SHORT COMMUNICATION

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Screening of individuals potentially exposed to *Encephalitozoon cuniculi* infection

Anna Wilczyńska[1,A-D] ✉, Anna Malm[2,B-E], Katarzyna Gębicka[2,B-C], Dariusz Duma[3,B-C], Beata Wojtysiak-Duma[3,B-C], Stanisław Winiarczyk[1,E-F], Maciej Skrzypczak[4,B,D-E], Michał Wójcik[5,D-E], Łukasz Adaszek[1,A-B,D,F], Marcin Kalinowski[1,C]

[1] *Department of Epizootiology and Clinic of Infectious Diseases, University of Life Sciences, Lublin, Poland*
[2] *Chair and Department of Pharmaceutical Microbiology, Medical University, Lublin, Poland*
[3] *Department of Laboratory Diagnostics, Medical University, Lublin, Poland*
[4] *Department of Gynecology, Medical University, Lublin, Poland*
[5] *Department of Animal Physiology, University of Life Sciences, Lublin, Poland*
A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation, D – Writing the article, E – Critical revision of the article, F – Final approval of the article

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Abstract

Introduction and Objective. *Encephalitozoon cuniculi* is a species of fungus belonging to the phylum Microsporidia, potentially pathogenic to rodents, rabbits, dogs, cats, horses and humans. The aim of the study is to conduct preliminary serological and molecular monitoring of encephalitozoonosis among persons coming into contact with rabbits, i.e., a potential reservoir of *E.cuniculi.*

Materials and Method. The study included 28 persons (Group 1), who had been in contact with rabbits (as the owners or veterinary staff), for a minimum of 2 years (from 2–20 years), and 20 persons who had had no such contact, who formed a control group (Group 2). Blood and serum samples were collected for molecular and serological testing for encephalitozoonosis from all the persons.

Results. Using the Real-Time HRM PCR, the DNA of *E. cuniculi* was detected in blood samples obtained from 3 person in the study group, but was not detected in the control group. The presence of antibodies to *E. cuniculi* was demonstrated in the 4 samples of sera collected from persons who had been in constant contact with rabbits.

Conclusion. The results obtained and the literature indicate that individuals working with animals are at increased risk of developing *Encephalitozoon ciniculi*, and that in the event of health problems, encephalitozoonosis should also be considered in the differential diagnosis.

Key words

zoonosis, encephalitozoon cuniculi, encephlitozoonosis

INTRODUCTION

Microsporidia are intracellular, opportunistic pathogens found in diverse environments. Originally classified as protozoa, they have been reclassified as fungi, although some researchers propose that they are a sister group to fungi [1, 2]. The National Institute of Allergy and Infectious Diseases have classified microsporidia as Category B Priority pathogens, which implies that these microorganisms spread moderately easily in the environment and have a moderate pathogenicity index [3]. Microsporidian spores are environmentally persistent and infect many animal species, being excreted in faeces, urine, or sputum, and spreading via airborne, alimentary, or vertical routes [4–7]. *E. cuniculi* spores withstand external conditions due to a two-layered wall and can survive up to 6 weeks at 22 °C, or longer in water [8–10]. Experiments show their infectivity duration depends on storage temperature, with a marked decline at higher temperatures, and *E. cuniculi* spores lose infectivity faster than those of other microsporidia.

Human infection most often occurs through food contaminated with microsporidian spores, including irrigated agricultural products, rabbit meat, and processed foods such as milk and fermented meat products. Notably, *E. cuniculi* spores remain active even after short-term high-temperature pasteurisation, and fermentation does not fully eliminate the risk of infection from pork products [11–13]. There are currently 17 known species of *Encephalitozoonidae* capable of infecting humans. The highest pathogenicity is exhibited by 4 species: *Encephalitozoon intestinalis, Encephalitozoon cuniculi,* as well as *Encephalitozoon hellem* and *Enterocytozoon bieneusi* [14]. In recent decades, these pathogens have been recognised as an emerging cause of disease in both humans and animals. The global prevalence of microsporidia infection in humans has been estimated to exceed 8%, with much higher rates (up to 50%) in immunocompromised populations such as HIV-positive patients or organ transplant recipients [15]. Those most at risk of infection are persons with compromised immunity, e.g., after organ transplants, suffering from viral infections (HIV), and undergoing immunosuppressive therapy [16–20], as well as owners of animals such as rabbits, dogs, cats or rodents, which may be carriers of microsporidia, excreting their spores in faeces and urine.

In humans infected with *Encephalitozoon* spp., the early stages of infection are characterised by diarrhoea and

✉ Address for correspondence: Anna Wilczyńska, Department of Epizootiology and Clinic of Infectious Diseases, University of Life Sciences, Lublin, Poland
E-mail: anna.wilczynska@up.lublin.pl

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weight loss. At a later stage, the following may develop: keratitis, urinary tract inflammation, hepatitis, encephalitis, peritonitis, prostatitis, sinusitis, pneumonia, rhinitis, urethritis and cholangitis [15, 21]. Among individuals, those who had undergone hip or knee replacement surgery; difficult wound healing was also associated with *E. cuniculi* spore contamination [22]. Infection in immunocompetent individuals is typically subclinical [23]. Given the potential hazard the disease poses to human health and life, research into its pathogenesis and treatment appears warranted.

In livestock, infections with Encephalitozoon species can reduce reproductive efficiency, impair growth, and increase mortality, resulting in measurable economic losses. Thus, microsporidia infections carry not only clinical, but also public health and socio-economic significance, highlighting the need for better epidemiological surveillance and diagnostic tools [24, 25].

