, causing encephalitozoonosis. Our objective in this study was to evaluate the seroprevalence of this parasite in rabbits and humans in China

Methods: Overall, 300 serum samples each from clinically healthy rabbit and human were collected from three regions of China (Sichuan Province, Chongqing Municipality and Jilin Province) from January to September 2013 and tested for anti- *E. Cuniculi* antibodies using an ELISA.

Results: An overall seroprevalence of *E. cuniculi* was recorded as 56/300 (18.76%) and 29/300 (9.76%) in rabbit and human sera, respectively. The seropositivity of rabbit samples collected from Jilin province was 41%, which was significantly higher ($P<0.01$) than Sichuan Province (9%) and Chongqing Municipality (6%). Three breeds of rabbit were used in the present study and antibody detection in Rex Rabbit was significantly ($P<0.01$) higher than Japanese White and New Zealand Rabbit. In human, Jilin province was more prevalent (18%) followed by Sichuan Province (6%) and Chongqing Municipality (5%).

Conclusions The *E. cuniculi* was present and widespread among healthy rabbits and humans in China

Introduction

The obligate intracellular microsporidium *Encephalitozoon cuniculi* is an zoonotic parasite that infects various mammals, such as rabbits, rats, mice, horses, foxes, cats, dogs, muskrats, leopards, baboons, and humans (1, 2). The most frequent route of transmission is through the ingestion of food or water contaminated by spores from urine of infected animals (3). However, a transplacental and respiratory route of infection has been reported in rabbits, too (4).

Rabbits suffering from encephalitozoonosis show various clinical signs, and most infections are initially asymptomatic until sudden death. Many rabbits infected with *E. cuniculi* subsequently develop renal failure, eye lesions, and neurological signs (5, 6). *Encephalitozoon cuniculi*, together with other microsporidia species, has emerged as an opportunistic infection in immunocompromised patients, i.e. persons suffering from AIDS (7-9). In addition, viable *E. cuniculi* was isolated from AIDS patients in many parts of the world (10-12).

A few is known about its prevalence in rabbits and human from East Asia. In Japan, high prevalence of *E. cuniculi* in not only diseased but also healthy rabbits and human was demonstrated (13, 14). Until now, nothing is known about the prevalence of this parasite in rabbits and humans in China, this study is the first survey to evaluate the prevalence of anti-*E. cuniculi* antibodies in rabbits and human in China by using the Enzyme-linked immunosorbent assay (ELISA), which might facilitate the development of rational strategies for disease control and management.

Materials and Methods

Sample collection

Overall, 300 serum samples each from clinically healthy rabbits and human were collected from January to September 2013. The collection of human and animal serum samples was approved by the Ethical Committee of the College of Animal Science, Henan Institute of Science and Technology, China (Approved No. 2010011). The samples were collected from three regions in China including Northeast China (Jilin Province) and Southwest China (Sichuan Province, Chongqing Municipality). The reason for choosing these locations was that these three regions are the major rabbit producing as well as consuming provinces. Due to the different climate and geographical environment, the Rex Rabbit, Japanese White Rabbit and New Zealand Rabbit were the main breed, respectively.

Parasite

The *E. cuniculi* used in the present study was a rabbit strain isolate and well conserved in liquid nitrogen in Henan Higher Education Engineering Technology Research Center for Animal Diseases Control and Residues Supervision, Henan Institute of Science and Technology, China.

Preparation of E. cuniculi antigen

E. cuniculi spores were produced on the RK 13 cell line in minimal essential medium with antibiotics (10 U penicillin/ml; 0.1mg streptomycin/ ml and 0.25mg amphotericine/ml) and 5% fetal bovine serum. The spores were harvested from the culture medium and stored at 4℃. Spores were purified by density gradient centrifugation with Percoll (Sigma-Aldrich, St. Louis, USA) using a standard procedure (Visvesvara et al., 1999). Following three cycles of freezing/thawing, spores of *E. cuniculi* were mixed with solid glass beads (400-600 micrometer diameter, Jencons Scientific Limited, West Sussex, UK) and sonicated (10 min, 60 W). The number of spore pre and post sonication was counted in a hemocytometer to ensure at least 95% spore disruption in the homogenate. The protein concentration of the supernatant was determined with a BCA protein assay kit (Bio-Rad,

Hercules, CA, USA). The soluble antigen solution was stored at -20℃ until use.

