Short communication

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

*Yaoqian PAN*¹, Shuai WANG², Xingyou LIU¹, Ruizhen LI¹, Yuqian SUN¹, Javaid Ali GADAHI²,³

¹. College of Animal Science and Henan Higher Education Engineering Technology Research Center for Animal Diseases Control and Residues Supervision, Henan Institute of Science and Technology, Xinxiang, Henan, PR China
². College of Veterinary Medicine, Nanjing Agricultural University, Nanjing, Jiangsu, 210095, PR China
³. Department of Veterinary Parasitology, Sindh Agriculture University, Tandojam, Pakistan

**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 a 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. 20100011). 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 amphotericin/ml) and 5% fetal bovine serum. The spores were harvested from the culture medium and stored at 4°C. 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,
The soluble antigen solution was stored at -20°C 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°C 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°C 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 X²-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           |
| Chongqing Municipality    | 100          | 6            | 6.00           |
| Jilin Province            | 100          | 41           | 41.00          |
| **Breed**                 |              |              |                |
| Japanese White Rabbit     | 100          | 9            | 9.00           |
| New Zealand Rabbit        | 100          | 6            | 6.00           |
| Rex Rabbit                | 100          | 41           | 41.00          |
| **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<sup>a</sup>|
| Chongqing Municipality | 100          | 5            | 5.00<sup>a</sup>|
| Jilin Province    | 100          | 18           | 18.00<sup>b</sup>|
| Gender            |              |              |                |
| Male              | 150          | 15           | 10.00<sup>a</sup>|
| Female            |              |              |                |
| 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.

Acknowledgements

This study is supported by the Nature Science Foundation of China (Project Number: 31372407) and Open fund of Henan Higher Education Engineering Technology Research Center for Animal Diseases Control and Residues Supervision (Project Number: 200901). The authors declare that there is no conflict of interests.

References

1. Didier ES, Didier PJ, Snowden KF, Shadduck JA. Microsporidiosis in mammals. Microbes Infect. 2000; 2:709-720.
2. Mathis A. Microsporidia: emerging advances in understanding the basic biology of these unique organisms. Int J Parasitol. 2000; 30:795-804.
3. Kunzel F, Joachim A. Encephalitozoonosis in rabbits. Parasitol Res. 2010; 106:299-309.
4. Baneux PJ, Pognan F. In utero transmission of Encephalitozoon cuniculi strain type I in rabbits. Lab Anim. 2003; 37:132-138.
5. Csokai J, Gruber A, Kunzel F, Tichy A, Joachim A. Encephalitozoonosis in pet rabbits (Oryctolagus cuniculus): pathohistological findings in animals with latent infection versus clinical manifestation. Parasitol Res. 2009; 104:629-635.
6. Kunzel F, Gruber A, Tichy A, Edelhofer R, Nell B, Hassan J, Leschnik M, Thalhammer JG, Joachim A. Clinical symptoms and diagnosis of encephalitozoonosis in pet rabbits. Vet Parasitol. 2008; 151:115-124.
7. Halanova M, Cislakova L, Valencakova A, Balent P, Adam J, Travnicek M. Serological screening of occurrence of antibodies to Encephalitozoon cuniculi in humans and animals in Eastern Slovakia. Ann Agric Environ Med. 2003; 10:117-120.
8. Weber R, Bryan RT, Schwartz DA, Owen RL. Human microsporidial infections. Clin Microbiol Rev. 1994; 7:426-461.
9. Tosoni A, Nebuloni M, Ferri A, Bonetto S, Antinori S, Scaglia M, Xiao I, Moura H, Visvesvara GS, Vago L, Costanzi G. Disseminated microsporidiosis caused by Encephalitozoon cuniculi III (dog type) in an Italian AIDS patient: a retrospective study. Mod Pathol. 2002; 15:577-583.

10. del Aguila C, Moura H, Fenoy S et al. In vitro culture, ultrastructure, antigenic, and molecular characterization of Encephalitozoon cuniculi isolated from urine and sputum samples from a Spanish patient with AIDS. J Clin Microbiol. 2001; 39:1105-1108.

11. Didier ES, Visvesvara GS, Baker MD, Rogers LB, Bertucci DC, De Groote MA, Vossbrinck CR. A microsporidian isolated from an AIDS patient corresponds to Encephalitozoon cuniculi III, originally isolated from domestic dogs. J Clin Microbiol. 1996; 34:2835-2837.