The aim of the study is to conduct preliminary serological and molecular monitoring of encephalitozoonosis among persons in contact with rabbits, a potential reservoir of *E. cuniculi*.

MATERIALS AND METHOD

The study included 28 persons (Group 1) who had been in contact with rabbits (owners or veterinary staff) for at least 2 years (range: 2–20 years), and 20 persons (Group 2) who had no such contact. The first group comprised veterinarians (n=21) working in general medical facilities (n=5) and facilities specialising exclusively in the treatment of exotic animals and small mammals (n=16)), veterinary technicians (n=1), students (n=1) and rodent owners (n=5). Each participant in the study was asked to report how often they come into contact with sick animals (Tab. 1) and whether they exhibit any symptoms of disease. Blood samples were collected for molecular, haematological, and biochemical testing from all individuals in both Groups 1 and 2 only once. Haematological and biochemical parameters were assessed in all subjects. Biochemical tests were performed using routine tests on a BioSystems A25 analyser (Barcelona, Spain). Concentrations of glucose, total protein, albumin, total bilirubin, urea, creatinine, uric acid, calcium, inorganic phosphate, iron, magnesium, and high-sensitivity C-reactive protein were determined. Haematological parameters were measured on a Sysmex XN1000 analyser. The blood samples were additionally subjected to molecular and serological testing for encephalitozoonosis.

The study obtained approval from the relevant Ethics Committee to collect and analyse blood samples with EC number 0254/75/2023. All persons provided informed consent to participate in the study.

Molecular testing. DNA was isolated from whole blood using a Genomic Mini set (A&A Biotechnology, Poland). PCR tests used a set of primers msp3, msp4a and msp4b, complementary to the ITS gene (internal transcribed spacer) (ITS) *Encephalitozoon* spp., with the following sequences: msp3 (5'GGAATTCACACCGCCCGTCACTAT3 '), msp4a (5'CCAAGCTTATGCTTAAG-TCCAAGGGGT 3') and msp4b (5 'CCAAG-CTTATGCTTAAGTCCAGGGAG 3') [24]. The sequences enabled amplification of a DNA segment approximately 300 base pairs long.

Real-time polymerase chain reaction with SYBR Green 1 dye was performed in thin-walled 100 µL tubes using the DyNAmo HS SYBR Green qPCR kit (Finnzymes), which enabled high specificity. The reaction mixture with a volume of 22.4 µL contained the following components: 10 mL of Master Mix containing modified hot start Tbr (Thermus brockianus) polymerase, a buffer for Tbr polymerase, dNTP, MgCl2 and intercalating SYBR Green 1 dye, 5 mL of de-ionised water, 0.8 mL of msp3 primer (final concentration of 25 pM/mL), 0.8 mL of msp4a primer (final concentration of 25 pM/mL), 0.8 mL of msp4b primer (final concentration of 25 pM/mL) and 5 mL of DNA.

The optimised real-time PCR consisted of 41 cycles, each comprising 3 steps: denaturation at 95 °C for 180 seconds, primer annealing at 95 °C for 5 seconds, and strand elongation at 60 °C for 15 seconds. The reactions were performed using a Rotor-Gene3000 thermal cycler (Corbett Research, Mortlake, NSW, Australia). For each reaction, the Ct values of the PCR products formed on the cDNA template were determined. To confirm amplification specificity, the melting temperature of the PCR products was determined by gradually increasing the reaction mixture temperature from 50 °C to 95 °C while continuously measuring fluorescence intensity. In the PCR reaction, *E. cuniculi* DNA obtained from a previous study [26] served as a positive control.

The PCR reaction products were sent for purification and sequencing to the DNA Sequencing and Synthesis Service of the Institute of Biochemistry and Biophysics of the Polish Academy of Sciences in Warsaw. The sequencing results were received via email and subsequently processed using the Lasergene DNA Star software.

Using the same program, the obtained sequences of the *Encephalitozoon cuniculi* isolates were compared with the sequences of *E. cuniculi* AJ005581.1, KJ941140.1 genotype 1, MF062430, genotype 2, KF736984.1 genotype 3, and HM045511 genotype 4 from the GenBank gene bank.

Serological testing. Blood samples were collected for serological testing from all individuals in groups 1 and 2. The serum obtained was stored at –70 °C until the time of analysis. The serological test for antibodies to *E. cuniculi* was performed using commercially available kits (Medicago, Uppsala, Sweden).

The serum samples were diluted 1:40 in PBS-T and applied to antigen-coated plates (both the positive and negative controls were diluted 1:100 in PBS-T). The plate was rinsed 3 times before use. Next, 100 µL of diluted negative control, 100 µL of diluted positive control, and 100 µL of diluted test samples were measured and added to the wells. The arrangement was incubated for 60 minutes at room temperature (20 °C – 25 °C).

The plate wells were then emptied and rinsed 3 times with 350 µL of PBS-Tween. 100 microlitres of diluted secondary antibody conjugated with HR was added to each well, and the mixture incubated for 30 minutes at room temperature of 20 °C – 25 °C. After 3 more rinses of the plate with PBS-Tween, 100 µL of liquid TMB substrate was added to each well, and the whole was incubated for 15 minutes at room temperature of 20 °C – 25 °C and 50 µL of stop solution was then added to each well. The absorbance of the samples was measured at 450 nm. The cut-off threshold was 2.1 (sample A_{450} EC coated/sample A_{450} control antigen coated), according to the test manufacturer's recommendations.

