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Short Communication

The First Identification of *Encephalitozoon cuniculi* Infection in an Animal Care Worker in Turkey

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Abstract

Background: As a zoonotic pathogen, *Encephalitozoon cuniculi* is a cause of serious disease in animals and people. The present study was to evaluate the health status examination of this seropositive animal care worker in our previous study.

Methods: Blood samples were taken from five workers. CIA test was applied to detect antibodies against *E. cuniculi* in blood serum. The indirect immunofluorescence antibody test was used as confirmation test. Seropositive worker had a complete medical examination.

Results: Only one worker was found to be seropositive according to the results of the serological test. Sera positive to *E. cuniculi* was confirmed with IFAT and spores were detected in the urine sample of the worker. The worker was treated with albendazole.

Conclusion: Rabbits should be examined routinely for the presence of anti-*E. cuniculi* antibody. People working with laboratory animal should avoid contact with urine and faeces of infected or pay attention to personal hygiene.

Introduction

The phylum Microsporidia consists of nearly 150 genera but only the most frequently identified human microsporidian pathogens are *Enterocytozoon bieneusi*, *E.intestinalis, E. cuniculi* and *E. hellem* in seven genera (1-3). As a zoonotic pathogen, *E. cuniculi* is a cause of serious disease - encephalitozoonosis in animals and people (4-8). In recent years, serological studies show that encephalitozonosis could be common but with-

out clinical significance. Subclinical *E. cuniculi* infection usually develops in immunocompetent hosts (9). In the world, the infections have been reported in Chile, Canada, France, Germany, Japan, Italy, Mexico, Spain, the United Kingdom, the United States, and in Switzerland in immunocompromised (without/with HIV infection) humans (2, 4, 5, 10). The detection of the parasite has increased considerably since the existence of infection in immunocopromised and immunocompetent human (4). In Turkey, only four studies have been reported to date of microsporidiosis in human, while animal encephalitozonosis has first been described histopathologically (11) and later in rabbits using the carbon immunoassay (CIA) test (12, 13) and histopathologically as the diagnostic method (14).

In our previous study, it was shown that rabbits (Fig. 1) were infected with *E. cuniculi* in a breeding colony and an animal care worker was also found to be seropositive in Turkey (13). The present study was to evaluate the health status examination of this seropositive animal care worker.

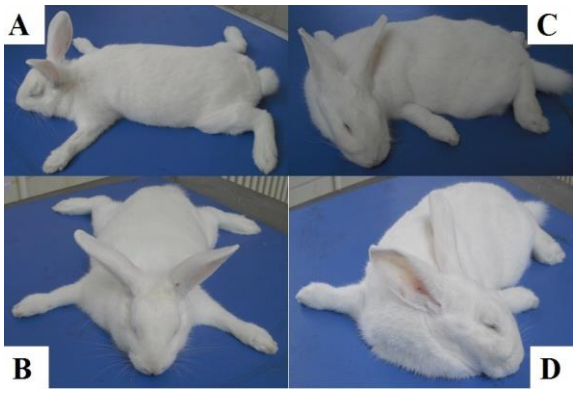

Fig. 1: Rabbits with torticollis (head tilt) due to cerebral infection with *E. cuniculi*

Materials and Methods

Serum samples

Blood samples were taken into evacuated tubes from the vena cubiti of the five workers. Serum was kept at –20ºC until testing.

Serological diagnosis

The Carbon Immunoassay (CIA) Test

The CIA test kit was purchased from Medicago (Medicago, Uppsala, Sweden). CIA was performed according to the manufacturer's instructions. With the positive sera, the *E. cuniculi* spores were stained greyish-black against the background of the carbon particles, and with negative sera, the parasite appeared white on the dark background. In assay, negative and positive control sera (supplied by Medicago) were run in each test.

The indirect immunofluorescence antibody assay (IFA).

The indirect immunofluorescence antibody test was used as confirmation test. The test was conducted as described in detail by Chalupský et al. (15). Following air-drying, the wells were covered with 15 µl of rabbit anti-human IgG marked fluorescein isothiocyanate conjugate (FITC, SIGMA) of 1:160 dilution. After 30 min, the slides were washed and then were mounted using Fluoromount-G. The reaction was examined using a fluorescence microscope (Olympus). Both a positive and negative rabbit serum (supplied by Medicago) were included in this test as control. In the case of a positive immunological reaction, the spores with strong peripheral fluorescence were observed as oval formations of 1.5–2.5 µm in size and were considered positive. In paralel of CIA test, a 1:40 cut-off was used to identify a sample as positive.

Detection of spores screening in the urine samples with light microscopy

A 24h urine sample was also collected from seropositive worker. Specimens were concentrated by centrifugation. Specimen of urinary sediment (10 µL) was stained with modified Trichrome stain (Ryan-Blue) for the detection of *E. cuniculi* spores as described by Ryan et al. (16). Diluted antigen from the CIA kit was used as a positive control for the accuracy of the stain method. Then one hundred observa-

tion fields were examined under oil immersion by light microscopy.