12. Rossi P, La Rosa G, Ludovisi A, Tamburini A, Gomez Morales MA, Pozio E. Identification of a human isolate of Encephalitozoon cuniculi type I from Italy. Int J Parasitol. 1998; 28:1361-1366.

13. Igarashi M, Oohashi E, Dautu G, Ueno A, Kariya T, Furuya K. High seroprevalence of Encephalitozoon cuniculi antibodies against microsporidian Encephalitozoon cuniculi polar tubes in sera from healthy and human immunodeficiency virus-infected persons in Japan. Clin Vaccine Immunol. 2007; 14:168-172.

14. Omura M, Furuya K, Kudo S, Sugiu M, Azuma H. Detecting immunoglobulin M antibodies against microsporidian Encephalitozoon cuniculi polar tubes in sera from healthy and human immunodeficiency virus-infected persons in Japan. J Vet Med Sci. 2008; 70:1301-1304.

15. Akerstedt J. An indirect ELISA for detection of Encephalitozoon cuniculi infection in farmed domestic rabbits in Italy. Acta Vet Scand. 1982; 43:211-220.

16. Akerstedt J. Serological investigation of canine encephalitozoonosis in Norway. Parasitol Res. 2003; 89:49-52.

17. Meng X, Zheng J, Gao Y, Zhang Y, Jia H. Evaluation of spore wall protein 1 as an alternative antigen for the diagnosis of Encephalitozoon cuniculi infection of farmed foxes using an enzyme-linked immunosorbent assay. Vet Parasitol. 2014; 203:331-334.

18. Neumayerova H, Jurankova J, Jeklova E et al. Seroprevalence of Toxoplasma gondii and Encephalitozoon cuniculi in rabbits from different farming systems. Vet Parasitol. 2014.

19. Hsu V, Grant DC, Zajac AM, Witonsky SG, Lindsay DS. Prevalence of IgG antibodies to Encephalitozoon cuniculi and Toxoplasma gondii in cats with and without chronic kidney disease from Virginia. Vet Parasitol. 2011; 176:23-26.

20. Cray C, Perritt E, Hughes C, Belgrave RL. Serological survey for antibody to Encephalitozoon cuniculi in horses in the USA. Parasitol Res. 2014; 113:2757-2759.

21. Okeowo EA. Seroprevalence of antibodies to Encephalitozoon cuniculi in domestic rabbits in Nigeria. Onderstepoort J Vet Res. 2008; 75:33-38.

22. Ashmawy KJ, Abuakkada SS, Awad AM. Seroprevalence of antibodies to Encephalitozoon cuniculi and Toxoplasma gondii in farmed domestic rabbits in Egypt. Zoonoses Public Health. 2011; 58:357-364.

23. Keeble EJ, Shaw DJ. Seroprevalence of antibodies to Encephalitozoon cuniculi in domestic rabbits in the United Kingdom. Vet Rec. 2006; 158:539-544.

24. Santaniello A, Dipineto I, Rinaldi L, Menna LF, Cringoli G, Fioretti A. Serological survey of Encephalitozoon cuniculi in farmed meat rabbits in Italy. Res Vet Sci. 2009; 87:67-69.

25. Dipineto L, Rinaldi L, Santaniello A, Sensale M, Cuomo A, Calabria M, Menna LF, Fioretti A. Serological survey for antibodies to Encephalitozoon cuniculi in farm rabbits in Italy. Res Vet Sci. 2009; 87:67-69.

26. Lonardi C, Grilli G, Ferrazzi V, Dal Cin M, Rigolin D, Piccirillo A. Serological survey of Encephalitozoon cuniculi infection in commercially reared rabbit does in Northern Italy. Res Vet Sci. 2013; 94:295-298.

27. Kucerova-Pospisilova Z, Ditrich O. The serological surveillance of several groups of patients using antigens of Encephalitozoon hellem and E. cuniculi antibodies to microsporidia in patients. Folia Parasitol (Praha). 1998; 45:175-179.

28. Halanová M, Valencáková A, Maleková B, Kvac M, Star B, Květonová D, Balent P, Cisláková L. Occurrence of Microsporidia as emerging pathogens in Slovak Roma children and their impact on public health. Ann Agric Environ Med. 2013; 20:695-698.