Table 1. Results of the study

Item	Age (years)	Gender	Facility type	Li-k	Length of service (years)	Freque-cy of contact with an-imals serving as E.c carriers	Frequency of contact with animals inf ected with E.c	Urinary system	nervous system	Respiratory system	Eyesight
1	35	♀	V	AC	8	F	F	-	-	-	-
2	25	♂	O	-	2	V F	M F	-	-	-	-
3	28	♂	VT	S	6	V F	V F	-	-	-	-
4	48	♂	V	S	20	V F	V F	-	-	Seasonal colds	-
5	38	♀	O	-	5	V F	V F	-	-	Frequent bronchitis	-
6	36	♀	V	AC	10	M F	M F	-	-	-	-
7	37	♀	V	AC	10	M F	M F	-	-	-	-
8	38	♀	V	S	11	V F	V F	-	-	-	-
9	27	♀	VS	S	2	V F	M F	-	-	-	-
10	29	♀	V	S	4	V F	F	-	-	-	-
11	34	♀	V	AC	10	M F	-	-	-	-	-
12	37	♀	V	S	9	V F	V F		-	-	-
13	30	♀	V	S	6	V F	V F	-	-	-	-
14	25	♀	W	-	4	V F	V F	-	-	-	-
15	28	♀	V	S	5	V F	V F	Urinary tract inflammation	-	-	-
16	38	♀	V	S	10	F	V F	-	-	-	-
17	38	♀	V	S	10	M F	M F	Urinary tract inflammation	Headaches, fatigue, muscle pain and numbness	Frequent bronchitis	-
18	39	♂	V	S	10	V F	V F	-	-	Bronchial asthma	-
19	29	♂	V	AC	4	V F	F	-	-	-	-
20	32	♀	V	S	6	M F	M F	-	-	-	-
21	28	♀	V	AC	4	V F	F	-	-	-	-
22	33	♀	V	S	8	V F	F	-	-	-	-
23	33	♀	V	S	5	V F	M F	-	Migraines	-	-
24	29	♀	V	S	4	V F	M F	-	-	-	-
25	34	♀	V	AC	8	V F	F	Urinary system infection	-	-	Abnormalities in the eye
26	27	♀	V	AC	3	V F	F	-	-	-	-
27	38	♂	O	-	2	V F	V F	-	-	-	-
28	35	♀	O	-	2	V F	V F	-	-	-	-

V – veterinarian; O – owner; VT – veterinary technician; VS- veterinarian student; AC – animal clinic; S – specialized animal clinic; F – Frequently; VF – very frequently; MF – Moderatly frequently; '–'– none; ♂ – male; ♀ – female

RESULTS

Using the Real-Time HRM PCR technique with SYBR Green I dye, DNA of *E. cuniculi* was detected in blood samples from 3 individuals in the study group, but was not detected in the control group. The individuals with fungal DNA detected in their blood had been in contact with rabbits for more than 2 years and had previously owned animals with active encephalitozoonosis.

The Ct values of the positive samples ranged from 28–38. Analysis of the amplicon melting curve showed that the melting temperature (Tm) of the obtained products ranged from 78.5 °C – 80.5 °C. The presence of products of the Real-Time PCR reaction was further confirmed by agarose gel electrophoresis. The size of the products referred to the mass standard was approximately 300 base pairs.

A comparison of nucleotide sequences from the isolates in the current study with reference sequences of *E. cuniculi* from the GenBank gene bank, using the DNA Star MegAligne programme, revealed that the highest similarity, approximately 100%, was found between the ITS gene fragments under study and the sequence MF062430 derived from fungi isolated from patients with postoperative wounds after hip replacement surgery (Fig. 1).

Antibodies to *E. cuniculi* were present in the samples of 4 sera collected from persons in Group 1 (1, 3, 6 and 7).

Table 2. Results obtained from Group 1

Item	Result of ELISA test	Ratio*	PCR	Sequence	Item	Result of ELISA test	Ratio*	PCR	Sequence
1	+	2.213	-	neg	**15**	-	0.840	-	neg
2	-	1.063	+	+	**16**	-	1.595	-	neg
3	+	2.351	-	neg	**17**	-	1.110	-	neg
4	-	1.127	-	neg	**18**	-	1.000	-	neg
5	-	1.383	+	+	**19**	-	0.826	-	neg
6	+	5.676	-	neg	**20**	-	0.886	-	neg
7	+	2.075	-	neg	**21**	-	0.794	-	neg
8	-	1.328	-	neg	**22**	-	1.127	-	neg
9	-	1.048	-	neg	**23**	-	1.000	-	neg
10	-	1.010	-	neg	**24**	-	0.887	-	neg
11	-	0.062	-	neg	**25**	-	0.919	-	neg
12	-	0.079	-	neg	**26**	-	0.716	-	neg
13	-	0.070	-	neg	**27**	-	0.962	-	neg
14	-	1.220	-	neg	**28**	-	1.069	+	+

* sample A_{450} EC coated/sample A_{450} control antigen coated

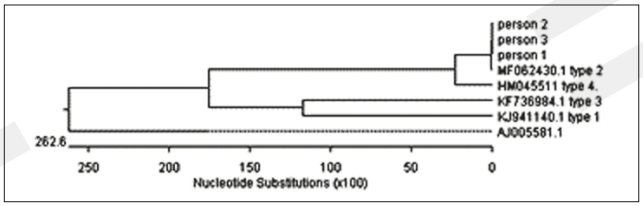

Figure 1. Phylogenetic tree showing the similarity of nucleotide sequences of the *E. cuniculi* ITS gene fragments obtained in the study

The absorbance values were 2.213, 2.351, 5.676 and 2.075, respectively. No disease symptoms were observed among any seroreactive individuals. All persons with positive serological test results were veterinarians (Tab. 2).