The health status of seropositive worker

Seropositive worker had a complete physical examination, medical history, serum biochemical parameters and hematological parameters, chest and sinus X-ray and total abdominal ultrasonography (USG) at the Ozkaya Medical Center, Ankara. The urine analysis was carried out using dip sticks.

Results

Serology and detection of spores

Blood samples were taken from all workers. CIA test was applied to detect IgG against *E. cuniculi* in blood serum. Only one worker was found to be seropositive according to the results of the serological test. Sera positive to *E. cuniculi* was confirmed with IFAT (Fig. 2A) and spores were also detected in the urine sample of the seropositive worker (Fig. 2B) after staining with modified trichrome stain.

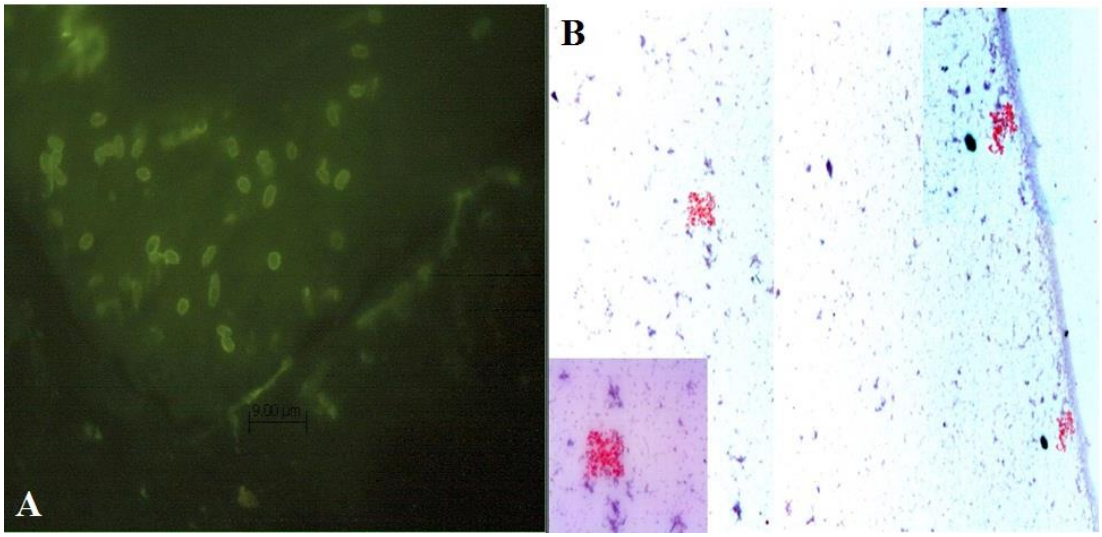

Fig. 2: A: *Encephalitozoon cuniculi* spores stained apple-green by indirect immunofluorescence with anti–*E. cuniculi* serum (Bar: 9 µm). **B:** Spores of *E. cuniculi* from urine stained with Trichrome blue (Magnification 1000×).

Health status

Laboratory tests were performed to evaluate different biochemical parameters and hematological parameters (Table 1). Laboratory examination shown in Table 1, the Complement 3 (C3), C4, IgA, IgM and IgG were measured within reference interval. The C-reactive protein concentration was 6 mg/L (0–10 mg/L), and the creatinine concentration was 0.95 mg/dL (0.5–1.4 mg/dL). The dipstick test was negative (-) for protein. Urine cultures showed no fungal or bacterial growth. The chest film, the frontal and maxillary sinuses and total abdominal USG did not reveal any lesions.

His visual acuity and visual field were normal, as were the results of funduscopy and there was no keratoconjunctivitis. The worker was treated with albendazole at 200 mg twice daily for 2 weeks. The urine samples at two weeks interval (3 times: the beginning, the third week beginning and the fifth week beginning) were collected to detect the spores using Trichrome staining after albendazole treatment. The result was found negative for *E. cuniculi* spore.