Determination of antibodies to E. cuniculi

An indirect enzyme-linked immunosorbent assay (ELISA) was used to detect *E. cuniculi* antibodies. The experimental procedure was adapted from two reference published (15, 16). Briefly, each well of a microtitre plate (Corning Inc., Corning, N.Y.) was coated with 1 µg soluble *E. cuniculi* antigen diluted in 100 µl carbonate bicarbonate buffer (100 mM, pH 9.6) and incubated at 4℃ for 3 days. The well surface was blocked with 5% BSA in 0.05% PBST (PBS containing 0.05% V/V Tween-20) at 37℃ for 2 h, followed by washing five times in 0.05% PBST. Serum samples were diluted at a 1:100 ratio in PBS. The conjugate was horseradish peroxidase (HRP) conjugated goat anti-rabbit IgG or goat anti-human IgG (Southern Biotechnology, Birmingham, Alabama, USA), and diluted 1:6,000 in PBS. Substrate tetramethylbenzidine (TMB, Tiangen Biotech, Beijing, China) was then added and incubated at room temperature for 20 min. 100 µl sodium acid (100 ng/ml) were added to stop the colour reaction. The optical density at 450 nm (OD450) was measured with a photometer. The serum with absorbance at least 2.1-fold higher than that of the negative control serum was considered positive.

Statistical analysis

Statistical analyses of *E. cuniculi* prevalence in different administrative regions and breed groups were performed by X^2-test. The differences were considered statistically significant if $P<0.05$. Correlations between *E. cuniculi* infection in humans and rabbits were tested with Pearson's rank correlation coefficient. Statistical analysis was performed using SPSS 16 software for Windows (SPSS Inc, Chicago, Illinois, USA).

Results

In the present study, 600 serum samples (300 each from rabbit and human) were collected and analyzed by ELISA to detect the antibodies against the *E. cuniculi*.

Seroprevalence in rabbits

An overall seroprevalence of 18.67% (56/300) was recorded in the rabbits. In case of breed wise seroprevalence of *E. cuniculi,* the Rex Rabbit from Jilin province was found more infected (41.00%, $P<0.01$) as compared with Japanese White Rabbit from Sichuan Province (9.00%) and New Zealand Rabbit from Chongqing Municipality (6.00%) (Table 1).

Table 1: Seroprevalence of *E. cuniculi* infection in rabbits in China

Variable	No. examined	No. positive	Prevalence (%)
Region			
Sichuan Province	100	9	9.00[a]
Chongqing Municipality	100	6	6.00[a]
Jilin Province	100	41	41.00[b]
Breed			
Japanese White Rabbit	100	9	9.00[a]
New Zealand Rabbit	100	6	6.00[a]
Rex Rabbit	100	41	41.00[b]
Total	300	56	18.67

Values bearing a different superscript letter (a, b) within a column differ significantly from one another ($P<0.05$)

Seroprevalence in humans

Twenty-nine (9.67%) out of 300 human serum samples were found positive for anti-*E. cuniculi* antibodies. The findings of the present study revealed highest prevalence of anti-*E. cuniculi* antibodies in Jilin Province (18.00%) followed by Sichuan Province and Chongqing Municipality with the prevalence rate of 6.00% and 5.00% respectively. The seroprevalence in males was 10.00% (15/150) and in females was 9.33% (14/150) (Table 2). Thus, the gender was not significantly associated with *E. cuniculi* infection in this study ($P>0.05$).