The results of biochemical and haematological tests showed no deviations from physiological norms in any of the persons qualified for the current study, i.e. from both Groups 1 and 2.

Statistical analysis. In the assessment of the prevalence of Encephalitozoon cuniculi infection in humans, statistical analysis performed using Fisher's exact test and Spearman's method enabled evaluation of the relationship between: permanent contact – pet owners (n = 5) or occasional contact – veterinarians involved only in the clinical examination of animals (n = 23), or the absence of such contact (n = 20), and a positive PCR result confirming E. cuniculi infection in humans. For humans in Groups I and II, statistical analysis showed no significant difference (p = 0.2553) between individuals who had contact with animals and the occurrence of a positive PCR results. In contrast, when only the group of humans who had contact with animal (Group I) was considered, a significant correlation (p = 0.0031) between the type of contact (occasional in veterinarians and permanent in animal owners) and the presence of a positive (in animal owners) or negative (in veterinarians) PCR results were noted.

DISCUSSION

Domestic animals, such as dogs, cats, rodents, and rabbits, can serve as reservoirs of *E. cuniculi*, which poses a risk to humans, and the source of infection may be contaminated food or water. Fungal spores were demonstrated to be capable of surviving for long periods in pasteurised milk derived from cows carrying *E. cuniculi*. An increasing number of potential sources of *E. cuniculi* has been found, with the parasite's DNA even detected in ticks [27, 28].

Rabbits are considered the primary source of the pathogen that affects humans, with the first cases of encephalitozoonosis in humans described in 1959 [29]. Literature data indicate that the disease primarily impacts humans with impaired immunity [30, 31], namely, patients with neoplastic diseases, such as lung cancer (8.4% of whom were tested positive for microsporidia in sputum, whereas all the results in the control group were negative), transplant recipients, HIV infected persons, the elderly and children [31–33]. Among patients after hip or knee replacement surgery, *E. cuniculi* was associated with wound-healing complications in 14.7% of 34 patients [22]. Infection in immunocompetent persons is typically subclinical [23]. Given the potential hazards that the disease poses to human health and life, it seems reasonable to conduct both molecular and serological monitoring.

Among the 28 participants in the study who had contact with rabbits, molecular testing detected fungal DNA in 3, all of whom had owned animals with an active form of the disease. The amplicon sequences revealed that, in all cases, infection with genotype II of the fungus was present. Based on the ITS (internal transcribed spacer) *E. cuniculi* genome regions, 4 genotypes of the fungus pathogenic to different species can be distinguished: genotypes I and II were isolated from humans, rabbits and rodents, donkeys and horses, genotype III from dogs and lemmings, while genotype IV was isolated from humans, cats and dogs [13, 32–35].

The detection of *E. cuniculi* DNA of genotype II, which is pathogenic to rabbits, in 3 persons who had contact with animals infected with encephalitozoonosis, may indicate that they were the source of infection in the owners. Unfortunately, in the study it was not possible to compare the amplicon sequences isolated from humans and rabbits. The animals that could have been potential sources of infection for PCR-positive persons with *E. cuniculi* died within 2 – 4 months