Table 1: Laboratory finding to evaluate different biochemical parameters and hematological parameters

Hematology			**Chemistry**					
Test	**Result**	**Reference Interval (Ri)**	**Test**	**Result**	**Reference Interval (Ri)**	**Bilirubin, direct**	**0.12**	**0-0.5 mg/dL**
WBC count	11.5	4.5–11 K/µL	Glucose	89	70–110 mg/dL	ALT	10	0-41 U/I
RBC count	5.35	4.5–5.9 M/µL	Uric acid	3.77	3.6–8.2 mg/dL	AST	31	0-41 U/I
Hemoglobin	15.8	13.5–17.5 g/dL	BUN	25	19–44 mg/dL	ALP	182	<258 IU/L
Hemotocrit	46.7	40–53%	Creatinine	0,95	0.5-1.4mg/dL	GGT	20	0-49 U/I
MCV	87.3	80–92 fL	Sodium	146	135–150 mmol/L	LDH	487	<480 IU/L
Platet count	299	130–400 K/µL	Potassium	4.3	3.5–5.5 mmol/L	CK	101	22-200 U/L
Neutrophils	75	40–75%	Protein, total	7.1	6.6-8.8 g/dL	Amylase	97	10-100U/L
Lymphocytes	20.5	20–40%	Albumin	5.04	3.5-5.2 g/dL	Lipase	20	0-60 U/L
Monocytes	4	2-4%	Bilirubin, total	0.4	0.4-1.35 mg/dL	C-reactive protein	6	0-10 mg/L
Immun Status			**Microbiology**			**Urine Analysis**		
C4	23.9	16–38 mg/dL	Anti-HCV	0.034 (-)	0–0.9 (-)	Bilirubin, Ürobilinogen, Protein, Nitrit,		Negative
C3	150	79–152 mg/dL	Anti-HBs	0.067 (-)	0–7 (-)	Blood, Leucocyte,		Negative
IgA	162	82–453 mg/dL	Anti-HIV	0.025 (-)	< (-)	Culture		None
IgG	1100	751–1560 mg/dL				Ph	1010	1000–1030 g/dL
IgM	255	46–304 mg/dL						

Discussion

Buget et al. (17) reported the first microsporidiosis in Turkey and it was detected in stool samples of an AIDS patient. Later, Yazar et al. (18) reported spores of Microsporidia in the brochoalveolar lavage sample of a patient with acute myeloblastic leukaemia-M3. In addition, Atambay et al. (19) investigated in no immunesuppressive problem patients for intestinal parasites and *Microsporidium*. After examination of 781 fecal specimens, the spores were detected at 6.5 %. Karaman et al. (20) found *Microsporidium* 10.9 % in the patients with cancer while 5.6 % in non-cancer patient. Species of microsporidia have not been identified in all reports; however microsporidiosis is reported in immunocopromised as well as in immunocompetent individuals. In Turkey, there was no data of *E. cuniculi* infection in human so far. To our knowledge, this is the first *Encephalitozoon* infection reported in an immunocompetent person.

First *Encephalitozoon* infection was reported in a boy and has been identified as a human pathogen in 1959 (2, 3, 21). In rabbits, *E. cuniculi* spores are finally localized in kidneys, eyes and brain causing clinical signs such as cataracts, head tilt, paralysis and death (22). Adult rabbits are infected by ingestion or inhalation of *E. cuniculi* spores, which are passed in the urine (23) but are excreted only sporadically (24). Urine examination is impractical as a diagnostic technique in encephalitozoonosis. Nevertheless, microsporidian spores are mostly isolated from the urine samples depend on immune status of patients according to previously case reports or studies. In our study, 24h urine sample was repeatedly collected from an animal care worker for detection of spores but only one sample was detected in urine.

In a recent serodiagnostic study, an immunocompetent laboratory worker was accidentally infected with *E. cuniculi* (2). In our study, general health status of the seropositive worker was investigated even though it had not been in any medical complaint.

Therefore *E. cuniculi* is a widespread microsporidian species infecting primates, rodents, lagomorphs and carnivores, and has also been found in immunocompetent humans (5). Direct transmission between animals and human has not been reported and the portal of entry of this pathogen remains unknown (7, 25). However, rabbits and dogs may play an important role as potential sources of *E. cuniculi* infection for humans according to histories of previously reported cases. *E. cuniculi* spores are also highly resistant in the environment and can survive several months under humid conditions. Therefore, oral transmission by contaminated drinking water or food and airborne transmission and it may be possible that all play a part in the infective process (5, 7, 26). In our study, the person had worked with rabbits in the breeding department for a long time. As a consequence, the disease may have been transmitted from infected rabbits to the worker because the results of the questionnaire also showed that he had ignored personal hygiene while washing cages and handling the animals during working time. Albendazole has been successfully used in patients with *E. cuniculi* infection (27). In our study, therefore the worker was given albendazole at 200 mg twice daily for 2 weeks. The urine sample of the worker was examined to identify the spores at three times in the different periods after treatment. Because of treatment, *E. cuniculi* spores were not detected in the urine samples.

Conclusion

(a) Rabbits should be examined routinely for the presence of anti-*E. cuniculi* antibody even if apparently healthy, especially considering its potential zoonotic risk; (b) People working with animal should avoid contact with urine and faeces of infected or healthy animals even though *E. cuniculi* spores were not detected in the worker urine sample and (c) always use good personal hygiene when handling animals.

Acknowledgements

The authors declare that there is no conflict of interest.

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