There was a direct correlation between *E. cuniculi* infection in humans and *E. cuniculi* infection in rabbits (r = 1.000, $P<0.01$).

Table 2: Seroprevalence of *E. cuniculi* infection in humans in China

Variable	No. examined	No. positive	Prevalence (%)
Region			
Sichuan Province	100	6	6.00[a]
Chongqing Municipality	100	5	5.00[a]
Jilin Province	100	18	18.00[b]
Gender	150	14	9.33[a]
Female			
Male	150	15	10.00[a]
Total	300	29	9.67

Values bearing a different superscript letter (a, b) within a column differ significantly from one another ($P<0.05$)

Discussion

Many serological surveys of *E. cuniculi* infections in different animals have been conducted using diverse methods in different areas in the world (17-20). However, very few studies have investigated the distribution of *E. cuniculi* infections in China. Meng et al reported that 16.7% of the fox serum samples collected in Liaoning province were positive, while 2% of the dog serum samples collected in Beijing, Shanghai, and Hunan were positive according to the ELISA using rSWP1(17). Until now, nothing is known about the prevalence of this parasite in rabbits and humans in China. The findings of the present study highlighted an alarming situation that the parasite is widespread in clinically healthy rabbits and human in China.

The present survey showed that the overall seropositivity for *E. cuniculi* infection in domestic rabbits was 18.67%, which was similar to that observed in Nigeria (16.5%) (21) and Egypt (15.0%) (22) but lower than that in the United Kingdom (52.0%) (23) and Italy (31.6-75.4%) (24-26). These differences may result from the different serological tests, rabbit populations, or climatic factors or to some combination of these conditions.

Interestingly, the breed seemed to influence the distribution of *E. cuniculi* seropositivity. In particular, the Rex Rabbit were showed to be associated to a higher frequency of infection respect to Japanese White Rabbit and New Zealand Rabbit ($P<0.01$). It is noteworthy to mention that also Lonardi et al reported a higher seroprevalence in rabbits of the X breed than Y breed and Z breed (26). These results indicate that there may be a potential association between the genetic line and the seropositivity against *E. cuniculi*.

The overall seropositivity for *E. cuniculi* infection in humans was 9.67%, which was similar to that observed in the Czech Republic (10-16%) (27) but lower than that in the Slovakia (26.4%) (28). In addition, viable *E. cuniculi* was isolated from AIDS Patients in many

parts of the world (10-12). The findings of the *E. cuniculi* from human cases from three regions of the China revealed zoonotic sources of infection involving rabbits to be major route of transmission in humans. In this study, the gender of human was not significantly associated with *E. cuniculi* infection ($P>0.05$), which was consistent with other reports (23, 26).

Among three provinces/municipalities, the highest prevalence of *E. cuniculi* infection in domestic rabbits and humans was detected in Jilin Province. *E. cuniculi* spores are often ingested or inhaled through food or soil contaminated with infected urine. The spores are environmentally resistant and can survive on the ground for several weeks or months (22). In addition, it is likely that the majority of rabbits are infected at a very early age from their mother. These results indicated that the environment of the Jilin Province had been seriously contaminated with infected *E.cuniculi* spores. In addition, close contact between owners and their rabbits could lead to an increased exposure to *E. cuniculi*.

Therefore, it is necessary for rabbit raisers, public health authorities to be aware of this problem in these regions. Comprehensive practical control approaches and measures, such as the improvement of feeding conditions and management of rabbit, serologic screening of breeding stock with elimination of *E. cuniculi* positive reactors, should be executed. Persons should avoid contact with the urine of infected or healthy rabbits, and always use good personal hygiene when washing cages and handling the rabbits.

Conclusion

This is the first survey to evaluate the prevalence of anti-*E. cuniculi* antibodies in rabbits and human in China. *E. cuniculi* is present and widespread among healthy rabbits and humans in China. Therefore, the fields of veterinary and human medicine in China should be aware about this zoonotic issue and precautionary measure should be taken to avoid the spread of encephalitozoonosis.

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