Table 3. Results of blood chemistry test

No.	ALBUMIN	ALP-AMP	ALT	AST	BILIRUBIN TOTAL	CALCIUM ARSENAZO	CHOL HDL DIRECT	CHOL LDL DIRECT	CHOLESTEROL	CREATININE	CRP – hs	g – GT	GLUCOSE – HK	IRON FERROZINE	MAGNESIUM	PHOSPHORUS	PROTEIN TOTAL	TRI-GLYCERIDES	UREA UV	URIC ACID
	g/L	U/L	U/L	U/L	mg/dL	mg/dL	mg/dL	mg/dL	mg/dL	mg/dL	mg/dL	U/L	mg/dL	ug/dL	mg/dL	mg/dL	g/L	mg/dL	mg/dL	mg/dL
1	49.6	68	20	15	0.56	14.58	86.4	53.6	151.7	0.51	0.5	20	85	146.0	2.22	4.24	73,0	85,7	30,1	5,85
2	55.9	74	14	19	0.97	14.91	77.0	80.2	199.2	0.78	0.5	10	95	131.2	2.58	2.77	79,1	101,9	24,8	6,48
3	47.5	63	12	16	0.75	13.78	95.1	51.2	165.9	0.72	0.7	17	88	173.0	2.26	3.72	68,9	42,7	26,4	4,24
4	46.6	46	15	15	0.45	14.64	93.6	88.6	232.6	0.76	2.3	16	67	134.2	2.44	4.95	72,9	132,3	33,8	5,67
5	51.7	58	20	17	0.92	13.79	103.9	56.8	193.2	0.70	0.4	12	100	175.2	2.16	3.70	73,6	104,3	19,0	4,17
6	45.6	50	14	17	0.42	13.77	60.3	66.5	167.3	0.70	0.7	9	87	45.4	2.26	2.81	67,2	69,6	27,5	5,73
7	46.1	63	15	15	0.65	13.49	102.0	72.0	187.0	0.63	2.8	9	78	117.9	2.05	3.65	72,5	85,1	19,8	3,91
8	42.0	43	21	13	0.40	13.59	86.8	59.8	171.2	0.70	0.7	7	140	67.9	2.03	3.81	59,7	41,9	29,5	4,33
9	48.0	123	38	25	0.22	13.51	56.5	99.3	199.1	0.35	0.7	34	90	49.9	1.91	3.20	74,4	164,7	16,9	6,15
10	39.5	44	14	13	0.15	12.97	90.5	69.3	218.8	0.64	2	11	71	69.0	2.30	3.07	58,6	115,1	27,5	4,23
11	45.4	50	15	13	0.72	13.35	85.0	64.2	177.4	0.66	1	10	94	85.8	2.04	3.99	65,0	44,9	24,1	3,28
12	48.7	55	23	19	0.46	14.4	58.8	114.5	250.8	1.11	1.3	47	83	100.1	2.34	3.48	75,1	171,2	36,6	6,03
13	46.6	46	15	15	0.45	14.64	93.6	88.6	232.6	0.76	2.3	16	67	134.2	2.44	4.95	72,9	132,3	33,8	5,67
14	45.3	79	15	15	0.86	13.47	93.7	84.8	214.5	0.59	1.7	16	74	208.1	2.10	3.86	67,9	76,3	21,2	3,89
15	50.2	67	29	24	0.53	14.19	98.4	119.8	263.4	0.98	2.9	67	124	96.1	2.07	3.14	71,9	127	27,5	8,14
16	50.1	61	14	18	0.40	13.94	86.4	102.6	249.3	0.57	0.3	10	85	94.9	1.88	3.87	71,5	102,2	25,5	3,64
17	41.5	62	13	11	0.18	13.35	98.9	41.3	160.7	0.74	0.4	10	74	60.1	2.20	3.03	72,3	71,1	22,2	4,92
18	55.0	50	19	21	0.80	14.13	90.5	29.5	133.8	0.61	0.4	16	84	151.3	1.98	3.15	77,0	53,4	18,3	4,54
19	49.1	88	24	19	0.30	13.73	58.0	116.4	228.9	1.00	0.5	42	80	62.5	2.56	2.90	69,7	150,9	25,2	7,91
20	44.9	55	9	13	0.32	13.42	75.7	81.9	192.7	0.63	0.3	8	91	57.2	2.27	4.30	68,0	64,9	35,6	4,43
standards	3.5–5.0	30–120	5–40	5–40	0.2–1.1	8.5–10.5	<130	<115	<200	0.7–1.4	0.08–3.1	5–55	70–100	10–150	1.60–2.60	2.5-5	60-80	<150	15-40	2,5-8,0

Table 4. Results cycological blood test

No.	WBC	RBC	HGB	HCT	MCV	PLT	NEUT	NEUT %	LYPH	LYPH %	MONO	MONO %	EO	EO %	BASO	BASO %
	10^3/uL	10^6/uL	g/dL	%	fL	10^3/uL	10^3/uL	%	10^3/uL	%	10^3/uL	%	10^3/uL	%	10^3/uL	%
1	5.60	5.32	15.9	48.1	90.4	258	3.03	54	1.8	32	0.53	9.5	0.17	3	0.07	1.3
2	5.93	4.19	12.9	38.8	93	277	2.67	44.9	2.49	42.1	0.51	8.6	0.22	3.7	0.04	0.7
3	7.33	4.36	12.8	37.6	86.2	245	3.7	50.6	2.73	37.2	0.77	10.5	0.09	1.2	0.04	0.5
4	3.96	4.54	13.3	42.6	93.8	201	1.98	50	1.6	39.1	0.34	8.6	0.06	1.5	0.03	0.8
5	5.19	4.24	12.1	36.6	86.3	208	2.66	51.8	1.75	34	0.47	4.3	0.22	4.3	0.04	0.8
6	6.79	4.46	14.0	41.9	93	260	3.7	54.6	2.4	35.2	0.56	8.2	0.07	1	0.05	0.7
7	3.36	3.78	12.0	36.5	96.6	226	1.47	43.8	1.5	44	0.25	7.4	0.12	3.6	0.03	0.9
8	9.21	4.84	14.3	42.5	87.8	259	6.52	70.8	1.8	19.5	0.69	7.5	0.13	1.4	0.07	0.8
9	8.34	4.43	12.8	39.5	89.2	288	4.89	58.6	2.1	25.5	0.56	6.7	0.64	7.7	0.09	1.1
10	6.27	4.78	14.9	43.7	91.4	183	3.33	53.2	2.36	37.6	0.46	7.3	0.09	1.4	0.03	0.5
11	6.15	4.52	13.2	38.9	86.1	185	3.42	55.5	1.48	24.1	0.56	9.1	0.65	10.6	0.04	0.7
12	6.95	5.39	16.2	47.2	87.6	235	3.91	56.2	1.88	27.1	0.78	11.2	0.3	4.3	0.08	1.2
13	3.74	4.06	12.3	37.4	92.1	206	1.52	40.6	1.6	42.8	0.37	9.9	0.21	5.6	0.04	1.1
14	6.01	4	12.8	38.1	95.3	329	3.22	53.5	2.2	36.1	0.43	7.2	0.15	2.5	0.03	0.5
15	4.39	4.69	14.5	40.8	87	178	2.63	59.9	1.27	28.9	0.44	10	0.03	0.7	0.02	0.5
16	5.70	5.3	15.8	46.1	87	252	3.94	69.1	1.3	22.8	0.34	6	0.07	1.2	0.05	0.9
17	5.49	4.07	11.1	35.7	87.7	310	2.9	52.8	1.9	34.8	0.45	8.2	0.15	2.7	0.07	1.3
18	6.31	4.61	13.5	39.3	85.2	156	4.07	64.5	1.32	20.9	0.45	7.1	0.42	6.7	0.05	0.8
19	6.77	4.57	13.3	39.8	87.1	277	3.27	48.3	2.77	40.9	0.62	9.2	0.09	1.3	0.02	0.3
20	5.55	5.15	15.9	46	89.3	286	2.78	50.1	2.3	41.4	0.36	6.5	0.06	1.1	0.04	0.01
Standards	4–10	3.5–5.2	12–16	37–47	82–92	150–400	1.5–7.4	70	1.1–3.5	15–40	0.03–0.8	3–8	0.2–0.67	2–4	0–0.13	0–3

prior to the commencement of the study. Interestingly, no antibodies to *Encephalitozoon* were detected in the serum of any PCR-positive individuals. This situation can be explained by the fact that the infection was diagnosed at an early stage, before the pathogen stimulated the host's immune system and specific antibodies were produced, as well as by a kind of camouflage involving the absorption of microorganisms by macrophages, and their non-presentation to the host's immune system [27]. The study performed in rabbits showed, that the results of serological testing are related to animal immune competency, circumstances of exposure, and the period prior to seroconversion. Relative to this point is that a few rabbits with lymphoma and very young rabbits have been described to be seronegative in the presence of infection [36]. The same situation may be noted in humans.

The serological test, which in addition to the physical examination, is a primary option for the diagnosis of *E. cuniculi* infection [37] and showed the presence of antibodies to *E. cuniculi* in the serum of 4 veterinarians (with a negative PCR result). No clinical signs of the disease were observed in any of them, which may suggest subclinical infection or a cross-reaction in serological tests between *E. cuniculi* antigen and antibodies to other species of the genus *Encephalitozoon* spp. [38, 39]. As indicated by literature data, the seroprevalence of *E. cuniculi* in the human population varies depending on the geographical region, ranging from 2.8% [40] in Slovakia, through 5.1–7.7% [41] in China [42], to 44.3% in Egypt [43]. The observations in the current study, despite being limited to a rather small study group, are confirmed by literature data, and indicate that persons working with animals are a risk group for developing Encephalitozoon, and, in the event of health problems, Encephalitozoonosis cuniculi should also be considered in the differential diagnosis [44]. The present study constitutes a pilot study in the context of human encephalitozoonosis caused by Encephalitozoon cuniculi infection.

Limitations of the study. The main limitations were the relatively small number of participants and the restriction of the analysis to a single microsporidian species. As outlined above, although positive ELISA results in patients could have reflected cross-reactivity between *E. cuniculi* antigens and antibodies for other microsporidia, the detection of *E. cuniculi* genetic material in the blood of the owners of affected animals may be evidence of infection. Encephalitozoon cuniculi is the principal microsporidian species infecting rodents and rabbits, which underpins the interest in the extent to which this organism may pose a risk to humans.

CONCLUSIONS

Notwithstanding the absence of clinical signs typical of encephalitozoonosis in any of the individuals in whom antibodies against *E. cuniculi* or fungal DNA were detected, infections caused by these pathogens should not be underestimated, as they may have a subclinical course and the clinical signs of the illness may be presented only in immunosuppressed patients.

In line with the concept of 'One Medicine, One Health, One World', it is advisable to conduct continuous monitoring of the occurrence of microsporidia in the environment, and to identify asymptomatic carriers of these microorganisms [45]. Diseases caused by microsporidia remain poorly studied, and the microorganisms in question are commonly found in the environment, thereby increasing the potential risk of human

and animal exposure to this pathogen. Encephalitozoonosis is a 'disease with many faces' that poses a serious hazard to animals and humans alike. Because their symptoms can be highly diverse, they are often not attributed to *E. cuniculi* infections as the primary cause. Understanding the potential clinical progression of encephalitozoonosis, as well as identifying animals that may serve as reservoirs of this pathogen, is crucial for effective diagnosis, prevention, treatment, and the protection of public health.

REFERENCES

1. Bojko J, Reinke AW, Stentiford GD, et al. Microsporidia: a new taxonomic, evolutionary, and ecological synthesis. Trends Parasitol. 2022;38(8):642–659. doi:10.1016/j.pt.2022.05.0074
2. Han B, Takvorian PM, Weiss LM. The Function and Structure of the Microsporidia Polar Tube. Exp Suppl. 2022;114:179–213. doi:10.1007/978-3-030-93306-7_86
3. Barton CE, Phalen DN, Snowden KF. Prevalence of microsporidian spores shed by asymptomatic lovebirds: Evidence for a potential emerging zoonosis. J Avian Med Surg. 2003;17(4):197–202. https://doi.org/10.1647/2002-011
4. Dalboni LC, Alvares Saraiva AM, Konno FTC, et al. Encephalitozoon cuniculi takes advantage of efferocytosis to evade the immune response. PLoS One. 2021;16(3):e0247658. Published 2021 Mar 5. doi:10.1371/journal.pone.0247658
5. Sak B, Vecková T, Brdíčková K, et al. Experimental Encephalitozoon cuniculi infection acquired from fermented meat products. Foodborne Pathog Dis. 2019;16(6):394–398. https://doi.org/10.1089/fpd.2018.2569
6. Wang S, Yao Z, Li L, et al. Seroprevalence of Toxoplasma gondii and Encephalitozoon cuniculi among domestic rabbits in central China. Parasite. 2018;25(9):1–7. https://doi.org/10.1051/parasite/2018010
7. Sobottka I, Schwartz DA, Schottelius J, et al. Prevalence and clinical significance of intestinal microsporidiosis in human immunodeficiency virus-infected patients with and without diarrhea in Germany: A prospective coprodiagnostic study. Clin Infect Dis. 1998;26(2):475–480. https://doi.org/10.1086/516328
8. Han B, Weiss LM. Microsporidia: Obligate Intracellular Pathogens Within the Fungal Kingdom. Microbiol Spectr. 2017;5(2):10.1128/microbiolspec.funk-0018-2016. doi:10.1128/microbiolspec.FUNK-0018-2016
9. Künzel F, Fisher PG. Clinical signs, diagnosis, and treatment of Encephalitozoon cuniculi infection in rabbits. Vet Clin Exot Anim Pract. 2018;21(1):69–82. https://doi.org/10.1016/j.cvex.2017.08.002
10. Li X, Palmer R, Trout JM, et al. Infectivity of microsporidia spores stored in seawater at environmental temperatures. J Parasitol. 2003;90(3):654–657. https://doi.org/10.1645/0022-3395(2003)089
11. Li J, Shi K, Sun F, et al. Identification of human pathogenic Enterocytozoon bieneusi, Cyclospora cayetanensis, and Cryptosporidium parvum on the surfaces of vegetables and fruits in Henan, China. Int J Food Microbiol. 2019;307:108292. doi:10.1016/j.ijfoodmicro.2019.108292
12. Javanmard E, Mirjalali H, Niyyati M, et al. Molecular and phylogenetic evidences of dispersion of human-infecting microsporidia to vegetable farms via irrigation with treated wastewater: One-year follow up. Int J Hyg Environ Health. 2018;221(4):642–651. doi:10.1016/j.ijheh.2018.03.007
13. Kváč M, Tomanová V, Samková E, et al. Encephalitozoon cuniculi in raw cow's milk remains infectious after pasteurization. Foodborne Pathog Dis. 2016;13(2):77–79. https://doi.org/10.1089/fpd.2015.2036
14. Brdíčková K, Sak B, Holubová N, et al. Encephalitozoon cuniculi Genotype II Concentrates in Inflammation Foci. J Inflamm Res. 2020;13:583–593. Published 2020 Sep 25. doi:10.2147/JIR.S271628
15. Han B, Pan G, Weiss LM. Microsporidiosis in Humans. Clin Microbiol Rev. 2021;34(4):e0001020. doi:10.1128/CMR.00010-20
16. Doshi N, Thet Z, Han T, et al. A Case of Intestinal Microsporidiosis in a Renal Transplant Recipient. J Med Cases. 2019;10(8):229–233. doi:10.14740/jmc3340
17. Brown M, Longano A, Dendle C, et al. Confirmed microsporidial graft infection in a HIV-negative renal transplant recipient: A case report and review of the literature. Transpl Infect Dis. 2018;20(3):e12888. doi:10.1111/tid.12888
18. Shah S, Jacob SS, Mani R, et al. Renal Microsporidiosis in Pediatric Bone Marrow Transplant Recipients: A Case Series. Renal Microsporidiosis in Pediatric Bone Marrow Transplant Recipients: A Case Series. Turk Patoloji Derg. 2020;36(1):68–72. doi:10.5146/tjpath.2017.01416
19. Smith RM, Muehlenbachs A, Schaenmann J, et al. Three Cases of Neurologic Syndrome Caused by Donor-Derived Microsporidiosis. Emerg Infect Dis. 2017;23(3):387–395. doi:10.3201/eid2303.161580
20. Wesołowska M, Szetela B, Kicia M, et al. Dual infection of urinary tract with Enterocytozoon bieneusi and Encephalitozoon cuniculi in HIV/AIDS patients. Ann Parasitol. 2019;65(1):77–81. https://pubmed.ncbi.nlm.nih.gov/31127886/
21. Weiss LM. 2020. Microsporidiosis, p 825–831. In Ryan ET, Hill DR, Solomon T, Aronson N, Endy TP (ed), Hunter's tropical medicine and emerging infectious diseases. Elsevier, Amsterdam, Netherlands.
22. Sak B, Gottliebová P, Nyčová E, et al. Microsporidia (Encephalitozoon cuniculi) in patients with degenerative hip and knee disease, Czech Republic. Emerg Infect Dis. 2024;30(3):469–477. https://doi.org/10.3201/eid3003.231263
23. Ditrich O, Chrdle A, Sak B, et al. Encephalitozoon cuniculi genotype I as a causative agent of brain abscess in an immunocompetent patient. J Clin Microbiol. 2011;49(7):2769–2771. https://doi.org/10.1128/JCM.00620-11
24. Stentiford GD, Becnel J, Weiss LM, et al. Microsporidia – Emergent Pathogens in the Global Food Chain. Trends Parasitol. 2016;32(4):336–348. doi:10.1016/j.pt.2015.12.004
25. Ruan Y, Xu X, He Q, et al. The largest meta-analysis on the global prevalence of microsporidia in mammals, avian and water provides insights into the epidemic features of these ubiquitous pathogens. Parasit Vectors. 2021;14(1):186. Published 2021 Apr 1. doi:10.1186/s13071-021-04700-x
26. Wilczyńska A, Komsta R, Szadkowski M, et al. Prevalence of Encephalitozoon cuniculi Infection in Guinea Pigs (Cavia porcellus) in Poland with Different Clinical Disorders-A Pilot Study. Animals (Basel). 2023;13(12):1992. Published 2023 Jun 14. doi:10.3390/ani13121992
27. Sak B, Fibigerová M, Mravcová K, et al. Tick-borne microsporidiosis: ticks as a neglected source of human microsporidian infections? Emerg Microbes Infect. 2024;13(1):1–3. https://doi.org/10.1080/22221751.2024.2384472
28. Keeble E, Künzel F, Montiani-Ferreira F, et al. Encephalitozoonosis in Pet Rabbits: Epidemiology, Pathogenesis, Immunology and Consensus on Clinical Management. Animals (Basel). 2026;16(2):346. Published 2026 Jan 22. doi:10.3390/ani16020346
29. Matsubayashi H, Koike T, Mikata I, et al. A case of Encephalitozoon-like body infection in man. AMA Arch Pathol. 1959;67(2):181–187.
30. Wang ZD, Liu Q, Liu HH, et al. Prevalence of Cryptosporidium, microsporidia and Isospora infection in HIV-infected people: a global systematic review and meta-analysis. Parasit Vectors. 2018;11(1):28. Published 2018 Jan 9. doi:10.1186/s13071-017-2558-x
31. Asghari A, Mohammadi MR, Norouzi R, et al. A global overview of microsporidia infection in HIV/AIDS patients: an updated systematic review and meta-analysis. Int Health. Published online January 28, 2026. doi:10.1093/inthealth/ihaf163
32. Ulusan Bagci O, Muftuoglu C, Guldaval F, et al. Molecular Prevalence of Microsporidia Infection in Patients with Lung Cancer. Am J Trop Med Hyg. 2023;108(5):895–900. Published 2023 Mar 20. doi:10.4269/ajtmh.22-0506
33. Seatamanoch N, Kongdachalert S, Sunantaraporn S, et al. Microsporidia, a Highly Adaptive Organism and Its Host Expansion to Humans. Front Cell Infect Microbiol. 2022;12:924007. 2022 Jun 16. doi:10.3389/fcimb.2022.924007
34. Pelin A, Moteshareie H, Sak B, et al. The genome of an Encephalitozoon cuniculi type III strain reveals insights into the genetic diversity and mode of reproduction of a ubiquitous vertebrate pathogen. Heredity (Edinb). 2016;116(5):458–465. doi:10.1038/hdy.2016.4
35. Panek J, Sendra K, Glenwright H, et al. Microsporidian obligate intracellular parasites subvert autophagy of infected mammalian cells to promote their own growth. MBio. 2025;(e01049–25):1–15. https://doi.org/10.1101/2022.08.15.503970
36. Csokai J, Joachim A, Gruber A, et al. Diagnostic markers for encephalitozoonosis in pet rabbits. Vet Parasitol. 2009;163(1–2):18–26. doi:10.1016/j.vetpar.2009.03.057
37. Montiani-Ferreira F, Joachim A, Künzel F, et al. Encephalitozoon cuniculi Infection in Rabbits (Oryctolagus cuniculus): Data from an International Survey of Exotic and Small Animal Veterinarians. Animals (Basel). 2024;14(22):3295. Published 2024 Nov 15. doi:10.3390/ani14223295
38. Jordan CN, Zajac AM, Snowden KS, Lindsay DS. Direct agglutination test for Encephalitozoon cuniculi. Vet Parasitol. 2006;135(3–4):235–240. doi:10.1016/j.vetpar.2005.09.009

39. Ghosh K, Weiss LM. Molecular diagnostic tests for microsporidia. Interdiscip Perspect Infect Dis. 2009;2009:926521. doi:10.1155/2009/926521
40. 40 Malčeková B, Valenčáková A, Molnár L, et al. First detection and genotyping of human-associated microsporidia in wild waterfowl of Slovakia. Acta Parasitol. 2013;58(1):13–17. https://doi.org/10.2478/s11686-013-0108-z
41. Luptáková L, Petrovová E. The occurrence of microsporidial infections and toxoplasmosis in Slovak women. Epidemiol Microbiol Immunol. 2011;60(4):156–160. https://pubmed.ncbi.nlm.nih.gov/22324244/
42. Pan Y, Wang S, Liu X, et al. Seroprevalence of Encephalitozoon cuniculi in humans and rabbits in China. Iran J Parasitol. 2015;10(2):290–295. https://pubmed.ncbi.nlm.nih.gov/26246829/
43. Abu-Akkada SS, El Kerdany EDH, Mady RF, et al. Encephalitozoon cuniculi infection among immunocompromised and immunocompetent humans in Egypt. Iran J Parasitol. 2015;10(4):561–570. https://pubmed.ncbi.nlm.nih.gov/26811722/
44. Santaniello A, Cimmino I, Cimmino L, et al. Zoonotic risk of Encephalitozoon cuniculi in animal-assisted interventions: laboratory strategies for the diagnosis of infections in humans and animals. Int. J. Environ. Res. Public Health 2021;18(17): 9333. https://doi.org/10.3390/ijerph18179333
45. Abdoli A, Olfatifar M, Zaki L, et al. The global prevalence of microsporidia infection in rabbits as a neglected public health concern: A systematic review and meta-analysis. Prev Vet Med. 2025;234:106380. doi:10.1016/j.prevetmed.2024.106380